MECHANISMS OF IMPAIRED DIURETIC RESPONSIVENESS IN CHRONIC HEART FAILURE

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Abstract

A series of 7 studies was performed on patients with chronic heart failure to determine more fully the mechanisms of decreased responsiveness to diuretics typically found in this condition. Furosemide, a diuretic agent acting on the thick ascending limb of the loop of Henle, was chosen for all the studies which follow. A steady diuresis was achieved by repeated bolus injections of furosemide.

In the first study described in Chapter 3, the effect of dietary salt restriction on diuretic responsiveness was assessed. It was found that sodium restriction impaired the diuretic and natriuretic response to intravenous furosemide.

The effect of a single acute-on-chronic oral dose of captopril, an angiotensin converting enzyme inhibitor, was compared with placebo, see Chapter 4. Captopril enhanced the natriuresis and diuresis produced by furosemide, despite causing a fall in blood pressure and glomerular filtration rate.

Dobutamine and sodium nitroprusside, agents which may improve cardiac output in heart failure, but by different mechanisms, were compared in the next study described in Chapter 5. Dobutamine potentiated furosemide induced natriuresis but had no effect on diuresis. Sodium nitroprusside diminished the diuretic response to furosemide, when compared with dobutamine, but did not significantly affect sodium excretion.

Digoxin was administered intravenously and increased natriuresis but not diuresis compared to placebo, see Chapter 6.

Atrial natriuretic peptide was infused at low, then high doses. Chapter 7 describes how this produced no significant effect on

furosemide induced diuresis and natriuresis, although a prolonged hypotensive response was observed.

The effect of indirect sympathetic nervous system activation by tyramine and alpha-adrenergic inhibition by phentolamine were compared. As Chapter 8 shows, neither agent produced any effect on diuresis or natriuresis.

In the last study, the relative diuretic and natriuretic effects of dopamine and dobutamine were compared, see Chapter 9. High dose dobutamine and dopamine enhanced furosemide induced natriuresis but did not significantly affect urine volume.

It is argued that agents which produce hypotension tend to acutely impair diuresis and natriuresis in response to intravenous furosemide in patients with heart failure, despite improvement in renal plasma flow and cardiac output or apparently beneficial alterations in plasma hormone concentrations. The only exception found was the response to captopril. Thus chronic supression of angiotensin converting enzyme modifies the usual renal response to a fall in arterial pressure and glomerular filtration rate associated with acute, intense suppression of angiotensin II.

The work presented in this thesis has formed a detailed, but by no means comprehensive examination of mechanisms of reduced renal responsiveness to furosemide in patients with heart failure. The interrelationship between the sympathetic nervous system, the renin-angiotensin-aldosterone system, the atrial natriuretic peptides and the systemic and renal haemodynamic status as they relate to diuretic responsiveness in patients with chronic heart failure have also been explored.

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Chapter 1 Introduction, historical review and aims

1.1 General introduction

Heart failure is a clinical syndrome recognised by a constellation of symptoms and signs, resulting from deranged cardiac function. Impaired pump function leads to activation of the sympathetic nervous system, the renin-angiotensin-aldosterone system, natriuretic peptides, endothelin-I, arginine vasopressin, kinins and renal prostaglandins (Dusting, Moncada, Vane 1979; Dzau, Packer, Lilly, et al 1984b; Lilly, Dzau, Williams, et al 1984; Francis 1985a, Creager, Faxton, Cutler, et al 1986; Laragh 1986; Rieger, Kromer, Kochsiek 1986a; Mancia 1990; Hiroe, Hirata, Fujita, et al 1991; Remes, Tikkanen, Fyhrquist, et al 1991).

The fall in cardiac output and decreased renal perfusion provide a powerful stimulus to activation of salt-retaining neuroendocrine systems (DiBona 1977; Cannon 1977; Cody 1989; Eiskjaer, Bagger, Danielsen, et al 1991). Compensatory retention of salt and water initially restores cardiac output and renal perfusion, at the expense of further load on the already weakened heart muscle. Declining cardiac function and activation of neuroendocrine systems leads to a vicious cycle of fluid retention and worsening heart failure. The loss of compensatory vasodilator influences, both in the blood vessels and in the kidneys, aggravates peripheral vasoconstriction and sodium retention and adds to the haemodynamic burden of the failing heart (Packer 1992).

In the present chapter conventional treatments for heart failure will be reviewed with special attention to their effects on salt and water retention. The role of activation of various neuroendocrine systems in the pathological

sodium and water retention of heart failure will be explored.

1.1.1 The kidney

The primary role of the kidney is to maintain the composition of the extracellular fluid within very narrow margins. This is achieved by regulation of both the volume and the composition of the fluid excreted as urine.

Vascular supply of the kidney

In man, each kidney weighs approximately 300g and contains some 1000-000 nephrons. Together they receive one fifth of the resting cardiac output, nearly 90% of which supplies the renal cortex. The main renal artery divides into anterior and posterior branches within the renal hilum just before entering the parenchyma. The anterior branch divides into 4 segmental arteries which together supply two thirds of the kidney. Segmental divisions of the posterior branch supply the posterior surface of the kidney.

The segmental arteries divide into interlobar arteries within the medulla. They ascend towards the renal cortex. At the cortico-medullary junction they give off arcuate arteries which run parallel to the surface of the kidney. The arcuate arteries then divide and ascend as cortical radial arteries towards and perpendicular to the renal capsule. Afferent arterioles branch directly from these vessels, carrying blood to glomerular capillaries which are distributed throughout the cortex. Cells and plasma exit the glomerular circulation not in venules but in efferent arterioles. Efferent arterioles from more superficial glomeruli give rise to the post glomerular, cortical peritubular microcirculation. Efferent arterioles from deeper glomeruli descend into the medulla. There they form the outer medullary peritubular capillary microcirculation and the vasa recta. The vasa recta accompany the

thin limbs of Henle's loops into the inner medulla.

The true venous circulation of the kidney begins as blood leaves the capillaries of the peritubular networks and the vasa recta. Venules from all cortical regions join to form cortical radial veins and these descend towards the cortico-medullary junction. Here they are joined by veins draining the medullary microcirculations. There are multiple anastomoses between veins at all levels of the venous circulation.

Autoregulation of renal vascular tone

Renal vascular tone is dynamic and can respond rapidly to a fall in renal perfusion with vasodilatation. This effect is most evident at renal perfusion pressures of 50-60mmHg. Below this range, renal blood flow diminishes in proportion to the fall in perfusion pressure. The myogenic reflex, a property of the renal arterioles to respond to a change in wall tension by dilating (in response to a fall in tone) or constricting (in response to a rise in tone) is rapid and appears to be an intrinsic property of the vessel wall. When mean systemic arterial pressure falls below 60mmHg, adequate renal perfusion can no longer be maintained and urine formation declines then ceases (Guyton 1991).

Hormonal and neural control of renal vascular tone

The renin-angiotensin-aldosterone system

The predominant sites of action of vasoactive compounds and the sympathetic nervous system are the afferent and efferent arterioles. The renin-angiotensin-aldosterone system plays a major role in both renal haemodynamics and regulation of salt and water balance. Angiotensin II is formed by a series of steps initiated by renin released from the kidney.

Renin acts upon angiotensinogen (synthesised in the liver) to form angiotensin I, which is subsequently converted to angiotensin II by the action of angiotensin converting enzyme, primarily within the pulmonary circulation. Angiotensin II is one of the most potent vasoconstrictor substances known and is also a specific stimulator of the release of aldosterone, a sodium-retaining hormone. In addition to the systemic pressor effects of angiotensin II, the local renal actions promote glomerular filtration. This is because angiotensin exerts more pronounced constrictor effects on the efferent than the afferent arterioles (Ichikawa, Miele, Brenner 1979), an effect which raises the pressure upstream in the glomerular capillary. The net effect of angiotensin II formation is to raise blood pressure and expand the intravascular volume. Renin is stored within the granules of specialised myoepithelial cells called juxtaglomerular cells located in the walls of the afferent arterioles. The rate of release is influenced by a fall in renal perfusion pressure, sensed by the renal baroreceptors located in the arterial wall. The sympathetic nervous system and circulating catecholamines influences renin release through beta adrenergic receptors (Davis 1973; Davis, Freeman 1976; Thames 1984). The composition of the fluid (pre-urine) delivered to the distal renal tubule also influences the rate of renin release. In addition there is a negative feedback from the consequences of renin release, that is a rise in blood pressure, intravascular volume and improved renal perfusion. Angiotensin II itself inhibits renin release (Wathen, Kingsbury, Stouder, et al 1965; Bunag, Page, McCubbin, et al 1967).

Circulating catecholamines and the renal nerves

The kidneys are well supplied with nerves from the coeliac plexus which innervate major intrarenal arteries, afferent arterioles and to a lesser extent,

the efferent arterioles, tubule structures and glomerular mesangium. As mentioned above, β-adrenergic stimulation activates the renin-angiotensin system but α1-adrenergic stimulation (and to a lesser effect, post-synaptic α2-adrenergic receptor stimulation) causes marked vasoconstriction of the interlobular arteries and the afferent arterioles and a fall in renal blood flow. Stimulation of the vasomotor centre in the medulla, parts of the cerebral cortex and parts of the brain stem results in similar vasoconstriction. Under resting conditions, renal neural stimulation is low and has little influence on renal function. In low output disease states such as heart failure, sodium depletion or haemorrhage, significant neurally mediated vasoconstriction can result from an increase in sympathetic neural stimulation (Levine, Francis, Goldsmith, et al 1982; DiBona, Sawin 1983; DiBona 1994a; DiBona, Sawin 1994b).

Dopamine is an endogenous renal neurotransmitter with specific dopamine receptors. It produces vasodilatation and an increase in renal blood flow at low doses but at larger doses also stimulates adrenergic receptors, thus causing vasoconstriction (Rosenblum, Tai, Lawson 1972; Robie, Goldberg 1975; Miller 1984).

Renal prostaglandins

Membrane-bound phospholipid is metabolised locally to prostaglandins via the cyclo-oxygense pathway (Dunn, Hood 1977). The vasodilator prostaglandins prostaglandin E_2 and prostacyclin are the primary vasoactive end products of arachidonic acid metabolism in the kidney, while thromboxane, a powerful vasoconstrictor, is produced to a much lesser extent. Production of renal prostaglandins is enhanced by renal

vasoconstrictors such as angiotensin II (Walker, Whorton, Smigel, et al 1978), arginine vasopressin (Usberti, Dechaux, Guillot 1980), endothelin 1 (Leppaluoto, Ruskoaho 1992), and α-adrenergic neural stimulation (Flamenbaum, Kleinman 1977; Needleman, Wycke, Bronson, et al 1979; Stahl, Paravicini, Schollmeyer 1984). Production is inhibited by cyclo-oxygenase inhibitors (non-steroidal anti inflammatory drugs and aspirin). Like the neural control of renal blood flow, prostaglandins exert little or no influence during basal conditions, but play a major part in maintaining renal blood flow during low output states (Dunn, Hood 1977; Iino, Imai 1978; Dusting, Moncada, Vane, 1979; Schor, Ichikawa, Brenner 1980; Chiu, Brown, Barnett 1984; Dzau, Packer, Lilly, et al 1984b; Gottlieb, Robinson, Krichten, et al 1992a; Motwani, Fenwick, Struthers 1992c).

A number of other vasodilator substances have been identified, including the kinins bradykinin and kallidin (released from inert precursor kininogens by the action of the proteolytic enzyme kallikrein), histamine, acetylcholine, serotonin and parathyroid hormone but their precise roles, if any, in regulation of renal blood flow are unknown (Münzel, Kurz, Holtz, et al 1992). The atrial natriuretic peptides are a group of peptides released primarily from the atrium and have vasorelaxant effects in addition to their powerful diuretic and natriuretic effects. They will be discussed later.

Structure and function of the nephron

Nephrons are described as superficial, mid-cortical, or juxtamedullary nephrons, depending on their origins from a superficial, midcortical or juxtamedullary glomerulus. They are also described as short-looped when Henle's loop turns back in the outer medulla (or cortex); cortical nephrons when Henle's loop turns back in the cortex and long-looped when Henle's

loop turns back in the inner medulla.

Each nephron consists of the renal corpuscle and the tubular portions of the nephron. The renal corpuscle comprises Bowman's capsule, the glomerulus (glomerular tuft) and the urinary space (Bowman's space). The glomerulus is composed of glomerular lobules.

The tubular portions of the nephron are divided into the proximal tubule, the intermediate tubule and the distal tubule. The tubular portion is separately and independently subdivided on microanatomical grounds into the proximal convolution, the loop of Henle (long loop, short loop, cortical loop) and the distal convolution. Thus the loop of Henle comprises the straight part of the proximal tubule, the intermediate tubule and the straight part of the distal tubule.

The renal corpuscle

The renal corpuscle consists of a tight bundle of capillaries situated in the proximal end of the renal tubule, also known as Bowman's capsule. The capillary walls are in intimate contact with specialised epithelial cells or podocytes, separated only by an acellular basement membrane. The endothelial cells of the capillaries are separated from one another by small gaps or fenestra and the epithelial cells of the tubule protrude numerous foot processes to cover the outer surface of the glomerular capillary loop, separated by slit-like diaphragms (Bulger, Dobyan 1982).

About 180 litres of fluid are filtered by the glomeruli per day but less than 1% is excreted as urine (Brenner, Beeuwkes 1978; Ganong 1987). The fluid is driven out of the capillaries and into the tubule by hydraulic pressure. The

oncotic pressure tends to pull fluid back into the capillary and the balance between the two determines the rate of filtration (Brenner, Coe, Rector 1986). Tubule fluid protein concentration is minimal, thus the oncotic pressure within the urinary space is negligible. In certain disease states, hyperproteinaemia elevates plasma oncotic pressure and thus reduces glomerular filtration. Likewise, urinary outflow obstruction raises the hydraulic pressure in the tubule and reduces glomerular filtration. As mentioned earlier, filtration can be enhanced in individual renal corpuscles by selectively constricting the efferent arteriole and thus raising capillary hydraulic pressure (angiotensin II). Contraction of the supporting cells or mesangium in the renal corpuscles in response to vasoconstrictor substances reduces the surface area available for filtration.

Tubuloglomerular feedback

The rate of fluid delivery to the area of the early distal tubule known as the juxtaglomerular apparatus also regulates glomerular dynamics. The juxtaglomerular apparatus consists of granular cells, extraglomerular mesangium and the macula densa. The macula densa is anatomically in close proximity to the efferent arteriole and the renal corpuscle and releases renin in response to reduced perfusion pressure, renal sympathetic nerve stimulation or altered delivery of sodium to the juxtaglomerular apparatus region of the distal tubule (Davis, Freeman 1976; Keeton TK, Campbell WB 1980; Kurtz, Bruna, Pratz, et al 1988; Osborn, DiBona, Thames 1991; Persson, Gushwa, Blantz 1984). High distal tubule fluid flows and salt delivery rates produce a fall in glomerular filtration, largely as a result of increases in afferent and efferent arteriolar resistance. The precise mechanism is not certain but may be related to angiotensin II induced contraction of mesangial cells, thereby reducing the calibre of the arterioles

(Ausiello, Kreisberg, Roy, et al 1980; Barnes, Guy, Lifschitz, et al 1981). This mechanism is very important as fluid transport mechanisms can be overwhelmed by excessive distal delivery of fluid and solutes with resultant excessive urinary losses.

The passage of solutes larger than 20 Angstrom molecular radius across the glomerular wall is limited (Brenner, Beeuwkes 1978), consistent with the presence of "pores" of specific size and configuration within the membrane. There is also a charge-selective barrier because of the fixed negative charges within the structural proteins of the surface coat of the epithelial foot processes and the slit diaphragms lying between the adjacent foot processes (Bulger, Dobyan 1982). Negatively charged macromolecules are impeded and positively charged ones facilitated in their passage across the capillary wall compared with neutral compounds of the same size (Brenner, Beeuwkes 1978).

In humans the maximum urine flow is about 1.5 l/hour (20% of the GFR or 36 litres per 24 hours). The remaining 144 liters of plasma filtered per day are coupled with solute reabsorption. This occurs primarily in the proximal tubule and the thin descending limb of the loop of Henle (intermediate tubule). The distal tubule and collecting tubule segments are not entirely impermeable to water thus some water reabsorption also occurs here.

The lowest osmolality achievable is finite because of obligatory solute excretion, because of the failure of the salt reabsorptive processes and because of the finite tubule water permeability. Likewise there is an obligatory 600mOsm of waste products to excrete per day and because the maximum concentrating power of the human kidney is 1300mOsm, the

minimum urine output per day is about 500ml.

The peritubular microcirculation

Fluid is returned to the circulation in a two-stage process which involves translocation of fluid and solute into the renal interstitium with subsequent uptake into the peritubular capillary blood. About two thirds of the fluid reabsorption takes place in the convoluted and straight portions of the proximal tubule, and the cortical peritubular capillary network lies in close proximity to these nephron segments. The blood in these capillaries has a high oncotic pressure, having already passed through the glomerulus, and a low hydraulic pressure because of the tone of the efferent arteriole (Berry, Rector 1980). Fluid reabsorption is thus favoured, indeed equilibrium is not reached at the distal end of the capillary network (Berry 1983). The precise composition of the reabsorbed fluid is determined by the selective transport of individual solutes across the renal epithelial cells. The renal corpuscular mechanisms which preserve glomerular filtration when renal perfusion is impaired also mitigate against excessive urinary loss of essential body fluids by augmenting the proportion of filtered water and solutes reabsorbed in the proximal nephron. As efferent artery constriction raises filtration pressure, more fluid is filtered and the oncotic pressure in the efferent arteriole is higher. The flow of blood into the cortical peritubular capillary network is reduced and slower flow allows time for complete reabsorption of solute (Brenner, Beeuwkes 1978).

The medullary microcirculation

The tubules and vascular supply to the medulla are arranged in close anatomic proximity and with precise alignment of ascending and descending tubules and vessels (Bulger, Dobyan 1982). This arrangement

is essential for the renal concentrating mechanism. A gradient of increasing interstitial solute concentration exists from the cortico-medullary junction to the papillary tip to allow maximum urinary concentration. The concentration gradient is maintained by the renal countercurrent exchange system. The hairpin shape of the tubule and of the vasa recta allow a gradient to be maintained between the cortico-medullary junction and the deeper medulla while the permeability properties of different parts of the tubule allow the gradient to be generated (Kokko, Rector 1972; Brenner, Beeuwkes 1978; Kokko 1979).

Urine is concentrated by countercurrent multiplication and countercurrent exchange, a complex interaction involving the loops of Henle, the medullary interstitium, the medullary blood vessels or vasa recta and the collecting tubule (Brenner, Beeuwkes 1978; Kokko 1979). Arginine vasopressin, a hormone excreted by the anterior pituitary, regulates concentration of urine in the medulla by altering the permeability of the distal renal tubules and collecting ducts to water (Kachdorian, Wade, DiScala 1975; Dousa, Valtin 1976; Wade 1985). The release of arginine vasopressin is stimulated by very small (1-2%) increases in plasma osmolality as well as by volume depletion (Morton, Connell, Hughes, et al 1985). The circulating hormone binds to specific receptors in the basal and lateral aspects of the distal tubules and collecting ducts and activates adenylate cyclase (Hays 1976; Levine, Franki, Einhorn, et al 1976; Wade 1985). The formation of cyclic AMP leads to phosphorylation of specific membrane proteins and rearrangement of the microtubules and microfilaments of the tubular cells to form pores (Schwartz, Schaltz, Kinne-Saffran, et al 1974; Kachadorian, Wade, DiScala 1975; Taylor, Maffly, Wilson, et al 1975; Wade 1985). The tubule becomes freely permeable to water, allowing it to diffuse passively

into the hypertonic renal medullary interstitium (Kokko 1979).

Countercurrent multiplication occurs in the loop of Henle and requires specific permeability and transport characteristics of the descending and ascending limbs. When the preurine reaches the loop of Henle, two thirds of the water and solute has already been reabsorbed. The remaining third which is still isosmotic to plasma, passes down the thin descending limb deep into the medulla. Being highly water permeable and virtually impermeable to salt and urea, passive water equilibration with the hypertonic medullary interstitium results in concentration of the pre-urine. The hypertonicity of the medullary interstitium is provided in equal parts by urea and sodium chloride (Kokko, Rector 1972; Jamison 1976; Kokko 1979; Brenner 1986). Thus the fluid entering the thin ascending limb has a higher sodium concentration and a lower urea concentration than the surrounding interstitium. The thin ascending limb is relatively more permeable to sodium chloride than to urea and is impermeable to water, thus sodium moves out of the tubule down its concentration gradient with a smaller amount of urea moving into the tubular fluid. The net effect is to dilute the tubular fluid and concentrate the medullary interstitium. The fluid passes up into the thick ascending limb which is also impermeable to water. Active chloride transport provides the energy for passive diffusion of sodium out of the thick ascending limb along the electrochemical gradient and further dilutes the fluid (Kokko 1979). Urea remains in the fluid as the thick ascending limb is relatively impermeable to it. Fluid entering the distal convoluted tubule is dilute, but relatively rich in urea. The tubule segment is impermeable to both water and urea so the fluid passes unaltered into the collecting duct (Brenner, Beeuwkes 1978; Kokko 1979) .

When concentrations of arginine vasopressin are high, the collecting duct is highly permeable to water, which diffuses freely into the hypertonic interstitium, resulting in a concentrated urine (Dousa, Valtin 1976; Hays 1976). When arginine vasopressin concentrations are low, the collecting duct is impermeable to water and highly dilute urine can be formed. The initial portion of the collecting duct is relatively impermeable to urea, thus with water equilibration, pre-urine reaching the terminal segments is rich in urea. It dissipates down its concentration gradient into the papillary interstitium, maintaining the hypertonicity of the medulla.

Countercurrent exchange

The hypertonicity generated in the papillary interstitium would rapidly be washed away by the blood flowing through it if it were not for the countercurrent exchange system in the vasa recta. Water and solutes are freely permeable across the capillaries and rapid equilibration occurs. Because the vasa recta follow the same hairpin bend that the loop of Henle does, and ascending and descending portions of the vasa recta are in close proximity, at any level of the loop, the concentrations in ascending and descending limbs are the same. This allows a gradual rise in concentration gradient from the top to the bend in the loops and because of their length, for substantial concentration gradients to develop between the bottom and top of the loops, without solute washout (Kokko 1979; Brenner, Beeukwes 1978; Brenner 1986).

Renal sodium reabsorption

99.4% of filtered sodium is reabsorbed, and all parts of the tubule and collecting duct play a part. In the proximal and distal tubules and in the collecting ducts, sodium diffuses passively down its concentration gradient

and electrical gradient from the tubular lumen into the tubular epithelial cells. Sodium is actively pumped from the epithelial cells into the interstitial space in all parts of the tubule except the thin portions of the loop of Henle (Herbert, Schafer, Andreoli 1981; Ganong 1987).

60% of filtered sodium is reabsorbed in the proximal tubule, powered mainly by the Na+-K+-ATPase pump system located in the basolateral membrane (Kokko 1979; Berry, Rector 1980; Ganong 1987). There are two phases for the transport of sodium from the proximal tubule. Initially it combines with specific carrier complexes using the energy derived from the electrical and/or chemical concentration gradient for sodium across the luminal membrane (Aronson 1981). This gradient is maintained by the Na^+-K^+-ATPase pump system (Berry, Rector 1980). The sodium-coupled reabsorption of glucose and amino acids in the early proximal tubule generates a lumen-negative potential difference in this part of the tubule (Kokko 1973; Barratt, Rector, Kokko, et al 1974; Fromter, Gessner 1974; Burg, Patlak, Green, et al 1976). In the latter part of the proximal tubule a positive potential difference is generated by the high chloride and low bicarbonate concentrations relative to the peritubular plasma (Berry, Rector 1980; Rector 1983). This serves to accelerate sodium movement out of the tubule. Movement from the renal interstitium into the peritubular capillaries is governed by Starling's forces (Rector 1983). The peritubular oncotic pressure is governed by the percentage of renal plasma flow which becomes glomerular ultrafiltrate. This usually amounts to 20%, however when more is filtered, the peritubular capillary oncotic pressure is increased proportionally and subsequently more fluid and solute is reabsorbed from the proximal tubule.

The distal tubule

Dilution of the distal tubule fluid occurs irrespective of the state of water balance as described earlier (countercurrent multiplication). Sodium reabsorption in the thick ascending limb of the loop of Henle is a secondary active transport system similar to that in the proximal tubule, but a lumen-positive potential difference is generated (Ganong 1987). The thick ascending limb of Henle's loop contains a cotransport mechanism for K^+ and Na^+ with two Cl ions using energy from the chemical concentration gradient for sodium and chloride. The gradient is maintained by the Na^+-K^+-ATPase pump. A parallel potassium diffusion channel replaces the tubular potassium. This mechanism is highly sensitive to loop diuretics such as furosemide, which act only from the lumen. Furosemide acts by combining with the symporter in the luminal membrane and preventing its translocation into the cell (Greger 1985; Wittner, DiStefano, Schlatter, et al 1986; Giebisch, Klein-Robbenhaar 1993a; Giebisch, Klein-Robbenhaar, Klein-Robbenhaar, et al 1993b). The distal convoluted tubule continues active dilution of the tubule fluid using the Na^+-K^+-ATPase pump. It generates a lumen-negative potential difference and has the highest activity of Na^+-K^+-ATPase of any of the nephron segments (Bulger, Dobyan 1982). It is highly impermeable to water thus the fluid becomes further diluted.

Collecting system

The collecting system comprises the connecting tubule, the cortical collecting duct, the outer medullary collecting duct and the inner medullary collecting duct. The final regulation of urinary solute and water excretion takes place in the collecting system.

The connecting tubule connects the distal convoluted tubule to the collecting duct and is functionally different from both. It shares some characteristics with the distal convoluted tubule, being relatively impermeable to water, with high Na^+-K^+-ATPase activity (Ganong 1987). It contains two cell types, the connecting tubule cells and the intercalated cells. The collecting duct has two cell types, the collecting duct cells or principal cells and the intercalated cells (Bulger, Dobyan 1982). The intercalated cells contain numerous large mitochondria and organelles while the principal cells have lighter cytoplasm and fewer organelles. In addition they possess a single long cilium projecting into the lumen from an apical membrane. As the collecting duct passes from cortical to medullary to papillary locations in the kidney, the number of intercalated cells decrease and the principal cells increase, with a dramatic fall in the carbonic anhydrase content of these cells (Bulger, Dobyan 1982). Sodium is reabsorbed from the collecting tubule to very low levels (1mEq during periods of salt deprivation) while chloride reabsorption is retarded, generating a large lumen-negative electrical potential difference.

An increase in the filtered load of Na and Cl ions to the loop of Henle stimulates NaCl transport, as does vasopressin, cell volume reduction, adrenergic agonists, and aldosterone (Ganong 1987). The opposite occurs after prostaglandin E2 administration, medullary interstitial hypertonicity and in the response to high levels of K^+ in the medullary interstitium surrounding the thick ascending limb (Giebisch, Klein-Robbenhaar 1993a).

Absorption of the principal salts in the collecting duct segments is controlled by mineralocorticoids such as aldosterone (Ganong 1987). This increases K^+ and H^+ secretion and Na reabsorption. Some of the effect on K^+

secretion is immediate while a 60 minute lag is required for H^+ and 90 minutes for Na^+ reabsorption.

Aldosterone acts by forming an active steroid receptor complex within the collecting duct cell cytoplasm. Transcription of m and r DNA follows, which leads to increased production of aldosterone induced protein. This protein directly stimulates active H^+ secretion. It also stimulates cell metabolism and this increases the Na^+ permeability of the luminal cell membrane (Brenner 1986). This leads to increased cell Na^+ concentration, which stimulates Na^+-K^+-ATPase activity. This stimulates Na^+ reabsorption and intracellular K^+ concentration. Enhanced cell K^+ amplifies the immediate, steroid-induced increased K^+ secretion (by its effect on K^+ permeability).

Stimulation of the renal nerves increases reabsorption of sodium in the ducts not only by enhancing renin release but by a mechanism which is independent of prostaglandins and the renin-angiotensin system. Neural control of sodium reabsorption is mediated by the α_1-adrenoceptor subtype (Gottschalk 1979, DiBona 1977; 1978; 1982; DiBona, Sawin 1983), however α_2-adrenoceptors modulate the antidiuretic action of arginine vasopressin and thus indirectly control renal excretory function (Gellai 1990). Sympathetic activation of the renal ß-adrenergic receptors stimulates the renin-angiotensin system while central adrenergic stimulation increases the nonosmotic release of arginine vasopressin (Abraham, Schrier 1994).

1.1.2 Treating heart failure

Loop diuretics

The introduction of powerful diuretic drugs such as furosemide acting on the loop of Henle had a dramatic effect on the treatment of heart failure and still remain the mainstay of therapy for most patients with chronic heart failure. The treatment of patients with decompensated heart failure requires a sustained reduction in total body salt and water. This can only be attained in severe cases when both salt and water are restricted, to prevent rapid reabsorption in the hours following the diuresis and natriuresis. Patients with compensated heart failure maintain a steady salt and water balance with the aid of a regular daily diuretic and can often maintain this without additional salt restriction. The price paid for diuresis and natriuresis is activation of neuroendocrine systems (Brown, Davis, Johnston 1966; Schaer, Covit, Laragh, et al 1983, Francis, Benedict, Johnstone, et al 1990, Anand, Kalra, Harris, et al 1991).

Intravenous furosemide administration leads to an increase in systemic arterial pressure, probably due to renin release, and a small decrease in cardiac output (Nelson, Ahuja, Silke, et al 1983; Francis, Siegel, Goldsmith, et al 1985b; Petersen, DiBona 1994). Renal effects of furosemide include vasodilatation and redistribution of blood towards the cortical nephrons, actions thought to be important for the drug's diuretic action. Renal prostaglandin E_2 release is also stimulated by furosemide (Fujimura, Ebihara 1988) through stimulation of renal papillary biosynthesis whether or not the renin-angiotensin system is activated at the same time (Katayama, Attallah, Stahl, et al 1984). Furosemide prevents retrieval of filtered Na^+ and K^+ by inhibition of a cotransport mechanism with two Cl^- ions located in the

thick ascending limb of Henle's loop (Greger 1985, Brenner 1986; Wittner, Di-Stefano, Schlatter, et al 1986; Giebisch, Klein-Robbenhaar, Klein-Robbenhaar, et al 1993b). It probably also has an additional inhibitory effect on fluid reabsorption in the proximal tubules (Knox, Wright, Howards, et al 1969; Shalmi, Petersen, Christiansen 1989; Petersen, Shalmi, Abildgaard, et al 1991). In order to inhibit this cotransporter, furosemide must first reach the luminal fluid.

Enhanced rates of sodium absorption follow chronic treatment with loop diuretics (Hropot, Fowler, Kalmark, et al 1985; Stanton, Kaissling 1988, Ellison, Velazquez, Wright 1989; Reyes, Leary 1993a&b) and are almost certainly tightly related to the maintained increase in sodium delivery and sodium uptake into the cells of the distal collecting tubule (Barlet-Bas, Cheval, Khadouri, et al 1990). The increased rate of sodium reabsorption by these distal tubule segments persists beyond the acute diuresis and is functionally significant because it reduces the sodium loss and attenuates the apparent potency of loop diuretics during their chronic administration (Giebisch, Klein-Robbenhaar, Klein-Robbenhaar, et al 1993b; Loon, Wilcox, Unwin 1989; Wilcox 1991; Stanton, Kaissling 1988). Not only does avid renal sodium retention take place, but an attenuated early diuretic response relative to the initial dose response develops with subsequent doses, both in normals and heart failure patients (Reyes, Leary 1992). As heart failure worsens, increasing doses of diuretics are required to maintain sodium balance with concomitant side effects.

Angiotensin converting enzyme inhibitors

Over the past decade, the use of angiotensin converting enzyme (ACE) inhibitors has become routine therapy for patients with heart failure and

have been shown in several large studies both to improve symptoms and functional status and to prolong survival in patients with moderate and severe heart failure. Unfortunately the use of ACE inhibitors has had little impact on the diuretic requirements of most patients, who generally continue to require substantial doses of loop diuretic agents to control fluid accumulation and symptoms of failure (Odemuyiwa, Gilmartin, Kenny, et al 1989; Fitzpatrick, Nicholls, Ikram, et al 1983; Cowley, Stainer, Wynne, et al 1986). Thus diuretics remain one of the mainstays of therapy in most patients with chronic heart failure, yet the development of reduced diuretic responsiveness or even refractory heart failure with long term treatment limits their usefulness. As progressively larger doses are required, side effects become more frequent. These side effects include hyponatraemia, hyperuricaemia, hypokalaemia, hypomagnesaemia, hyperglycaemia and cramp. Tachyphylaxis does not develop to these adverse effects.

Vasodilators

Vasodilator drugs should, in theory, be beneficial for the treatment of heart failure by reversing the intense vasoconstriction which characterises more severe disease. This should interrupt the vicious cycle of further damage to the cardiac myocytes and additional neuroendocrine activation (Riegger, Liebau 1982; Riegger 1985). Patients treated with conventional vasodilators such as hydralazine and isosorbide dinitrate have demonstrated beneficial short term haemodynamic responses in several clinical studies (Cogan, Humphreys, Carlson, et al 1980; Franciosa 1992b; Franciosa, Jordan, Wilen, et al 1984) but only the combination of isosorbide dinitrate and hydralazine has been shown to improve survival in patients with heart failure, and this effect was found to be significantly less than that achieved by ACE inhibitors (Cohn, Johnson, Ziesche, et al 1991). Tachyphylaxis develops to the effects

of either agent administered alone (Packer, Meller, Medina, et al 1982; Franciosa, Weber, Levine, et al 1982c; Packer, Lee, Kessler, et al 1987). The clinical course of patients treated with the vasodilator minoxidil was worse than that of those on placebo, despite sustained improvements in left ventricular ejection fraction (Franciosa, Jordan, Wilen, et al 1984). Wilcox, Guzman, Mitch, et al (1987) showed that prazosin in conventional doses has no effect on the renal response to furosemide in normal man although Lang, Choy, Rahman, et al (1993c) demonstrated a natriuretic effect of a low (non-pressor) dose of prazosin in patients with heart failure undergoing a water-diuresis and after conventional doses of furosemide had been administered.

Calcium channel antagonists

Agents such as nifedipine and diltiazem have potential benefits in the treatment of diastolic dysfunction but can precipitate acute deterioration in patients with significantly impaired left ventricular function due to negatively inotropic properties (Elkayam, Amin, Mehra, et al 1990; Goldstein, Boccuzzi, Creuss, et al 1991; Packer 1989) and thus the use of vasodilators is often restricted to the acute management of heart failure.

Dopamine

Dopamine was first introduced for the treatment of decompensated heart failure in the early 1970's. It stimulates renal dopamine-1 receptors, dopamine-2 receptors, systemic β1-adrenoceptors and α-adrenoceptors and thus has both inotropic and vasodilator properties (Beregovich, Bianchi, Rubler, et al 1974; Brown, Lorenz, Erdmann 1985; Brodde 1991). In the kidney, dopamine preferentially dilates the renal arterioles at low dose (<5μg/kg/min). At higher doses (5-10μg/kg/min), its vasoconstrictor effects become more pronounced, with constriction of systemic and renal arterioles

and increased peripheral vascular resistance developing .

Dopamine has been shown to restore diuresis in cardiac patients following surgery (Hilberman, Maseda, Stinson, et al 1984; Rosenblum, Tai, Lawson 1972), shock (Loeb, Winslow, Rahimtoola, et al 1971; Talley, Goldberg, Johnson, et al 1969) and in patients with renal impairment (Talley, Goldberg, Johnson, et al 1969), but reports of diuretic properties with dopamine in patients with heart failure are largely anecdotal (Beregovich, Bianchi, Rubler, et al 1974; Goldberg, McDonald, Zimmerman 1963; Goldberg 1972; McDonald, Goldberg, McNay, et al 1964). A recent study of low-dose dopamine infusion in elderly patients with congestive heart failure showed no diuretic or natriuretic effect (Robinson, Gariballa, Fancourt, et al 1994) while the effect in burns patients was inconsistent (Graves, Cioffi, Vaughan, et al 1993). Duke, Briedis and Weaver (1994) suggest that low-dose dopamine may even be harmful by masking signs of renal ischaemia. The oral dopamine analogue ibopamine causes a diuresis but not natriuresis in patients with heart failure (Wehling, Zimmermann, Theisen 1990; Kasmer, Cutler, Munger, et al 1990; DeiCas, Metra, Visioli 1992) but whether tachyphylaxis will develop with chronic use due to down-regulation of the dopamine receptors, as occurs with β-adrenergic receptors, remains to be seen.

Dobutamine

Dobutamine is a synthetic catecholamine which stimulates α1, β1 and β2-adrenoceptors. The α-adrenergic stimulation results in renal artery vasoconstriction, while ß2-adrenergic stimulation causes renal vasodilatation (Insel, Snavely 1981). Overall the effect on renal blood flow is

negligable (Robie, Goldberg 1975). Dobutamine also causes peripheral vasodilatation and cardiac inotropy. Because of its balanced inotropic and vasodilator properties and preservation of myocardial metabolic function, dobutamine is widely used as the inotrope of choice in hypotensive patients with left ventricular failure. There are some anecdotal reports and uncontrolled studies describing diuresis and natriuresis with dobutamine in patients with heart failure (Leier, Webel, Bush 1977; Leier, Heban, Huss, et al 1978; Hilberman, Maseda, Stinson, et al 1984; Kiyingi, Field, Pawsey, et al 1990) but controlled studies comparing the effect of dobutamine and furosemide with furosemide on its own have not been reported. Dobutamine may have diuretic effects due to improvement in cardiac output but whether the putative diuretic effects of dopamine and dobutamine differ in this group of patients is not clear. Diuresis may depend on stimulation of the renal dopamine receptor, or on restoration of renal perfusion pressure, through an inotropic action. Unfortunately, with chronic use, the beneficial haemodynamic effects of β-adrenergic agonists are lost (Unverferth, Blanford, Kates, et al 1980; Krell, Kline, Bates, et al 1986) and survival studies both with the oral partial agonist xamoterol and using intermittent outpatient infusions of dobutamine have demonstrated increased mortality with respect to placebo (Dies 1988; The Xamoterol in Severe Heart Failure Study Group 1990; Uretsky, Jessup, Konstam, et al 1990; Packer 1990).

Phosphodiesterase inhibitors

Phosphodiesterase inhibitors improve cardiac contractility by increasing concentrations of cyclic AMP and are used for the acute treatment of severe decompensated heart failure not responding to beta adrenoceptor agonists. These drugs were hailed as the breakthrough for outpatient management of cardiac failure as, unlike dopamine and dobutamine, they can be

administered orally. Unfortunately survival studies have raised concerns that long-term therapy with phosphodiesterase inhibitors can enhance the frequency and complexity of ventricular arrhythmias, provoke myocardial ischaemia and shorten survival (Kinney, Carlin, Ballard, et al 1982; DiBianco, Shabetai, Silverman, et al 1984; Packer, Medina, Yushak 1984a; Massie, Bourassa, DiBianco, et al 1985; Likoff, Weber, Andrews, et al 1985; Jessup, Ulrich, Samaha, et al 1987; Packer, Carver, Rodeheffer, et al 1991) thus their use is restricted to short term treatment of decompensated heart failure.

Digoxin

Digoxin, a cardiac glycoside with inotropic and chronotropic properties, has been used in the treatment of heart failure for centuries (Withering 1785; Greeff, Schadewaldt 1981). Controversy still remains as to its role in the treatment of patients with chronic heart failure in sinus rhythm. William Withering in his book "An account of the foxglove, and some of its medical uses: with practical remarks on dropsy, and other diseases" reported increased excretion of urine with digitalis although it is probable that many of his patients had untreated atrial fibrillation and that the beneficial effects he noted related to slowing of rate and inotropic properties rather than to a specific diuretic effect. Gavey and Parkinson (1938) subsequently reported the effect of digitalis in patients with heart failure in sinus rhythm and showed that some 50% of patients did exhibit a diuresis but that it occurred more frequently in patients in atrial fibrillation. Rader, Smith, Berger, et al (1966) found no additional diuresis when digoxin was added to optimal diuretic therapy but Hull and Mackintosh (1977) showed that some patients required an increase in diuretic therapy when digoxin was withdrawn.

Digoxin produces its clinical effects by binding to the extracellularly exposed recognition site on the sodium pump, sodium-potassium-adenosine triphosphatase (Schatzmann 1953). A diuretic and natriuretic action could be explained by inhibition of Na^+-K^+-ATPase in the basolateral membrane of renal tubuloepithelial cells with inhibition of sodium reabsorption. The degree of inhibition would theoretically relate to the local density of Na^+-K^+-ATPase in different parts of the nephron and its affinity for digoxin. Sensitivity to the cardiac glycoside ouabain has been reported to differ markedly in different portions of the nephron (Doucet, Barlet 1986). A specific digitalis binding site of high affinity and selectivity has been conserved on Na^+-K^+-ATPase in all animal species in the course of evolution. It has recently been discovered that the endogenous regulator of Na^+-K^+-ATPase in animals, as in several plants, is ouabain (Hamlyn, Blaustein, Bova, et al 1991; Lloyd, Sandberg, Edwards 1992; Kelly, Smith 1992; Blaustein 1993).

There has been a resurgence of interest in the use of digoxin for the management of patients with heart failure in sinus rhythm, as it was shown that most, if not all other available inotropic agents have been associated with increased mortality with long term use in heart failure (Xamoterol in Severe Heart Failure Study Group 1990; Packer, Carver, Rodeheffer, et al 1991; Likoff , Weber , Andrews, et al 1985; Massie, Bourassa, DiBianco, et al 1985). Some of the beneficial long term effects of digoxin may be due to inhibition of the sympathetic nervous system (Gheorghiade, Hall, Lakier, et al 1989), the renin-angiotensin system (Covit, Schaer, Sealey, et al 1983; Ribner, Plucinski, Hsieh, et al 1985) and modulation of autonomic tone as digoxin has been demonstrated to modulate baroreceptor function (Ferguson, Berg, Sanders, et al 1989). Guyatt, Sullivan, Fallen, et al (1988)

showed benefit from digoxin in patients with heart failure in sinus rhythm and Packer, Gheorghiade, Young et al (1993) demonstrated that withdrawal of digoxin from patients with chronic heart failure treated with ACE inhibitors resulted in deterioration and weight gain, suggesting some diuretic effect, even in this patient population, although Gheorghiade and Beller found a neutral effect (1983).

Beta-adrenergic antagonists

β-adrenergic antagonists have favourable effects on symptoms of patients with idiopathic dilated cardiomyopathy and on survival in patients with heart failure due to ischaemic heart disease (Swedberg, Hjalmarson, Waagstein, et al 1980; Chadda, Goldstein, Byington, et al 1986; Engelmeier, O'Connell, Walsh, et al 1985; Sethi, Nair, Arora, et al 1990). However treatment with the partial beta agonist xamoterol was associated with increased mortality in the "Xamoterol in severe heart failure study group" (1990) and several authors have described worsening heart failure developing in patients on being prescribed β-adrenoceptor antagonists (Ikram, Fitzpatrick 1981; Binkley, Lewe, Lima, et al 1986; Shanes 1987; Waagstein, Caidahl, Wallentin, et al 1989; R Lang 1990; Packer 1992).

Arginine vasopressin antagonists

Elevated arginine vasopressin concentrations have been reported in patients with congestive heart failure (Yamane 1968; Rouleau, Moye, de-Champlain, et al 1991) and thus extensive work with antagonists is in progress. At the time of writing, no suitable antagonist is available for clinical use but research projects in patients with heart failure are underway (Nicod, Waeber, Bussien, et al 1985; Creager, Faxon, Cutler, et al 1986;

Laszlo, Laszlo, De Wied 1991).

1.2 Possible mechanisms for the loss of diuretic responsiveness found in heart failure

1.2.1 Pharmacokinetics of furosemide

In severe heart failure, furosemide absorption from the gastrointestinal tract may be reduced due to mucosal congestion, however studies in patients with stable heart failure showed no change in bioavailabilty of furosemide after intravenous or oral doses (Van-Meyel, Gerlag, Smits, et al 1992a), thus resistance to furosemide in congestive heart failure manifests as a change in pharmacodynamics of response, related probably to altered solute haemostasis (Brater, Chennavasin, Seiwell 1980; Brater, Seiwell, Anderson, et al 1982). Even in patients with reduced gastric absorption, the pharmacokinetics of intravenously administered furosemide appear little altered. The small reduction in renal clearance of the drug can be accounted for by the reduced glomerular filtration rate in patients with heart failure. Patients with more severe cardiac failure show lower glomerular filtration rate (GFR) and furosemide renal clearance than those with mild failure (Nomura, Yasuda, Minami, et al 1981).

Despite similar furosemide levels in the plasma and the urine, patients with heart failure have a reduced renal response to a given urine furosemide concentration when compared with controls. Thus in one series, the maximum sodium excretion rate was 30% of that in normal subjects and the total natriuretic response was reduced to 50% of normal. Excretion of furosemide in heart failure is qualitatively the same as in normal subjects but the time course is delayed (Andreason, Mikkelsen 1977; Brater,

Chennavasin, Seiwell 1980). Nomura, Yasuda, Minami, et al (1981) demonstrated a moderate correlation between renal furosemide clearance and urinary sodium concentration. They also found that urinary furosemide excretion correlated with natriuretic effect in patients with heart failure.

The mechanisms for the reduced responsiveness to furosemide in heart failure are complex, but include activation of the sympathetic and renin-angiotensin systems and thus opposing sodium retaining forces. In addition, chronic furosemide therapy leads to local renal adaptation, in health or disease. Tubular segments distal to the site of furosemide's action in the thick ascending limb of Henle's loop respond to the increased load of fluid and electrolytes with enhanced reabsorption of sodium and sharply augmented secretion of potassium (Stanton, Kaissling 1988; Stanton, Giebisch 1992; Giebisch, Klein-Robbenhaar, Klein-Robbenhaar, et al 1993b). Even in the absence of volume contraction, reabsorption of sodium by downstream nephron segments is enhanced (Stanton, Kaissling 1988). This transport stimulation is associated with a striking amplification of the basolateral membrane area of distal convoluted cells, connecting tubule cells and principal cells (Kaissling, Stanton 1988). There is also a striking increase in the ATPase content of these tubule segments (Garg, Kapturczak 1987; Scherzer, Wald, Popovtzer 1987).

1.2.2 Sodium homeostasis in health and in heart failure

Normal volunteers initially respond to salt loading with weight gain (Rovner, Conn, Knopf, et al 1965; Solomon, Atherton, Bobinski, et al 1987) and a rise in blood pressure (Guyton 1980; Guyton 1991; Blaustein, Hamlyn 1991). Despite continued dietary sodium loading, weight and blood pressure stabilise at a new level. When sodium loading is withdrawn, blood pressure

and body mass rapidly return to baseline levels. In the same fashion, an intravenous sodium load is quickly excreted by the kidneys in health (Blaustein, Hamlyn 1991). Administration of mineralocorticoids (DOCA) to animals and normal volunteers causes initial fluid and salt retention, usually without oedema formation followed by an "escape" from the sodium-retaining effects after approximately 5 days (August, Nelson, Thorn 1958; Rovner, Conn, Knopf, et al 1965; Davis, Urquhart, Higgins, et al 1966). Patients with heart failure do not "escape" from the sodium retaining effects of mineralocorticoid steroids and continue to retain sodium and water despite total body sodium excess (Warren, Stead 1944; Chonko, Bay, Stein, et al 1977). Spironolactone, a competitive inhibitor of aldosterone, causes little or no increase in sodium excretion in patients with cardiac failure (Sanders, Melby 1964), and avid sodium can persist in patients in whom a high sodium intake suppresses aldosterone concentrations (Chonko, Bay, Stein, et al 1977). Aldosterone is thus not the only factor responsible for sodium retention in heart failure but it plays a permissive role. Davis, Holman and Hyatt (1955) and Davis, Howell and Southworth (1958) showed that adrenalectomised dogs were able to retain salt and develop oedema in two separate models of heart failure provided a small, fixed dose of mineralocorticoid replacement was given.

1.2.3 Response to diuretics in heart failure and health.

Healthy volunteers, in normal sodium balance, develop a brisk natriuresis and diuresis after a single dose of diuretic. This is rapidly followed by avid sodium retention until neutral sodium balance has been regained. The natriuretic response to a diuretic depends on the level of external sodium balance at which it is assessed (Reyes 1991; Reyes, Leary 1992; Reyes, Leary 1993a) and is greater during states of salt excess. During regular

once-daily administration, the early-after-dosing diuretic effect persists but becomes attenuated with respect to the diuresis following the initial dose (Loon, Wilcox, Unwin 1989). The increased rate of sodium reabsorption by the distal tubule segments is functionally significant because it reduces the sodium loss and attenuates the apparent potency of loop diuretics during their chronic administration (Stanton, Kaissling 1988; Loon, Wilcox, Unwin 1989; Wilcox 1991; Giebisch, Klein-Robbenhaar 1993a). The compensatory sodium reabsorption occurs distal to the site of action of furosemide in the loop of Henle where sodium is being lost (Reyes, Leary 1993b).

Enhanced rates of potassium secretion also follow chronic treatment with loop diuretics such as furosemide (Hropot, Fowler, Karlmark, et al 1985; Stanton, Kaissling 1988; Ellison, Velazquez, Wright 1989). This transport stimulation in the thick ascending limb of the loop of Henle is associated with a striking increase in the basolateral membrane area of distal convoluted cells, connecting tubule cells and principal cells (Kaissling, Stanton 1988). They are also accompanied by a sharp increase in ATPase content of these tubule segments (Garg, Kapturczak 1987; Scherzer, Wald, Popovtzer 1987). These morphological and functional changes persist and are independent of aldosterone and AVP level alterations (Stanton, Kaissling 1988; Kaissling, Stanton 1988). Transport stimulation is almost certainly tightly related to the maintained increase in sodium delivery and sodium uptake into cells of the distal convoluted tubule (Barlet-Bas, Khadouri , Marsy, et al 1990).

The observation that thiazides given in conjunction with loop diuretics during chronic diuretic therapy exert a significantly larger sodium diuresis than in untreated controls, suggests that a key site of enhanced Na^+ reabsorption is the distal convoluted tubule (Loon, Wilcox, Unwin 1989).

In heart failure, a similar, though blunted diuretic and natriuretic response occurs after diuretic administration, followed by avid sodium retention. Fluid losses are rapidly replaced after diuretic dosing, unless salt and water intake are restricted. Although patients with heart failure are traditionally managed with both diuretics and salt restriction, it is not clear that this policy is more advantageous than diuretics alone, in stable patients.

1.2.4 The renin-aldosterone-angiotensin system in heart failure

The renin-aldosterone-angiotensin system (RAAS) is activated at an early stage in experimental heart failure and after myocardial infarction in man, with circulating concentrations of angiotensin II and renin falling as a stable, compensated state is achieved by salt and water retention. Studies of untreated heart failure patients (Anand, Kalra, Harris, et al 1991; Remes, Tikkanen, Fyhrquist, et al 1991) have shown that concentrations of renin and angiotensin II are usually within normal limits until progressive fluid retention is evident or until diuretics are administered.

Plasma concentrations of renin may increase in heart failure in response to a number of stimuli, including decreased renal tubular sodium load, reduction in renal perfusion pressure or an increase in sympathetic activity. Angiotensin II causes proximal tubular sodium reabsorption, contraction of the glomerular arterioles (predominantly the efferent arterioles), stimulation of secretion of the antidiuretic hormone and aldosterone and alteration of the distribution of blood flow in the kidney (Cannon 1977; Navar, Carmines, Huang, et al 1987; Cleland, Dargie 1987b). Angiotensin II also stimulates thirst via a central effect and has indirect action on sodium retention through stimulation of noradrenaline release from nerve endings (Antonaccio, Kerwin 1981). The hormone facilitates sympathetic nervous system

ganglionic transmission (Purdy, Weber 1988), may modulate baroreceptors and has vascular remodelling properties. These effects generally favour sodium and water retention. It was thus anticipated (supported by animal experiments) that blocking the effects of the activated RAAS should correct the pathological sodium and water retention found in heart failure.

Earlier attempts to inhibit the renin-angiotensin system with the aldosterone antagonist spironolactone proved to be a disappointment as only 40% of the patients with heart failure tested developed a diuresis (Sanders, Melby 1964) and there was little relationship between the measured level of aldosterone and subsequent diuresis with spironolactone. The drug did cause increased levels of renin and angiotensin II and prevented the hypokalaemia usually associated with loop diuretics. These results suggest that aldosterone is not an overriding factor in the promotion of sodium reabsorption in man.

In addition to the sodium-retaining actions on the sympathetic nervous system and through the formation of aldosterone, angiotensin II has powerful direct tubular sodium retaining properties. Angiotensin converting enzyme (ACE) inhibitors prevent the formation of angiotensin II. This results in profound suppression of circulating angiotensin II, at least initially, with high concentrations of active renin and angiotensin-I and low levels of aldosterone. The fall in circulating angiotensin II results in a fall in glomerular filtration rate (GFR) and a rise in renal plasma flow (ERPF) due to loss of efferent arteriolar constriction by angiotensin II. Filtration fraction (GFR/ERPF), usually elevated in heart failure as a consequence of decreased renal perfusion, falls towards normal levels. Thus, in theory, ACE inhibition should have powerful natriuretic and diuretic actions by improving renal haemodynamics and by decreasing levels of aldosterone and

angiotensin II. In practice, several investigators have demonstrated that patients with heart failure are extremely sensitive to the hypotensive effects of ACE inhibitors and antidiuresis and antinatriuresis is the usual acute response (Fitzpatrick, Nicholls, Ikram, et al 1983; Cleland, Dargie, Hodsman, et al 1984; Cleland, Dargie, Gillen, et al 1986; Cleland, Dargie, Robertson, et al 1987a; Flapan, Davies, Waugh, et al 1991a&b; Motwani, Fenwick, Morton, et al 1992b).

In experiments with normal subjects where diuresis is induced by furosemide after salt restriction or in the salt replete state, after a brisk diuresis, the RAAS is activated and avid sodium retention occurs until sodium balance is restored (Wilcox, Mitch, Kelly, et al 1983). Administration of the ACE inhibitor captopril does not prevent the antinatriuresis, nor does it produce a negative sodium balance, despite suppression of the activated RAAS (Kelly, Wilcox, Mitch, et al 1983; Wilcox, Guzman, Mitch, et al 1987). ACE inhibition has been shown to enhance diuresis in animals (Harris, Navar, Ploth 1984; Navar, Carmines, Huang, et al 1987) and MacDonald, Craig and Watson (1989) demonstrated enhanced natriuresis but not diuresis in salt replete normal volunteers when ramipril was added to furosemide, without any effect on urinary prostaglandin E_2 or 6-keto prostaglandin F_{1a}. Flapan, Davies, Waugh, et al (1991a&b) showed that captopril lowered diuresis and natriuresis after 40mg of furosemide in patients in heart failure. They also investigated the effect of posture on the response to ACE inhibitors in patients with heart failure and showed that captopril blunted the diuretic response to furosemide in the supine position while the diuresis was decreased by the erect position with no further decline when captopril was added. Double-blind studies of total body sodium in patients with heart failure did not demonstrate any natriuresis over

6-8 weeks of treatment with ACE inhibitors (Cleland, Dargie, East, et al 1985b; Cleland, Dargie, Robertson, et al 1987c), probably the mechanism for correction of hyponatraemia which is reported with ACE inhibitors (Packer, Medina, Yushak 1984b). Introduction of an ACE inhibitor leads to a reduced renal response to furosemide initially, supported by the finding of enhanced phosphate reabsorption, suggesting enhanced proximal tubular sodium reabsorption (Cleland, Dargie 1987b). However studies which have examined the longer term response to ACE inhibitors in patients with heart failure have shown that the impaired ability to excrete a salt load can be restored (Volpe, Tritto, DeLuca, et al 1992) or diuretic requirements diminished, suggesting enhancement of natriuresis (Dzau, Hollenberg 1984a; Kubo, Nishioka, Nishimura, et al 1984; Mujias, Fouad, Textor, et al 1984).

Motwani, Fenwick, Morton, et al (1992b) demonstrated that the initial sodium retention may be dose-related, with natriuresis being seen for a few hours after 1mg of captopril and sodium retention after 25mg. They were not able to correlate the sodium retention with fall in blood pressure, suggesting that this was not the primary factor accounting for the difference in natriuretic response to furosemide observed with different doses of captopril. He postulated that the lower dose of captopril preserved enough circulating angiotensin II to oppose the post glomerular vasodilator effect of high dose diuretic and thus preserve GFR, while antagonising some of the tubular salt-retaining actions of angiotensin II.

Thus, despite theoretical considerations, the renal interaction of furosemide and ACE inhibitors does not appear particularly beneficial at first sight. The interaction of chronically administered ACE inhibitors and diuretics has yet

to be assessed.

Some authors have suggested that the long term beneficial effects of captopril may relate more closely to tissue levels of renin, aldosterone and angiotensin II than to circulating levels of these hormones. Work in hypertensive patients has shown that the extent of individual response to ACE inhibitors usually depends on basal circulating levels of active renin (MacGregor, Markandu, Banks, et al 1982) but elderly patients, theoretically having low renin levels, may still respond to ACE inhibitors as monotherapy (Pannier, Garabedian, Madonna, et al 1991; Cox, Duggan, O'Boyle, et al 1989). Several studies in patients with heart failure have failed to demonstrate a relationship between baseline renin levels and response to an ACE inhibitor (Davis, Ribner, Keung, et al 1979; Packer, Medina, Yushak 1984a; Packer, Medina, Yushak, et al 1985; Mettauer, Rouleau, Bichet, et al 1986). The discrepancy between haemodynamic effects and circulating levels of ACE inhibitors (Sakaguchi, Chai, Jackson, et al 1988; MacFadyen, Lees, Reid 1991) raises the possibility that some of the effects of ACE inhibitors are due to local inhibition of the renal angiotensin system, supported by the finding in animals that renal ACE activity is profoundly suppressed by ACE inhibitor therapy (Veltmar, Gohlke, Unger 1991). There is some evidence to suggest that the circulating renin-angiotensin system is activated in response to acute changes in circulating volume, while the local renal "tissue" RAAS is responsible for local renal haemodynamic effects and perhaps vascular remodelling. In spontaneously hypertensive rats the fall in blood pressure with an ACE inhibitor correlates better with the inhibition of the tissue than the circulating renin-angiotensin system (Cohen, Kurz 1982; Unger, Ganten, Lang, et al 1984; Weishaar, Panek, Major, et al 1991). Circulating concentrations of angiotensin II, whilst initially profoundly

suppressed by ACE inhibitors, may "escape" in the long term, despite continuing clinical benefit, suggesting that there is continued suppression of angiotensin II locally in the kidney, as has been demonstrated in rats (Kohzuki, Johnston, Chai, et al 1991). The precise contribution of the tissue and circulating RAAS to diuresis and natriuresis is still debated.

1.2.5 Atrial natriuretic peptide

The diuretic and natriuretic properties of extracts of atrial tissue was described by De Bold, Borenstein, Veress, et al in 1981 and subsequently atrial natriuretic peptide (ANP) was isolated from the atrial secretory granules responsible. Unlike conventional diuretic agents, ANP suppresses the formation of aldosterone, renin, catecholamines and arginine vasopressin (AVP) in man (Maack, Marion, Camargo, et al 1984; Samson 1985; Cody, Atlas, Laragh, et al 1986a).

ANP stimulates production of cyclic GMP by particulate guanylate cyclase. Cyclic GMP is the second messenger which mediates the vasodilatation, diuresis and natriuresis (Appel, Dunn 1987; Brenner, Ballermann, Gunning, et al 1990; Wilkins, Settle, Needleman 1990). ANP produces a diuresis, natriuresis, suppression of renin and aldosterone as well as a fall in blood pressure in normal volunteers and in hypertensive patients. Secretion of ANP is regulated by atrial stretch. Concentrations of ANP are raised in patients with heart failure by as much as ten to twenty times, but the renal response to endogenous or exogenously administered ANP is blunted (Cody, Atlas, Laragh, et al 1986a; Moe, Canepa-Anson, Armstrong 1992). This may be due to receptor down-regulation, decreased receptor affinity or sensitivity, renal tubular changes or antagonism by other neuroendocrine or renal haemodynamic factors. Honrath, Chong, Wilson, et al (1994) showed

that the renal response to ANP infusion is greatly influenced by the sodium intake, varying from zero to supranormal. The effect of enhancing renal cyclic GMP excretion with ANP on diuretic responsiveness has not been studied in heart failure.

Invasive studies in patients with heart failure with elevated right atrial, pulmonary and capillary wedge pressures showed that beneficial haemodynamic changes were evident during infusion of ANP with falls in these pathologically elevated pressures. In addition there was a significant fall in blood pressure and increased renal blood flow (Cody, Atlas, Laragh 1986a; Molina, Fowler, McCrory, et al 1988). There may be differences between the renal and haemodynamic responses to ANP in heart failure. Binding of ANP by antibodies and thus inactivation of the peptide resulted in significant anti-natriuresis and anti-diuresis in in animal models of heart failure, raising the possibility that ANP continues to play a beneficial role in heart failure (Awazu, Imada, Kon, et al 1989; Shepperson, Barclay, Bennett, et al 1991).

Atrial natriuretic peptide is inactivated by neutral endopeptidase which is found in large quantities on the brush border of the renal tubule. Recently the introduction of pharmacological agents which antagonise the action of neutral endopeptidase and thus prevent degradation of ANP has generated intense clinical research. One interesting finding has been that animals with elevated basal circulating concentrations of ANP respond to administration of neutral endopeptidase inhibitor with more profound diuresis and natriuresis than those with normal basal concentrations. There is some doubt as to whether this is the case in heart failure (Münzel, Kurz, Holtz, et al 1992). In addition, significant quantities of ANP appear in the urine after

administration of neutral endopeptidase inhibitors which is not the case when infusions of ANP are administered (Trippodo, Gabel, Harvey, et al 1991; Richards, Wittert, Espiner, et al 1991; Suzuki, Hirata, Matsuoka, et al 1992; Münzel, Kurz, Holtz, et al 1992). This raises the potential of pharmacological manipulation of ANP activity for therapeutic benefit.

1.2.6 The sympathetic nervous system

The sympathetic nervous system is activated in heart failure and, in man, plasma concentrations of noradrenaline correlate with prognosis (Cohn, Levine, Olivari, et al 1984; Rector, Olivari, Levine, et al 1987). Increased efferent renal sympathetic nerve activity has major effects on several renal functions: it stimulates renin release, constricts the glomerular afferent arteriole, alters the pattern of intrarenal blood flow, decreases glomerular filtration rate and enhances renal tubular sodium and water reabsorption (Cody, Covit, Schaer, et al 1986b; Mancia 1990; DiBona, Sawin 1994). These effects, by restoring circulating volume, blood pressure and cardiac output, may be both life-saving in states of hypovolaemia and of benefit in the early stages of heart failure. By increasing the load on the failing heart, however, ventricular function may further decline.

Although experiments in hypertensive men suggest that lowering sympathetic activity with ganglion blocking agents such as guanethidine can produce a diuresis, and prior renal denervation can prevent the impaired renal excretory responses in animal models of heart failure, sympathetic ganglion blockade results in sodium retention in patients with heart failure (Gaffney, Braunwald 1962; Gill, Mason, Bartter 1965). α1-antagonists also results in sodium retention, which cannot be reversed by ACE inhibitors

(Riegger, Haeske, Kraus, et al 1987b). The acute effects of β-adrenergic blockade are usually detrimental in patients with heart failure and may result in sodium retention (Ikram, Fitzpatrick 1981; Binkley, Lewe, Lima, et al 1986; Shanes 1987; Waagstein, Caidahl, Wallentin, et al 1989; Lang (R) 1990; Packer 1992). Pharmacological interruption of the sympathetic efferent pathways, despite theoretical advantages, generally results in sodium retention in heart failure.

Administration of isoprenaline to normal rats raises fractional sodium reabsorption in the duct to the same level as that seen in salt-deplete rats (Honrath, Wilson, Sonnenberg 1991). In normal subjects, phenylephrine or noradrenaline infusions decrease sodium excretion (Lang, Rahman, Balfour, et al 1993a&b), presumably by renal arterial constriction. In patients with heart failure however, dobutamine, a synthetic catecholamine which stimulates β1, β2 and α1-adrenergic receptors, may increase sodium excretion (Leier, Webel, Bush 1977; Leier, Heban, Huss, et al 1978). This could be due to increasing cardiac output, as in contrast to dopamine, no specific dopamine receptor stimulation or renal vasodilatation occurs. The enhancement of systemic pressure may override the effects of local renal arterial constriction or alternatively, dobutamine may have less renal vasoconstrictive effects that noradrenaline at the doses used in clinical practice. Tyramine has been shown to increase sodium excretion in normal subjects (Lang, Rahman, Balfour, et al 1993b) suggesting that circulating noradrenaline and neuronally released noradrenaline may have opposite effects on renal sodium handling in man.

Furosemide increases sympathetic activity (Attman, Aurell, Johnsson 1975)

which may be largely due to volume contraction,however a specific effect on adrenergic inhibition or stimulation cannot be excluded.

1.2.7 Abnormalities in haemodynamic responses

The renal consequences of heart failure are a fall in renal blood flow (ERPF) and glomerular filtration rate (GFR). The fall in ERPF exceeds the fall in GFR, as constriction of the efferent renal arteriole distal to the glomerulus by angiotensin II preserves GFR. This results in a rise in filtration fraction (GFR/ERPF) and an increase in proximal tubular forces favouring sodium reabsorption, as follows:

a) Efferent arteriolar constriction lowers the hydrostatic pressure in the peritubular capillaries.

b) Plasma proteins are filtered less and thus become more concentrated in the blood in the peritubular capillaries.

c) The combination of diminished hydrostatic pressure and increased colloid oncotic pressure favour the return of renal interstitial fluid into the peritubular capillaries.

d) Changes in the intrarenal distribution of blood flow, with diversion of blood flow from the outer cortex to the juxtamedullary nephrons, may also contribute to sodium retention.

As heart failure becomes more severe, renal blood flow declines further and afferent, rather than efferent arteriolar constriction becomes predominant, resulting in a steeper decline in GFR and pre-renal uraemia. ACE inhibitors, by releasing the efferent arteriole from the effects of angiotensin II, reduces renovascular resistance, but usually result in a decline in GFR.

Sodium nitroprusside (SNP) is a powerful arterial and venous vasodilator which increases cardiac output by decreasing systemic vascular resistance (Berkowitz, McKeever, Croke, et al 1977; Cogan, Humphreys, Carlson, et al 1980; Franciosa, Silverstein 1982a). It causes accumulation of cyclic GMP in the renal papillary collecting tubule (Appel, Dunn 1987) but has variable effects on diuresis in animals (Hamet, Tremblay, Pang, et al 1984; Chiu, Vemulapalli, Sybertz 1991) and man (Cogan, Humphreys, Carlson, et al 1980), depending on whether heart failure is present and on the extent to which blood pressure falls in response to the SNP infusion. In the intact dog, SNP was found to decrease total peripheral resistance and mean arterial pressure without increasing cardiac output and at the same time resulted in a fall in GFR, ERPF and sodium excretion. Patients with heart failure tend to respond to SNP infusion by increasing ERPF and decreasing filtration fraction (Cogan, Humphreys, Carlson, et al 1980) although changes in sodium excretion are variable. Miller, Fennell, Young, et al (1982) reported that SNP increases ERPF but does not alter GFR in heart failure. Minute urine flow, total cation excretion and filtration fraction increased and renal vascular resistance fell. It is likely that filtration pressure disequilibrium exists, because increases in ERPF produced by vasodilator agents are not accompanied by increases in GFR (Krakoff, De Guia, Vlachakis, et al 1973). These results suggest that the net hydrostatic pressure gradient across the glomerular capillary membrane is the primary determinant of GFR in both normal men and in patients with congestive heart failure (Cogan, Humphreys, Carlson, et al 1980).

Pressure natriuresis

As has been mentioned earlier, urine output is dependent on systemic arterial pressure (Guyton 1991) and although intrarenal mechanisms can

compensate for falls in renal perfusion pressure it is well known that anuria develops when the mean systolic blood pressure falls much below 60mmHg. In addition, there is circumstantial evidence, at least in essential hypertension, that the development of hypertension is a compensatory response to a reduced ability of the kidney to excrete a salt load, and that elevation of blood pressure results in enhanced natriuresis (Guyton 1991; Blaustein, Hamlyn 1991). Many of the agents used to treat heart failure have hypotensive actions (nitroprusside, phentolamine, ACE inhibitors, nitrates, prazosin). One could predict that a balance must exist between the benefits of vasodilatation on cardiac function and the deleterious effects of excessive hypotension on renal perfusion. Clinicians use inotropic agents such as dobutamine to restore urine output in patients with heart failure and it has been the author's experience that restoring an adequate blood pressure by this means is usually successful in restoring the urine output, without the need for specific renal artery vasodilatation. For this reason tyramine, dobutamine and dopamine were chosen as pressor agents to explore the role of elevating the blood pressure on diuresis and natriuresis.

1.3 Possible methods of restoring diuretic responsiveness

Possible ways of restoring the renal effects of furosemide in patients with heart failure are listed below. In practice, most available treatment modalities potentially influence more than one neuroendocrine or circulatory system. For example digoxin has actions on baroreceptors, the renin-angiotensin system, inotropism and renal plasma flow.

1.3.1: Enhancement of natriuretic factors such as ANP and renal prostaglandins and thus counteracting the vasoconstrictor and salt retaining actions of arginine vasopressin, the renin-angiotensin system and the

sympathetic nervous system.

1.3.2: Suppression of antinatriuretic systems such as the sympathetic nervous system, the renin-angiotensin system and arginine vasopressin.

1.3.3: Optimisation of renal haemodynamics by improving renal perfusion pressure, glomerular filtration rate and renal plasma flow or by correcting the pathological elevation of filtration fraction (GFR/ERPF) found in heart failure.

1.3.4: Improving cardiac output either by inotropic support or by vasodilatation.

1.3.5: By direct inhibition of renal tubular sodium reabsorption.

1.4 Problems with extrapolating the results of experiments in animal models of heart failure to patients

Various animal models of heart failure exist, including rapid ventricular pacing to induce left ventricular failure (with the added advantage of being completely reversible), clamping the pulmonary artery or aorta, clamping the inferior vena cava, occluding the left coronary artery or the creation of an arterio-venous fistula. Whilst these models have many similarities with heart failure in man and the animals respond in a similar fashion with salt and water retention and neuroendocrine activation, elevation of filtration fraction may not occur, despite being characteristic of heart failure in man. Another major difficulty experienced with these animal models is reproducibility of the experimental results in man. (Experiments which have given rather different results in animals and man include the effect of guanethidine on natriuresis and the effect of ACE inhibitors on left ventricular hypertrophy). Because of these factors, precisely controlled and repeatable studies in animal models of heart failure are not a substitute for controlled clinical studies of patients. Small pilot studies, with all their limitations, continue to have relevance in the management of heart failure.

1.5 Objectives

Diuretic resistance is an important clinical problem in patients with heart failure. There are a number of possible mechanisms by which response to diuretics could be improved.

1.5.1 Objectives of the individual experiments

1.5.1.1: To assess the effect of salt restriction on :

(a) the diuretic and natriuretic response to furosemide, comparing a sodium intake of 20mmol/day with 150mmol/day.

(b) body mass in heart failure.

(c) further neuroendocrine activation.

(d) renal perfusion.

1.5.1.2: to explore the role of ACE inhibition by captopril on:

(a) arterial pressure

(b) renal haemodynamics

(c) diuresis and natriuresis

(d) neuroendocrine activation.

1.5.1.3: to examine the role of increasing cardiac output using a vasodilator compared to an inotropic agent to assess the relative importance of arterial pressure and cardiac output in the diuretic response to furosemide with increases in cardiac output.

1.5.1.4: to assess the role of digoxin:

(a) to determine whether it has diuretic or natriuretic effects in heart failure

(b) to assess its effects on neuroendocrine activation

(c) to assess its effects on renal haemodynamics

(d) to assess its inotropic effects

1.5.1.5: to determine the effect of atrial natriuretic peptide on:

(a) diuresis and natriuresis.

(b) whether any effects correlate with changes in urinary cyclic GMP excretion.

1.5.1.6: to examine the effect on diuretic responsiveness of sympathetic nervous system activation using tyramine compared to antagonism with phentolamine infusion.

1.5.1.7: to determine whether dopamine augments a furosemide induced diuresis and if so, whether the effect is seen at dopamine concentrations compatible with a renal vasodilator effect alone, or only at pressor concentrations.

1.5.2 To determine the most important mechanism responsible for alteration in the renal response to furosemide

While the author recognises the impossibility of isolating individual systems in clinical studies such as these, an attempt has been made to achieve the objectives by accurate recording of arterial pressure, renal plasma flow, urinary furosemide excretion, urinary cyclic GMP excretion, plasma hormone concentrations and urinary electrolyte excretion. It should be possible to reach a conclusion about the most important or overriding factor responsible for diuretic resistance in heart failure, even if the precise contribution of and interaction of the other systems remains obscure.

1.5.3 To propose appropriate treatment strategies.

Chapter 2 Methods

2.1 Inclusion criteria

Patients were invited to participate in studies if they were between the age of 18 and 80 years, of either sex, requiring maintenance diuretic therapy for control of symptoms of heart failure. All had objective evidence of left ventricular systolic dysfunction as assessed by echocardiography (fractional shortening <20%) or radionuclide ventriculography (ejection fraction <40%).

Patients with obstructive valvular pathology, hypertrophic cardiomyopathy, constrictive pericarditis, frequent or unstable angina pectoris or a history of significant ventricular arrhythmia were excluded. Included were patients with idiopathic dilated cardiomyopathy, left ventricular failure as a consequence of ischaemic heart disease or heart failure secondary to valvular regurgitation. Patients with significant renal dysfunction (elevation of serum creatinine to greater than 200mmol/l), or hepatic dysfunction (elevation of aspartate transaminase, alkaline phosphatase or total bilirubin by more than twice the upper limit of normal) were excluded. All patients provided written informed consent.

2.2 Study design (Figure 2.1)

Studies were conducted on the day surgery ward at the Hammersmith Hospital. All patients acted as their own controls and were studied on a minimum of two occassions. All patients were interviewed by the senior hospital dietician and were provided with individual diet sheets to follow and diaries to complete during the three day period prior to each study. Diet sheets were collected during each study day and the details checked with the patient by the hospital dietician. The prescribed diet contained 100mmol of sodium and 40mmol of potassium per day for the three days prior to each

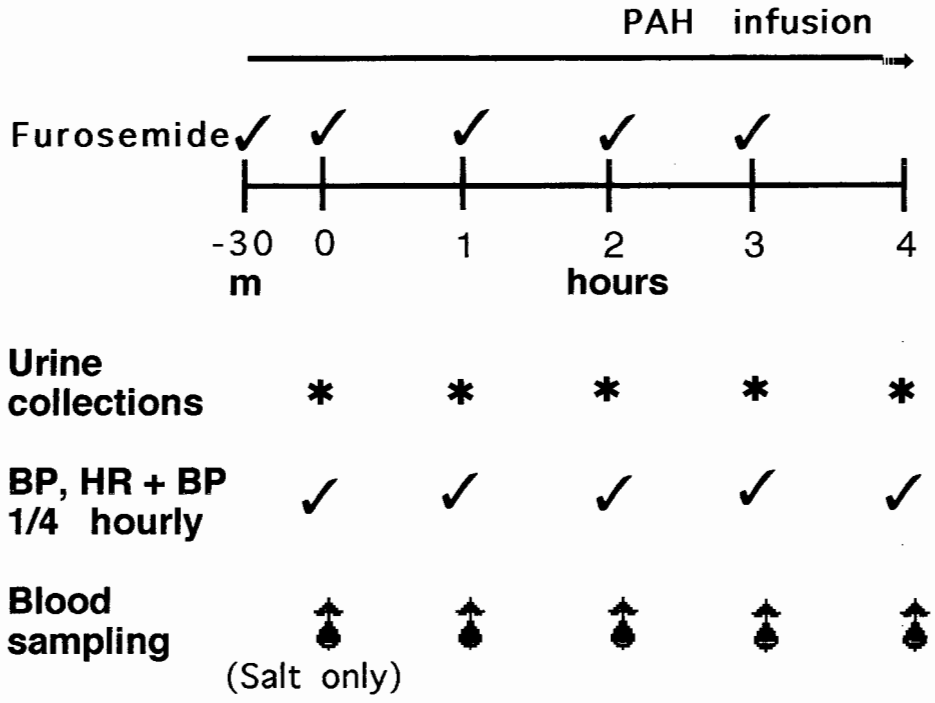
study (with the exception of the study investigating the effect of sodium restriction on diuresis, where 3-day diets of 150mmol of sodium or 20 mmol of sodium were adhered to in random fashion). Caffeine-containing beverages and nicotine were avoided on study days.

Usual therapy was omitted on study days. Patients partook of a light breakfast and fluids (excluding caffeine) at least two hours prior to studies and subsequently fasted until completion of the study. They were studied supine, over four hour periods, and hourly identical intravenous boluses of furosemide were administered to maintain a constant, moderate diuresis. Each patient received identical doses of furosemide on all study days, but individual furosemide requirements varied depending on the patients' usual diuretic requirements.

Plastic cannulae were placed in antecubital veins bilaterally for infusions and sampling respectively and initial loading boluses of sodium hippurate 0.8g (PAH) and furosemide 5mg were given. PAH was subsequently infused at 30mg/min for the duration of the study. Steady state was assumed to have occurred after 90 minutes of PAH infusion, when the first blood samples for plasma PAH were collected. After 30 minutes, patients passed urine and the study began. Further furosemide boluses were given at hourly intervals (time 0, 1, 2 and 3 hours) after each urine collection. The dose was constant for each patient for each study and timepoint, but varied between patients, depending on their usual diuretic requirements. Our aim was to achieve a steady diuresis of 100 - 300ml/hour and a preliminary assessment was made prior to the studies. Patients were brought to the day ward and reclined in the same chairs as were used for the studies subsequently. Hourly boluses of furosemide were administered

intravenously and urine output measured. Urinary losses were replaced orally each hour, with allowance made for insensible losses, to a maximum of 200mls per hour. The initial urine sample was measured for the purpose of accurate volume replacement but discarded. Hourly urine volume was measured using graduated cylinders and recorded. After four urine collections had been made, lines were removed, patients were examined and discharged.

Figure 2.1: Study design



2.3 Measurements

2.3.1 Hourly urine collections

Urine was saved for measurements of:

- cyclic GMP
- electrolytes:
  - sodium
  - potassium
  - chloride
  - urea
  - creatinine
  - magnesium
  - phosphate
- furosemide
- para-aminohippurate (PAH)
- inulin (for captopril study only).

2.3.2 Blood samples

The following electrolytes were analysed at one, two and three hours after dosing:

- sodium
- potassium
- chloride
- urea
- creatinine
- magnesium
- phosphate

2.3.3 Plasma hormones

Three or more of the following were measured in each study:

atrial natriuretic peptide (ANP)

active renin concentration (plasma renin)

noradrenaline

adrenaline

aldosterone

arginine vasopressin (AVP)

angiotensin II (A-II)

Effective renal plasma flow was calculated from para-aminohippurate (PAH) clearance in all studies. After a bolus of 800mg, an infusion of 30mg/min was commenced. After 90 minutes, hourly blood and urine samples were collected for PAH estimation.

Glomerular filtration rate was calculated from inulin clearance in the captopril study only. After a bolus of 50mg/kg, inulin was infused at 32.5mg/min. After 90 minutes, hourly urine and blood samples were collected for inulin estimation.

2.3.4 Haemodynamic measurements

Blood pressure was measured at 30 minute intervals using a mercury sphygmomanometer for the salt and captopril studies.

For the remaining studies blood pressure was measured at 15 minute intervals using an automated oscillomanometric device ("Dynamap-Criticon").

Heart rate was monitored continuously from the electrocardiogram.

Effective renal plasma flow was estimated from clearance of sodium hippurate

(PAH) using the formula ERPF = urine flow (mls/min) x urinary PAH concentration / plasma PAH concentration

Although the original equation referred to renal arterial plasma, the concentration in systemic venous plasma is used in clinical practise as the concentrations are virtually identical.

Glomerular filtration rate was estimated from clearance of inulin (captopril study) or clearance of creatinine using the formula

inulin clearance = urine flow rate x urinary inulin concentration. / plasma inulin concentration.

Changes in ejection fraction and cardiac index were assessed at 15 minute intervals by thoracic bioimpedence using the "Bomed NICOM3" (an automated ZCG-related non-invasive technique for measurement of cardiac output by means of electrical impedance). A detailed description of the technique follows (2.8.1).

2.4 Interventions

All the studies began with a 30 minute run-in period to ensure that patients could empty their bladders before the start. During the first hour there were no active interventions to allow steady state clearance of PAH and inulin to be achieved. Agents with a relatively long half life (captopril and digoxin) were administered at the start of the second hour. The remaining infusions (nitroprusside, dobutamine, ANP, tyramine, phentolamine and dopamine)

took place during the second and third hours only. The exception was the salt study, where the intervention consisted of the normal or salt deplete diet which had been followed for the preceeding 3 days. Studies were all at least 48 hours apart. A steady, moderate diuresis was maintained by hourly intravenous furosemide injection. This hourly dose was arrived at after an initial assessment on the ward when hourly boluses were administered in incremental fashion starting at 3mg if the patient was taking 40mg of furosemide per day, 4mg if the daily dose was 80mg and 5mg if the daily dose was 120mg or more. The final dose chosen was that which resulted in two consecutive hourly urine outputs of between 100 and 300ml.

Agents were infused at very low dose for one hour, then increased incrementally at five minute intervals to a predetermined maximum, that rate being continued for the duration of the following hour. This sequence was not followed with digoxin, because of its relatively long plasma half life, nor with captopril as it was administered orally. Thus digoxin was administered intravenously over 30 minutes and captopril was taken orally at the beginning of the second hour.

A variety of agents were compared with respect to the effect they had on a furosemide induced diuresis.

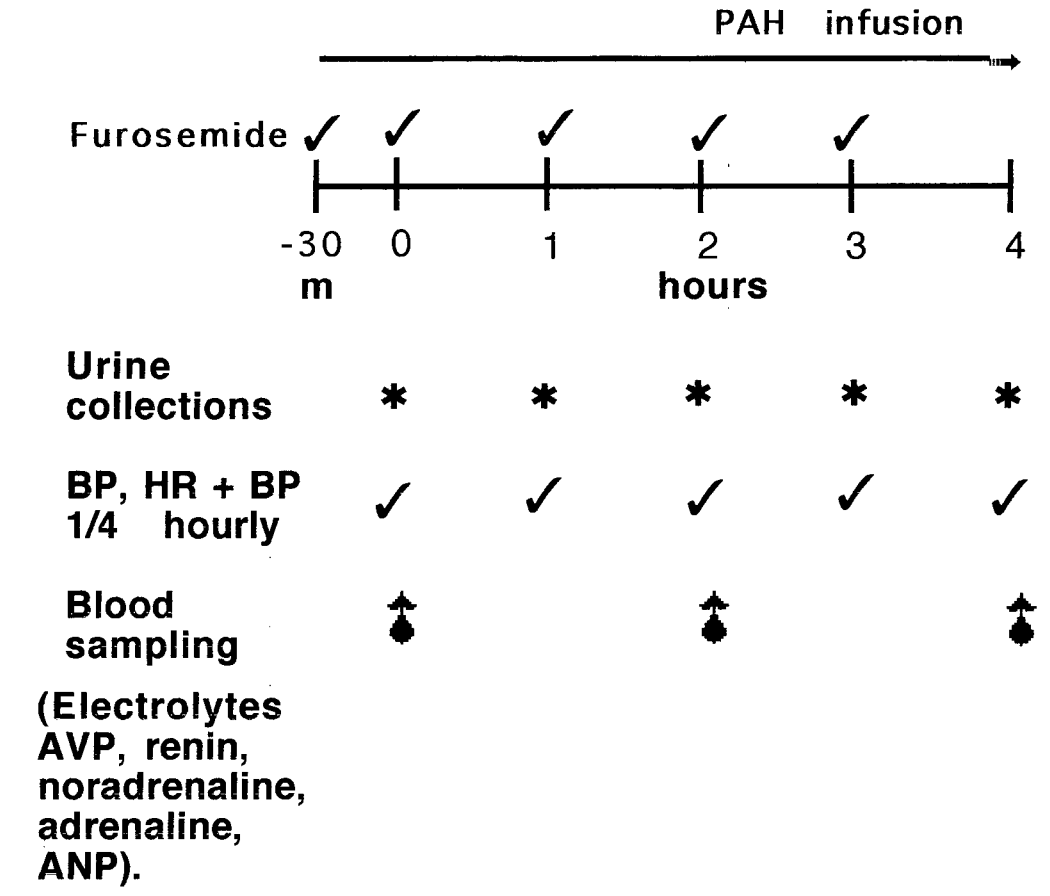
a) Salt restriction versus normal salt intake.
b) Acute-on-chronic suppression of angiotensin converting enzyme with captopril.
c) Atrial natriuretic peptide infusion.
d) Stimulation the efferent sympathetic outflow with tyramine or blockade with phentolamine.
e) Increasing cardiac output using an inotropic agent (dobutamine) as

compared to a vasodilator (sodium nitroprusside).

f) Comparing the effect of dobutamine with that of dopamine, which stimulates the renal dopamine receptor.

g) Assessing the effect of digoxin on diuresis and natriuresis.

Figure 2.2 Study design (SALT)



2.4.1 The effect of dietary sodium restriction on diuretic responsiveness to frusemide (Figure 2.2).

Patients were studied on two occasions, but the two study days were preceeded by 3 days of 150mmol of sodium/day (normal sodium intake) or three days of 20mmol of sodium/day (low sodium diet) in random fashion.

The following measurements were made:

a) Heart rate and blood pressure were measured at half hourly intervals

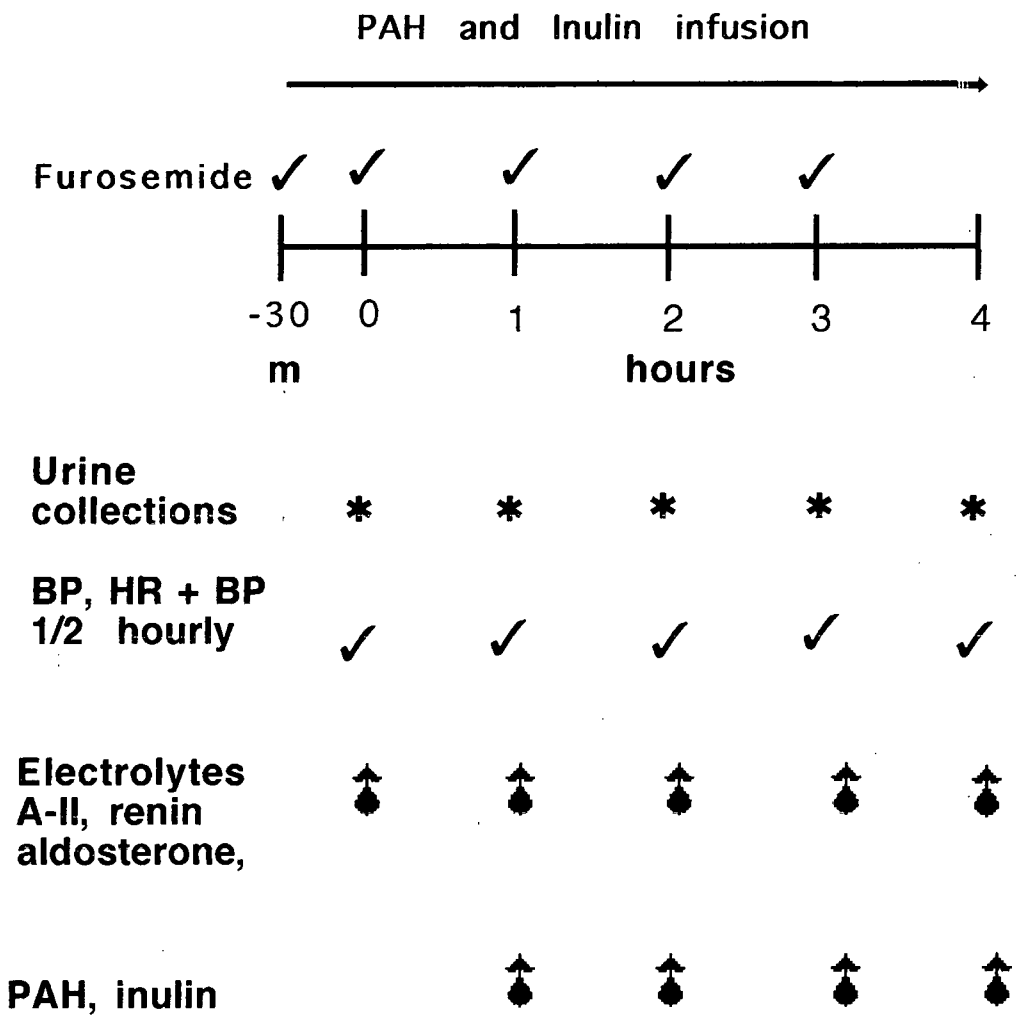
b) Urine volume, electrolytes, PAH and cyclic GMP were measured at the end of each hour.

c) Plasma PAH was measured at the end of each hour.

d) Plasma electrolytes were measured at 0, 2 and 4 hours.

e) Active renin concentration, argenine vasopressin, noradrenaline, adrenaline and atrial natriuretic peptide concentrations were measured at 0, 2 and 4 hours.

Figure 2.3 Study design (Captopril)



2.4.2 The effect of maximal suppression of angiotensin converting enzyme on diuretic responsiveness to intravenous furosemide (figure 2.3)

Patients were asked to adhere to a diet containing 100mmol of sodium and 40mmol of potassium per day for the three days prior to each study. All had been prescibed an ACE inhibitor for at least 3 months and this was substituted for captopril 12.5mg tds for at least 2 weeks prior to the study. Patients omitted their usual medication on the morning of each study but in addition omitted three doses of captopril prior to each study to allow circulating angiotensin II concentrations to return to baseline. In addition to infusion of PAH, inulin was administered as a bolus (50mg/kg) then infused at 32.5mg/min to calculate glomerular filtration rate from inulin clearance. At the end of the first hour of the study, captopril 12.5mg or matching placebo was given in random fashion.

The following measurements were made:

a) heart rate and blood pressure at half hourly intervals.

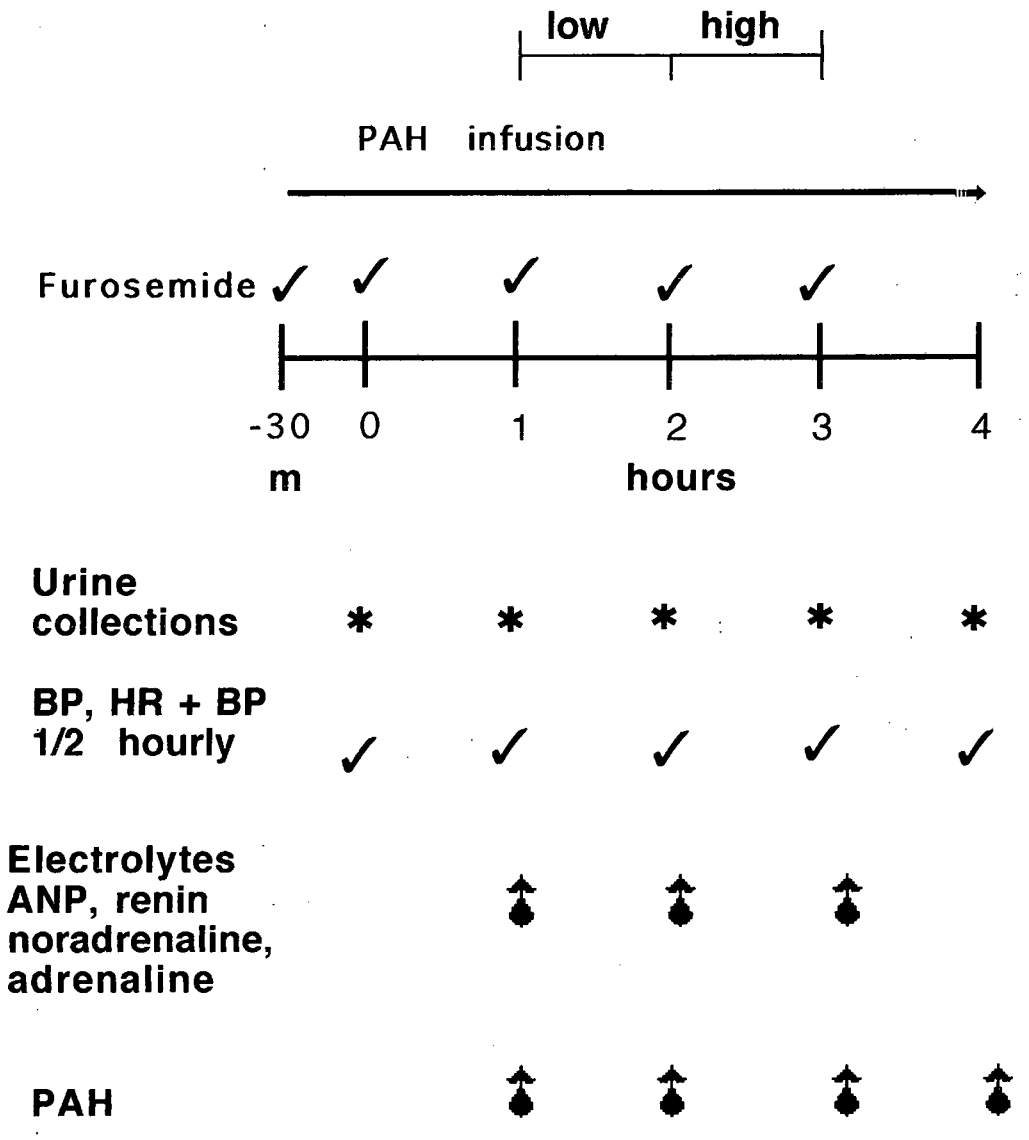
b) urine volume, electrolytes, PAH, cGMP and inulin

c) plasma PAH and inulin

d) electrolytes and haematocrit at 0, 1, 2, 3 and 4 hours.

e) plasma hormones at 0, 1, 2, 3 and 4 hours (aldosterone, angiotensin II and active renin concentration).

Figure 2.4. Study design (Dobutamine, nitroprusside)



2.4.3 The effect of agents which improve cardiac performance on the diuretic response to furosemide (figure 2.4)

Patients adhered to the standard diet as set out above. PAH was infused for the duration of the study and intravenous furosemide was injected at hourly intervals. After the first hour, infusion of sodium nitroprusside, dobutamine or normal saline was commenced, in random fashion and continued over the following two hours.

Sodium nitroprusside was infused at 0.2μg/kg/min during the 2nd hour then increased incrementally at 5 minute intervals during the 3rd hour to a maximum dose of 0.6μg/kg/min.

Dobutamine was infused at 1μg/kg/min during the 2nd hour then increased incrementally at 5 min intervals during the 3rd hour to a maximum of 10μg/kg/min.

Placebo (normal saline) was infused at 30ml/hour during the 2nd hour then at 60ml/hiur for the 3rd hour.

The following measurements were made:

Heart rate and blood pressure

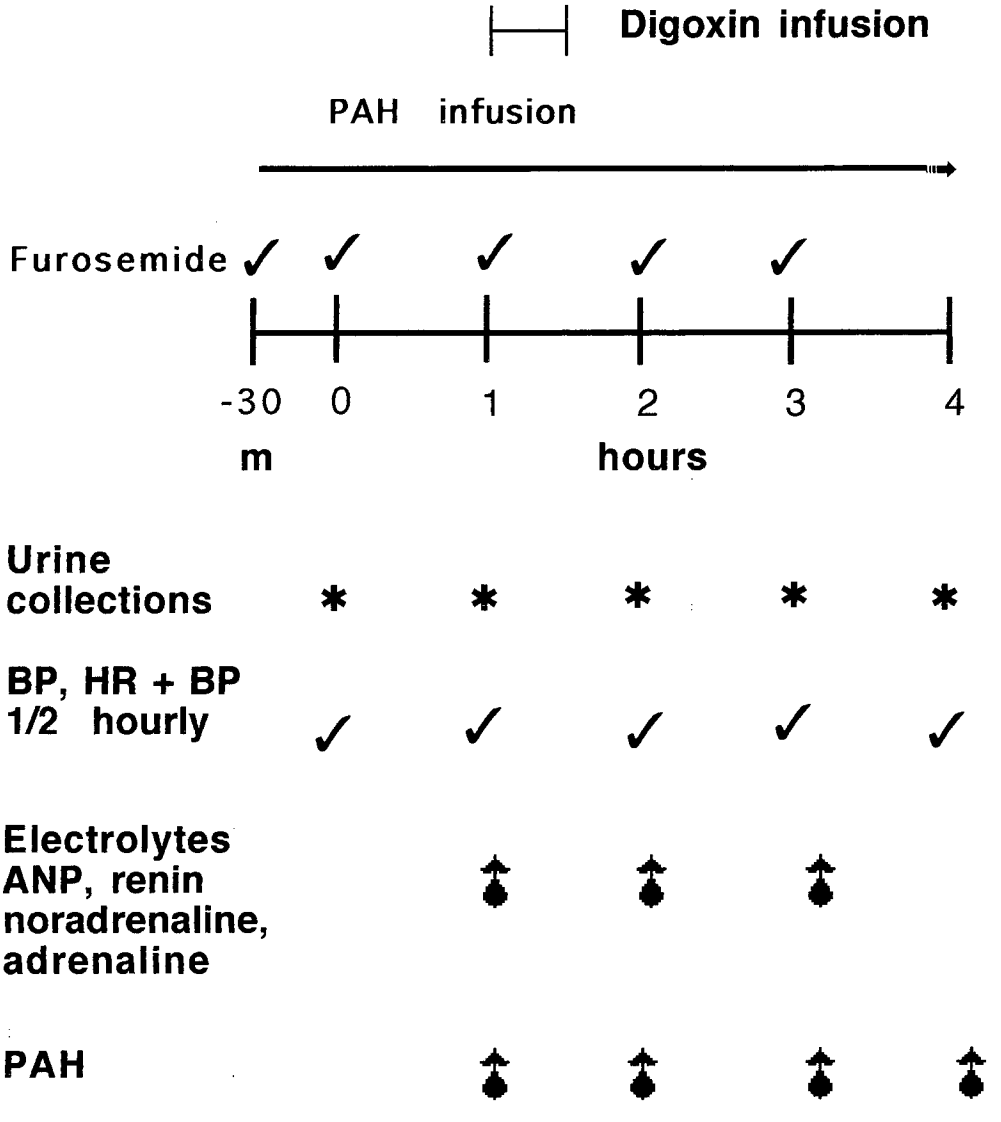
Cardiac index, ejection fraction, stroke volume and thoracic fluid index.

Urine volume, electrolytes, PAH, cGMP and furosemide.

Plasma PAH

Plasma electrolytes and hormones (atrial natriuretic peptide, noradrenaline, adrenaline and active renin concentration) at 1, 2 and 3 hours.

Figure 2.5. Study design (Digoxin)



2.4.4: The effect of intravenous digoxin infusion on a furosemide induced natriuresis and diuresis (figure 2.5)

Patients adhered to the standard diet and had PAH infused. At the end of the first hour, digoxin 500μg was infused over 30 mins.

The following measurements were made:

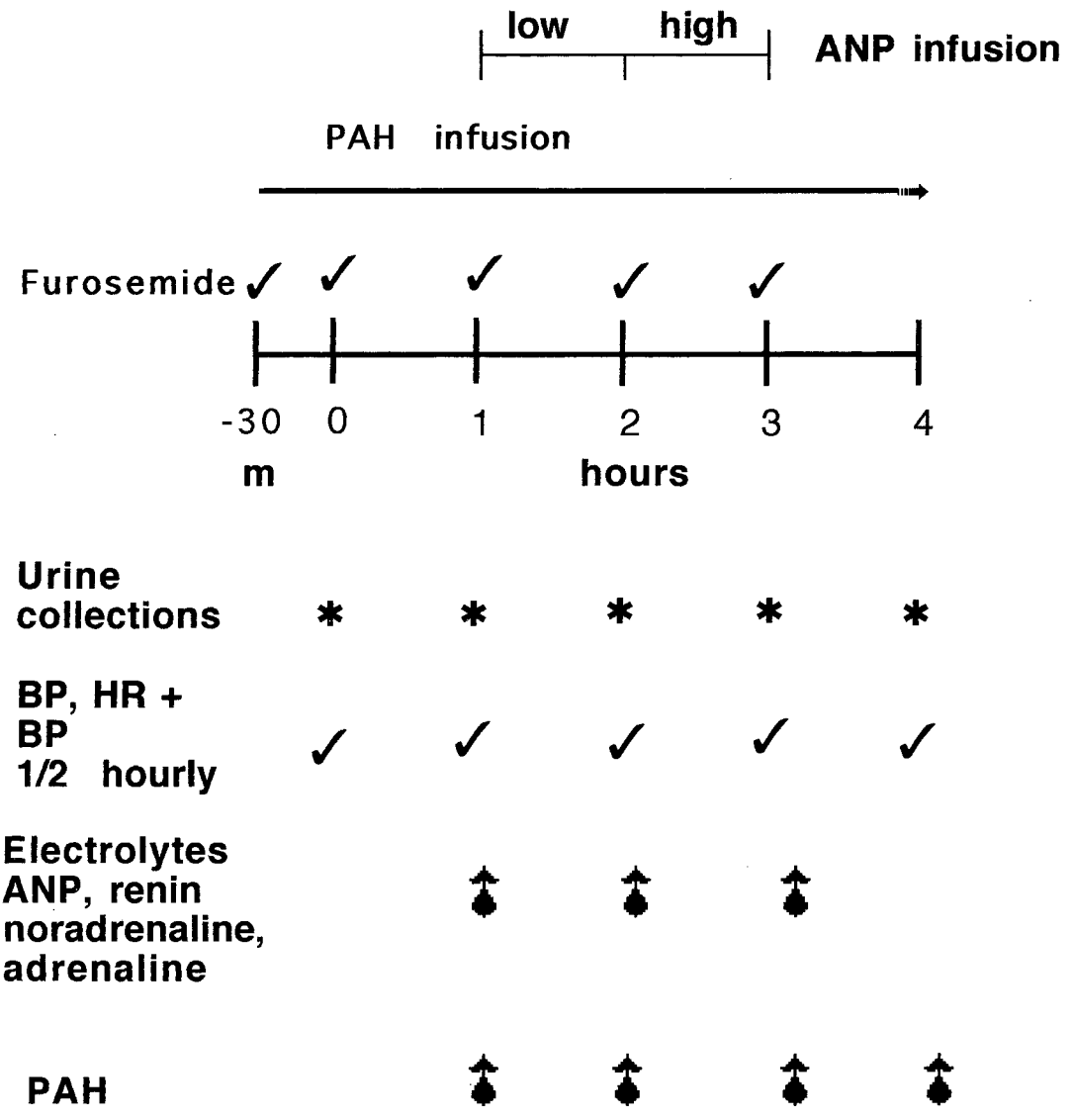
a) heart rate and blood pressure

b) urine volume, electrolytes, PAH, cGMP and furosemide.

c) plasma PAH

d) plasma electrolytes and hormones (atrial natriuretic peptide, active renin concentration, noradrenaline and adrenaline) at 1, 2 and 3 hours.

Figure 2.6. Study design (ANP)



2.4.5: The effect of atrial natriuretic peptide (ANP): Figure 2.6.

Patients adhered to the standard diet and had hippurate infused. At the end of the first hour, ANP infused at 0.05 mcg/kg/min for an hour and increased at 5 minute intervals incrementally to 0.15mcg/kg/min for the third hour was compared with placebo (normal saline).

The following measurements were made:

a) heart rate and blood pressure

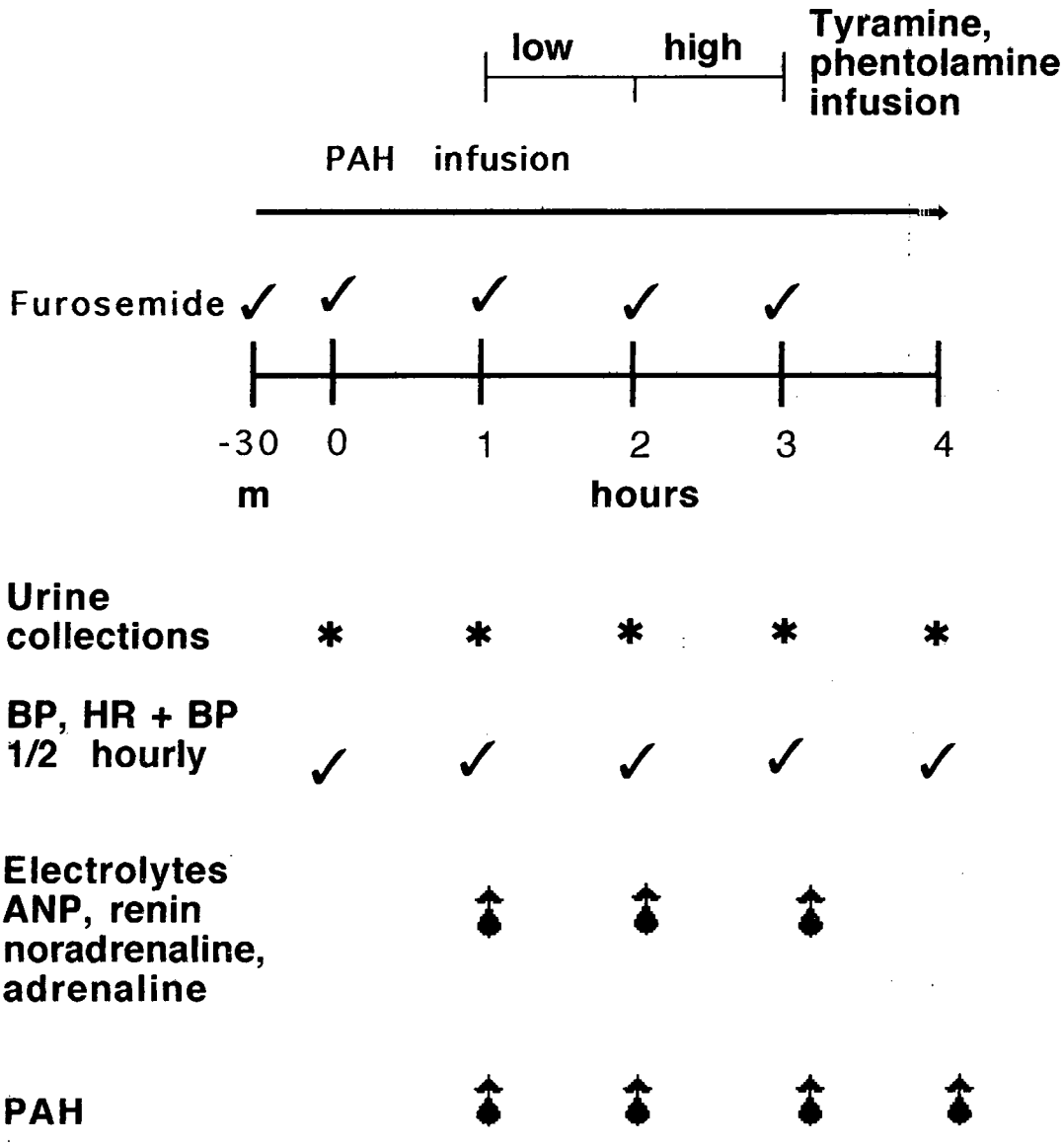
b) cardiac index, ejection fraction, stroke volume and thoracic fluid index.

c) urine volume, electrolytes, PAH, cGMP and furosemide.

d) plasma PAH

e) plasma electrolytes and hormones (atrial natriuretic peptide, active renin concentration, noradrenaline and adrenaline) at 1, 2 and 3 hours.

Figure 2.7. Study design (Tyramine, phentolamine)



2.4.6 The effects of sympathetic activation and inhibition on diuretic responsiveness (figure 2.7)

The study was identical to the preceeding one apart from the interventions: Tyramine or phentolamine were infused at 1mcg/kg/min from the begining to the end of the 2nd hour, then increased at 5 minute intervals incrementally to a maximum of 10mcg/kg/min for the 3rd hour, or placebo (normal saline) infused at 30ml/hour during the 2nd hour and 60ml/hour during the 3rd hour. Treatments were given in random fashion on 3 separate occassions, at least 48 hours apart.

The following measurements were made:

heart rate and blood pressure

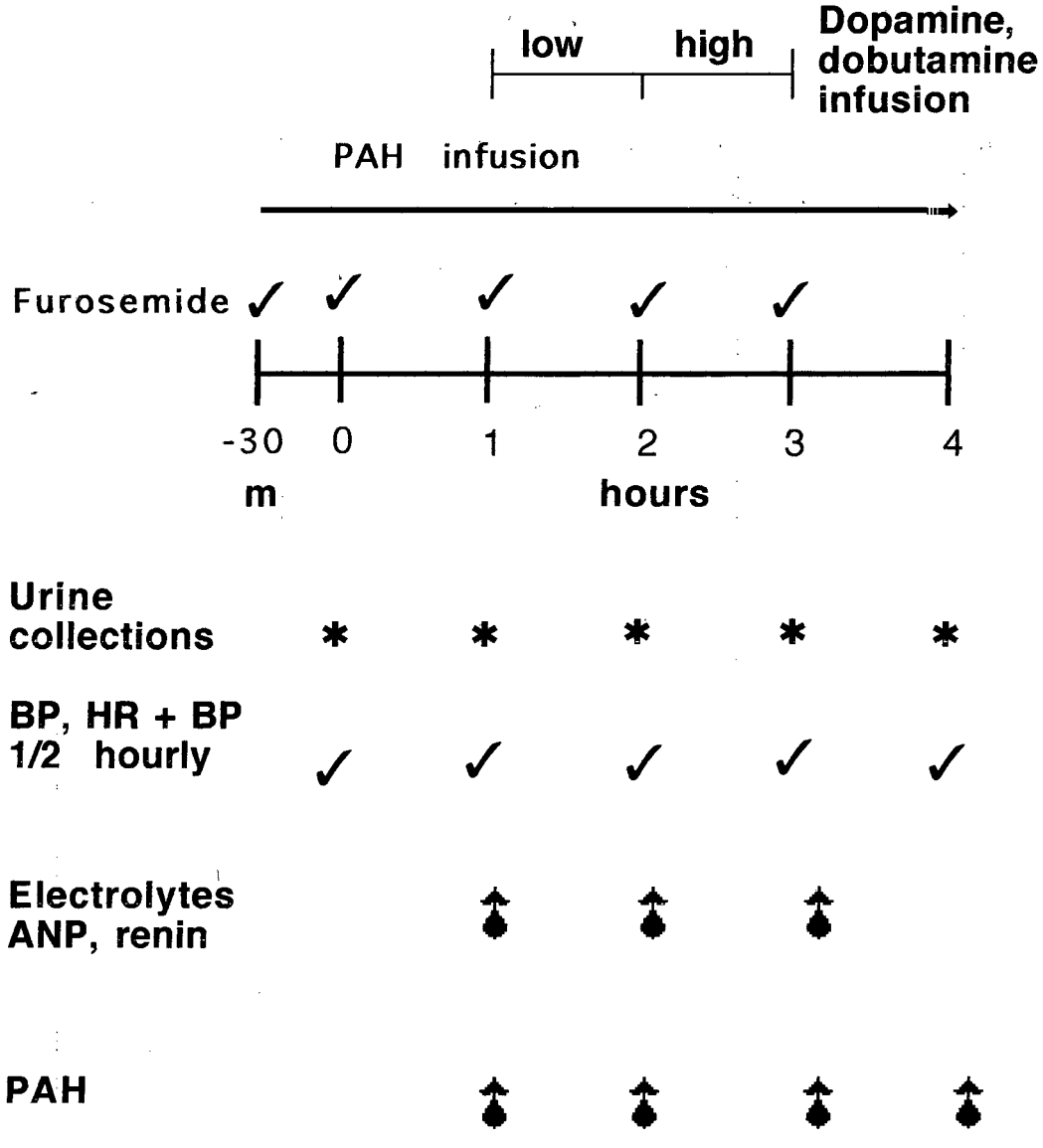
cardiac index, ejection fraction, stroke volume and thoracic fluid index.

urine volume, electrolytes, PAH, cGMP and furosemide.

plasma PAH

plasma electrolytes and hormones (atrial natriuretic peptide, active renin concentration, noradrenaline and adrenaline) at 1, 2 and 3 hours.

Figure 2.8. Study design (Dobutamine, dopamine)



2.4.7 The effect of a dopamine receptor agonist on diuretic responsiveness (figure 2.8)

The study followed the same design as the previous two, with interventions consisting of dopamine or dobutamine infused at 1μg/kg/min during the second hour and increasing incrementally at 5 min intervals to a maximum of 10μg/kg/min during the 3rd hour, or placebo (normal saline) infused at 30ml/hour for the 2nd hour and at 60ml./hour for the 3rd hour.

Measurements were as in the previous studies, excluding measurement of catecholamines.

2.5 Analysis of samples

2.5.1 Electrolyte analysis. The following kit manufacturers, methodological codes and analysers were used for **plasma sample analysis**:

Analyte	Kit man.	Meth.code	Analyser
Mg	Wako	999-83909	RA-XT (Technicon)
Cl	Chemlab	proprietary	CCM1 chloride meter (Chemlab Sci Equipt)
PO_4	Bayer diag	SG4-0004J81	SMAC-2 Technicon
Na	Bayer diag	SG4-0033J81	SMAC-2 Technicon
K	Bayer diag	SG4-0034J81	SMAC-2 Technicon
Urea	Bayer diag	SG4-0001J81	SMAC-2 Technicon
Creat	Bayer diag	SG4-0011B83	SMAC-2 Technicon

Urine sample analysis:

Na	Beckman	proprietary	E2A ISE analyser (Beckman)
K	Beckman	proprietary	E2A ISE analyser (Beckman)

Magnesium, chloride, phosphate, urea and creatinine were assayed as blood was.

Assays were kindly performed by Dr Jeremy Beacham, Mr John Meek and Mr Andrew Mashford in the chemical pathology laboratory, The Royal Postgraduate Medical School, London.

2.5.2 Hormonal analysis

2.5.2.1 ANP

Plasma atrial natriuretic peptide was measured after pre-extraction from plasma by radioimmunoassay (Richards et al; 1987). Blood was collected in chilled tubes containing EDTA and aprotinin and centrifuged at 3000rpm within 10 minutes of collection. Samples were stored at -80 ^{0}C until assay by Dr JJ Morton's team at the MRC laboratories in Glasgow. In brief, ANP was extracted from 1-4ml of plasma on C18 reverse phase columns ('Sep-Pak', Waters Associates). Standard solutions were prepared by serial dilution of synthetic alpha-ANP in Buffer A from 100 to 0.4pg/tube. 100μl of the sample, 100μl of the antibody at a dilution of 1/10 000 and 2pg ^{125}I-alpha hANPin 50μl of the buffer, were incubated at 4^{0}C for 24 hours. All assays were set up in duplicate. Non-specific binding of radioligand was estimated for standard solutions, plasma and plasma extracts by the addition of excess synthetic alpha-hANP (10ng) to duplicate samples and also by incubation and separation in the absence of antiserum. Non-specific binding in both standard solutions and plasma extracts was consistently less than 4%. Sensitivity of the assay was 2pg/ml of plasma. Intra-assay and inter-assay variations were 3.6% and 1.4% respectively.

2.5.2.2 Methodology for aldosterone assay

Aldosterone was kindly assayed by Dr Jeremy Beacham, Department of Chemical Pathology, Royal Postgraduate Medical School.

Plasma aldosterone was measured using a commercial solid-phase (coated tube) radioimmunoassay (Count-A-Coat Aldosterone kit; DPL, (UK) LTD). Venous samples were collected in chilled tubes on ice containing lithium heparin and spun down in a cooled centrifuge within 10 minutes of

collection. Cross-reactivity to other compounds was extremely low, as set out in the table below. Compounds listed as 'ND' were below the detection limit of the assay, even when assayed at great concentrations. Intra-assay variation was less than 8.3%, sensitivity was 15pg/ml and the normal range for recumbent subjects on a fixed sodium intake is <25ng/100ml of plasma.

Table 2.1 Cross-reactivity of the aldosterone assay

Aldosterone	100%	Estriol	ND
Androstendione	ND	Estrone	ND
Androsterone	0.0005%	Fludrocortisone	ND
Corticosterone	0.002%	Prazosin HCl	ND
18-OH-Corticosterone	0.033%	Prednisolone	0.00003
Cortisol	ND	Prednisone	ND
Cortisone	0.0003%	Pregnenolone	ND
11-Deoxycorticosterone	0.006%	Progesterone	0.007%
11-Deoxycortisol	0.0004%	17a-Hydroxyprogesterone	ND
Dexamethasone	0.00005%	Spironolactone	0.06%
DHEA	0.0005%	Testosterone	ND
Estradiol	ND		

2.5.2.3 Methodology for plasma renin concentration

Samples were kindly assayed by Dr JJ (Ian) Morton's team at the Medical Research Council laboratories in Glasgow. Blood was collected in chilled tubes containing disodium EDTA as anticoagulant and separated within 10 minutes. Plasma was stored at -80 degrees until assay. No converting enzyme or angiotensinase inhibitors were added. Plasma active renin concentration was measured by a modification of an antibody trapping

technique in the presence of added excess renin substrate (Miller, 1980). Radioimmunoassay of angiotensin-I generated during the incubation of plasma and excess ox renin substrate was performed. Results for its assay were calibrated with the International Standard Renin. In order to measure active renin concentration, ox renin substrate was included in the reaction mixture at concentrations sufficient to ensure zero-order reaction kinetics with respect to substrate. Reaction velocity was therefore proportional to renin concentration. External calibration against the International Standard Renin allowed results to be expressed in enzyme concentration units rather than velocities. Units are expressed in μU renin/ml plasma as derived from a calibration graph constructed using pure human renin. The intra-assay variation was 3.4% with a sensitivity of 1μU/ml of plasma. The normal range for recumbent subjects on a fixed sodium intake is <40μU/ml.

Buffers and reagents:

a) Incubation buffer: 3 mol/l Tris/HCl, pH 6.9, containing 0.005mol/l disodium ethylenediamine tetraacetate (EDTA).

b) Radioimmunoassay buffer: 0.25 mol/l Tris /HCl, pH 7.4, containing 0.01% human serum albumin (HSA).

c) Ile^5-angiotensin I (National Institute for Biological Standards and Control, London House) was used as an antigen for antibody production, as a routine radioimmunoassay standard, and in the calibration of reaction velocity with the International Standard Renin.

d) International Reference Standard Renin was obtained from the National Institute for Biological Standards and Control, London. House standard human kidney renin was prepared from kidneys obtained post mortem.

20μl of plasma was incubated at 37^0 C for 30 minutes with 20μl of a premixed solution consisting of ox or sheep renin substrate, incubation buffer and antibody at appropriate trapping concentration. This concentration is unique to each antibody and was determined by formal titration as described. Abnormally high or low renin samples were assayed by longer incubations or in dilution, respectively. The enzyme reaction was terminated by dilution and cooling, obtained by addition of 500μl of ice-cold buffer followed at once by trace ^{125} I-angiotensin I (5pg, 5000-10000cpm). The radioimmunoassay for angiotensin I was completed by further incubation at 4^0 C for 18 hours. Separation of free and bound ligand was achieved by addition of Dextran-coated charcoal. Standard curves for angiotensin I were prepared by serial dilution of Ile^5 -angiotensin I in 20μl buffer from 200-3.1 pg per tube in duplicate. Pooled human plasma was used as assay quality control. Control high and low renin samples were run with each assay.

2.5.2.4 Methodology for angiotensin II estimation (A-II)

Samples were kindly assayed by Dr JJ Morton's team at the MRC laboratories in Glasgow. Plasma angiotensin II was measured by radioimmunoassay using pre-extraction of angiotensin II from plasma prior to assay (Morton & Webb1985). The normal range for recumbent subjects on a fixed sodium intake was 2-12pg/ml plasma. Venous blood samples were taken into chilled tubes containing EDTA / o-phenanthroline to inhibit converting enzyme and angiotensinase enzymes. The samples were extracted by passage through Sep-pak C_{18} cartridges (Waters Associates, MA, USA). The cartridges were pretreated with methanol (5ml) and then

water (5ml). Plasma (5ml) was then passed through the cartridge under gentle vacuum. After washing with water (5ml), angiotensin II was eluted from the column with 20% aqueous methanol (2ml). The extracts were dried and redissolved in Tris buffer (50mmol/l; pH 7.5) for assay. Sensitivity of the assay was 2pg/ml of plasma with an intra-assay variation of 10%.

2.5.2.5 Methodology for PAH estimation

Samples were kindly assayed by Dr JJ Morton's team at the MRC laboratories in Glasgow. Plasma and urinary PAH were measured using the colourometric method of Waugh and Beall (1974), incorporating a buffered protein-precipitating reagent of optimal and controlled pH for the formation of coloured product. The buffered protein precipitant of pH 1.4 was prepared by dissolving in distilled water 129.0g of 1.0M dichloroacetic acid, 57.0g of p-toluenesulfonic acid and 34.0g of NaOH pellets to a 1ml volume. One percent p-dimethylaminobenzaldehyde-57% ethanolic solution was prepared by dissolving 5g of highly purified p-dimethylaminobenzaldehyde in 300ml of 95% ethanol and distilled water was added to yield a final volume of 500ml. The standard solution was prepared by dissolving free p-aminohippuric acid in distilled water at a concentration of 100mg/100ml.

A preliminary dilution was performed where neccessary to attain a urine/plasma ratio near 1.0 and an estimated plasma concentration below 6mg/100ml. One volume of plasma or urine was mixed with 10 volumes of the buffered 1.0M DCA-0.3M TSA reagent. After standing for a few minutes, the protein-containing samples were centrifuged. Aliquots of the 1:11 supernatants or samples were then mixed with an equal volume of 1%p-dimethylaminobenzaldehyde-57% ethanolic reagent. After 5 or more minutes standing, absorbances are measured in a photometer at 450nm,

using the reagent blank to set the photometer at zero absorbance. Concentrations of PAH were calculated from the measured absorbances, with reference to a standard working curve. Sensitivity of the assay was 1mg/100ml of plasma and 50mg/100ml of urine with an intra-assay variation of 6.2%.

Effective renal plasma flow was calculated from the formula $\frac{U_{PAH}V}{A_{PAH} - V_{PAH}}$

where U_{PAH} = the urine PAH concentration and V = urine flow rate and

$A_{PAH} - V_{PAH}$ = the arterio-venous PAH concentration difference.

About 90% of PAH is extracted in a single pass across the renal vasculature in normal humans when plasma PAH is allowed to range from 1-6mg/dl. Under these circumstances the renal venous PAH concentration is low, and neglecting this term introduces an error of only about 10% in the calculation of renal plasma flow. Although serial samples were not tested, 90 mins were allowed for equilibration of the PAH at which point steady state was anticipated.

2.5.2.6 Methodology for inulin estimation

The assays were kindly performed by Dr JJ Morton and his laboratory staff. Plasma and urinary inulin were measured using a slight modification of the method of Higashi and Peters (1950). In brief, 2.5 ml of water was mixed with 0.5 ml of plasma or urine. 1.3 ml CdSO4 solution (prepared by dissolving 17.34g CdSO4,8H2O in 84.6ml of 0.5mmol/l sulphuric acid and diluting to 500ml with water) and 0.5ml of NaOH solution (1.1mmol/l) were added sequentially and mixed. After standing for 5 minutes the sample was centrifuged and 1ml of the supernatant or standard solution was transferred

to a test tube with a ground glass joint, to which 1.5 ml of resorcinol was added and mixed. Ferric chloride 1.5 ml was then added and mixed. Samples were held in a water bath for 40 minutes at 80oC then cooled and read against the reagent blank within 20 minutes. Sensitivity of the assay was 20mg/l of plasma and 200mg/l of urine with the intra-assay variation 3%.

2.5.2.7 Plasma vasopressin

Plasma vasopressin was measured by radioimmunoassay of pre-extracted plasma. The assays were kindly performed by Dr JJ Morton and his laboratory staff.

Blood samples for AVP measurement were collected in chilled tubes containing heparin and aprotinin and separated within 10 minutes. Plasma samples were stored at -80^{0}C until assay. AVP was extracted from the plasma using prepacked C_{18} reverse-phase cartridges (Sep-pak, Waters Associates, Milford, Mass., USA). Cartridges were initially washed with methanol (5ml) followed by distilled water (5ml). Plasma (3-5ml) was applied to the cartridge under gentle vacuum followed by washing with 1% acetic acid (5ml). AVP was eluted from the column by washing with methanol (2ml). The methanol extracts were dried under a stream of air and the dried extracts dissolved in 50mmol/l Tris pH7.4 for assay. A specific AVP antiserum was raised in New Zealand white rabbits by immunization with AVP conjugated to bovine thyroglobulin. Standard AVP (International Institute for Biological Standards and Control) or unknown was incubated with AVP antiserum at a final dilution of 1:75 000 and ^{125}I-AVP (0.5pg, New England Nuclear, Dreich, West Germany) for 48 hours at 5^{0}C. Bound from free AVP was separated using dextran-coated charcoal. The inta-assay

variation was 6.1% and values for normal recumbent subjects after overnight fluid restriction are 0.2-1.0pg/ml plasma. (Morton, Connell, Hughes, et al 1985b). Sensitivity of the assay was 0.1pg/ml of plasma.

2.5.2.8 Plasma noradrenaline and adrenaline

Plasma noradrenaline and adrenaline were extracted from plasma and assayed by high performance liquid chromatography and electrochemical detection after the method of Goldstein, Feuerstein, Izzo, et al (1981). The assays were kindly performed by Dr JJ Morton and his laboratory staff.

Venous samples were collected in chilled glass tubes with heparin and separated within 10 minutes. Plasma samples were stored at -80^{0}C until assay. The reagents and apparatus for liquid chromatography with electrochemical detection (LCED) were all commercially available.

For assay of plasma catecholamines, 1-1.5ml of freshly thawed plasma was added to 25mg acid-washed alumina and 50μl of of 5mM sodium metabisulphite in a 4ml plastic tube. To this was added 50μl of 10ng/ml DBHA, the internal standard, and 400 μl of 1M Tris-2gm% EDTA which had been adjusted with HCl to a pH of 8.6. The sample was shaken for 10 minutes, centrifuged, and the supernatant discarded. The alumina was washed twice with 1.5ml of 0.2%tris/EDTA, pH 8.1 and the catecholamines then desorbed with 100μl of 0.1% acetic acid containing 0.1mM sodium metabisulphite. The tube was shaken on a vortex mixer for 10 seconds and in order not to inject particles of alumina, was spun in a microcentrifuge and the supernatant transferred to an empty plastic tube. 40μl of the supernatant was analysed using a Waters automated HPLC system for catecholamine analysis with electrochemical detection.

Recovery was calculated from the ratio of DHBA peak heights in the two injections, since 500pg of DHBA had been added to the plasma sample, and 50 of the 100μl of solution used to elute the catecholamines had been injected. The concentrations of noradrenaline and adrenaline were then calculated by dividing the peak adrenaline or noradrenaline height by the recovery, multiplying by 500 and dividing by the peak height for the corresponding 250pg standard, with the results expressed in pg/ml. Recoveries from the alumina extraction averaged about 65%.

The assays were kindly performed by Dr JJ Morton and his laboratory staff. Sensitivity of the assay was 0.1nmols/l of plasma for both adrenaline and noradrenaline with an intra-assay variation of 5.3% and 5.7% respectively.

2.5.2.9 Urinary Cyclic GMP

Guanosine cyclic monophosphate (cGMP) was assayed by radioimmunoassay, kindly performed by Dr Jane Kirk and her laboratory assistants in Dr Martin Wilkins's laboratory at the Royal Postgraduate Medical School. A commercial antibody to rabbit cGMP from Sigma Chemical Co was used as the radioligand. 5μl of urine (or appropriate dilution; 1:1, 1:10 or 1:50) was incubated overnight in 300μl sodium acetate buffer (pH 5.9) with primary antibody (final titer 1:75 000), goat antirabbit antibody (final titer 1:12 500) and radioligand (10 000cpm). The following day, each tube received 2ml of distilled water (4^0C) and was centrifuged at 3000 rpm for 30 minutes. The supernatant was discarded and the pellet counted by automatic gamma counter. The limit of detection of the assay was 0.25 pmol/tube with intra-and interassay variability of 6% and 14% respectively. There was no crossreactivity with cyclic AMP (Wilkins, Settle, Needleman 1990).

2.5.3 Urinary concentrations of furosemide

Furosemide concentrations were determined using a spectro-fluorimetric HPLC method. These assays were kindly performed by Mr Faruq Noormohamed in Prof Landt's laboratory at the Westminster Hospital. Internal standard (bumetanide, 250ng) was added to 0.25ml urine sample which was subsequently made up to 1 ml with distilled water. The samples were mixed and centrifuged at 6000 rpm to remove any particulate matter. The supernatent was injected (30μl) via an autosampler (WISP 710B, Waters Millipore, Milford, Mass.) on to a 25 cm mixed alkyl-cyano bonded reverse phase column (Sherisorb 5μ ODS/CN;PhaseSep, Deeside, UK). The peaks were eluted with an isocratic buffer (0.1M $H_3 PO_4$: Acetonotrile; 60:40, pH 3.5 with NaOH) pumped at 1ml/min. Furosemide (5.5 min) and bumetanide (8.5 min) peaks were detected with a Kratos FS970 fluorimeter (Applied Biosystems, Manchester UK; excitation wavelength 220nm, emission cut-off at 379nm) and quantified with an integrator (model SP4270, Spectra Physics, San Jose, California.) using peak height ratios. The urinary furosemide curve was linear from 150 ng/ml to 5000 ng/ml with an intra-assay and inter-assay coefficient of variation of 1 and 7% respectively. The minimum detection limit of the assay was 40ng and the minimum quantifiable limit was 100ng. With the exception of patients on nifedipine, whose urine samples exhibited multiple peaks, none of the other drugs administered concomitantly interfered with determination of furosemide.

2.6 Statistical analysis of data

A visual impression of the data was displayed graphically using means with error bars representing ±1 standard error of the mean (S.E. mean).

The data was tested for normal distribution using the Kolmogorov-Smirnov

test for goodness of fit (Conover 1971). Without exception the data was not normally distributed, thus non-parametric tests were used. The Friedman test for analysis of variance was used to test significance of any differences between groups at different time points where time points and factor levels (active and placebo) were regarded as within-subject factors. Differences between time points were analysed using Wilcoxon matched pairs signed ranks tests. All probabilities were two-tailed. One-tailed t-tests were avoided as there was a greater likelihood of introducing a Type 1 error. Values of $p<0.05$ were considered significant. Data was imput to a computer database and analysed using Statview SE software and Apple Macintosh.
Mrs Julie Jeacock's help with revision of the statistics is gratefully acknowledged (Senior Statistician, Trafford NHS Trust).

2.7 Ethical and safety considerations

All studies were approved by the research and ethics commitee of the Hammersmith Hospital. Informed written consent was obtained from all patients.

It was clearly stated that the studies were unlikely to be of direct benefit to the patients. In addition it was made clear that the studies would be terminated at any stage, at the patient's request, in the event of discomfort, chest pain, breathlessness, dizziness, or indeed any other symptom. No patient requested that a study be stopped, but the infusion rate was decreased in several patients, in accordance with the protocol, when heart rate or arterial pressure rose or fell above or below the predetermined limits set for each patient.

The main potential dangers involved in these studies are secondary to the inotropic and chronotropic effects of adrenergic agonists, with the risk of

provoking angina pectoris, breathlessness or arrhythmias in patients with heart failure, some with underlying ischaemic heart disease, and that of precipitating hypotension or even syncope by administration of the powerful vasodilators sodium nitroprusside and phentolamine in patients with reduced circulating volume. These risks were minimised by excluding patients with a history of recent angina pectoris, or of ventricular tachycardia within the last two years requiring maintenance therapy. All patients who received tyramine infusions had urine collections tested for catecholamine breakdown products to exclude phaeochromocytoma. With the exception of ANP and tyramine, all these drugs are licensed for therapeutic use in patients with heart failure and have well documented actions and side effects.

2.8: Description of the BOMED NCCOM-3 with a detailed explanation of the methodology.

BOMED NCCOM3 (revision 7) Bomed Medical Manufacturing LTD, Irvine, California was used to measure thoracic bioimpedance. The NCCOM3 is a computer which extrapolates measures of cardiac filling and contractility from measurement of transthoracic impedence during cardiac ejection, using electrocardiogram electrodes both to sense and to deliver the current. A small sinusoidal current is applied to electrodes at the base of the neck and inferior aspect of the thorax (see diagram). A set of electrodes 5cm inside the stimulating electrodes record the changing impedance over that length of thorax. The electrocardiogram is monitored concurrently to time the cardiac cycle. The thorax is treated as a conducting cone, with adjustments made for height and weight of the individual. The equation of Berstein calculates cardiac output from the equation $\mathbf{SV=V_{EPT}\ xt_{LVE}x(dZ/dt)_{max}/Z_0}$, where SV =stroke volume; V_{EPT}= volume of

electrically participating tissue calculated from height and weight, t_{LVE}= left ventricular ejection time (s); $(dZ/dt)_{max}$= maximum rate of impedance change during systolic upstroke; and Z_0= baseline thoracic impedance.

Ag/AgCl (Kimal) monitoring electrodes were positioned carefully according to the manufacturer's instructions after prior preparation of the skin with abrasion and alcohol. Averaged recordings over 16 cardiac cycles were collected at 15 minute intervals.

2.8.1 Parameters

The NCCOM3 displays 12 cardiodynamic parameters: cardiac output or index (CO/CI), heart rate (HR), stroke volume or stroke index (SV/SI), peak flow or peak index (PF/PFI), ejection fraction (EF), end diastolic volume or index (EDV/EDI), index of contractility, thoracic fluid index (conductance), acceleration index, ventricular ejection time, ejection ratio (preload index) and systolic time ratio.

Peak flow: this is defined as the highest rate of left ventricular volumetric delivery during the ejection phase (ml/sec). The flow reaches its peak value in the first half of systole, typically 65msec after opening of the aortic valve. PF time remains unchanged with alterations in heart rate. PF is linked directly to the ejection phase contractility and thus is dependent on the volaemic state and is a good index of ventricular performance.

Ejection fraction is directly related to measurement of systolic time intervals and is a non-linear inverse function of heart rate. Ventricular ejection time, however, is related to stroke volume (it takes less time to eject a smaller volume).

The systolic time ratio: this is the pre-ejection period (PEP) / ventricular ejection time (VET). PEP is the duration of isovolumic contraction.

Ejection fraction is estimated by the following equation based on the results of a comparative study with Bomed and Muga EF: **EF= 0.84-(0.64 x (PEP / VET))**

This assumes that the PEP and /or VET do not change without a change in haemodynamics, and this may not be the case in states of adrenaline surges such as is found with acute myocardial infarction, which shorten both PEP and VET, (underestimates EF) or left bundle branch block (LBBB) which artificially prolongs the the PEP (overestimates EF).

End diastolic volume (EDV) is calculated from SV / EF. EDV is thus an estimate from the calculated EF, (however the widely accepted measurement of the 'wedge pressure' by occlusion catheter (pressure) is used to estimate left ventricular end diastolic volume, ie conversion of pressure to volume).

Index of contractility: Maximum ejection flow (peak flow) or index is fluid-volume dependent and can be used to determine the patient's volaemic status.

Thoracic fluid index (TFI) is an impedance measurement which varies inversely with the conductance of the chest. It is influenced by age, sex and posture. Changing from the standing to the supine position leads to a 10% increase in TFI due to decreased venous return, resulting in less fluid in the chest and thus less conductance. Pulmonary oedema causes a decrease in

TFI (ie increased conductance) but non cardiac pulmonary oedema and cardiac pulmonary oedema can be distinguished by the indices of cardiac function, that is changes in contractility index (ACI). A decrease in contractility index indicates impaired cardiac function.

Acceleration index is the initial acceleration of blood in the left ventricle, which takes place 10-20 msec after the AV valve opens and is much less load-dependent than PFI.

Ventricular ejection time is a direct measurement of mechanical systole which is heart rate and pump status dependent. The absolute value of VET does not have a specific use as it varies widely with different circumstances.

Ejection ratio eliminates the effect of heart rate on VET, giving ER=VET/HRP.
(Heart rate period).

Systolic time ratio is calculated from PEP/VET and is a sensitive indicator of ventricular performance.

Summary

a) Global blood flow related parameters: CO, SV, EDI, PFI, HR
b) Left ventricular performance: SV, PF, IC, AI, STR
c) Heart chamber sizes, efficiency: SV, EDV, EF
d) Volaemia-related parameters: TFI, IC, ER

2.8.2 Validation of the technique

a) Validation in normal volunteers.

Moore (1992) compared the technique with rebreathing of CO_2, at rest and during bicycle steady state exercise, in normal subjects. He found that results were acceptably in agreement at mild or moderate levels of exercise but found that there was no acceptable agreement between the techniques at rest, or at high workload exercise. He found that impedence measurements seemed less reliable at high workloads, while rebreathing of CO_2 appeared less reliable at rest.

b) 24 hours following myocardial infarction, Northridge (1990) compared bioimpedence with thermodilution and Doppler in 25 patients. The mean cardiac output by Doppler was 4.03 (range 2.2-6.0), by thermodilution 3.95 (range 2.1-6.2) and by bioimpedance 3.79 (range 1.1-6.2). In three patients the Doppler result differed from thermodilution by more than 1l/min and in 3 patients thermodilution and bioimpedance differed by more than 1l/min. The 95% limits of agreement for bioimpedance and thermodilution were -1.43 to +1.11l/min. The coefficient of variation for the repeated measurements of cardiac output using bioimpedance by the same observer was 2.7%. Thus both non-invasive methods were accurate and reproducible in most patients with acute myocardial infarction.

3) Comparison of thoracic bioimpedance with thermodilution in normal volunteers and intensive care patients (Jewkes, Sear, Verhoeff, et al 1991). Variability: there was good correlation for individual subjects at rest with repeated measurements on the same day. SV variation on 4 different days had coefficient of variation of 10.9%. This was similar to the variation in heart rate and blood pressure that was measured at the same time. For the patients, there was a correlation coefficient of 0.72 for cardiac output, $p<0.001$. The mean difference in SV for the 2 methods was -0.86l/min. TBI

tended to underestimate stroke volume at higher cardiac outputs and overestimated stroke volume in the lower cardiac output ranges. It is accepted that a 10% variability between thermodilution and dye test (which itself has a variability of aproximately 8%) measurement of cardiac output exists, so the difference between thermodilution and thoracic bioimpedence was of the same order of magnitude. Thus TBI was able to follow individual patient trends.

4) De Mey, Matthews, Butzer, et al (1992) examined agreement and reproducibility of thoracic bioimpedance versus M-mode echocardiography in healthy subjects. They found that the methods were not interchangable as coefficients of reproducibility between the two methods were 25% of baseline.

5) Ng, Walley, Tsao, et al (1991) compared Doppler and thoracic bioimpedance in healthy volunteers. There was no systematic difference between the methods but individual differences were wide.

2.8.3 The present studies

Thoracic bioimpedance (TBI) was chosen to assess changes in haemodynamic parameters because of its simplicity and the ability to take repeated measurements. As an in-house control, changes in SV and EF were compared during 20 minutes of incremental dobutamine infusion and sodium nitroprusside infusion in 8 patients. After the dobutamine or nitroprusside study was completed, patients were transferred to the echocardiography laboratory and received an additional infusion, while simultaneous TBI and Doppler measurements were made. (It was not possible to make measurements in duplicate throughout the individual

studies because of the limited availability of Doppler echocardiography and the layout of the hospital).

Although there was some agreement between stroke volumes measured by the two techniques during dobutamine infusion (figure 2.2), this was not the case during nitroprusside infusion and individual reproducibility of baseline data was poor for both methods. One reason for the wide baseline variability could have been residual effects from the 2 hour infusion although there was a minimum delay of 90 minutes between stopping the high dose infusion and starting the next infusion in the echocardiography suite to allow washout. When the changes in all TBI parameters during all the definitive infusion studies were analysed there were no significant changes in stroke index or thoracic fluid index in the group during either intervention (table 2.4). Looking at individual patient data, some individuals (the minority) did show a clear rise or fall. The mean plasma concentrations of ANP of the group were elevated, indicative of elevated left atrial pressure and significant falls in blood pressure occurred with nitroprusside infusion, which would be anticipated to predict a rise in cardiac output as changes in arterial pressure of a similar magnitude in a group of patients with heart failure treated with nitroprusside were associated with clear changes in haemodynamics measured invasively (Young, Leon, Pratt, et al 1988).

Subsequent analysis of results for the remainder of the studies showed that no parameter changed significantly in any study, despite significant changes in arterial pressure and heart rate (Tables 2.4-2.6). In view of these findings, the validity of the thoracic bioimpedance technique in these patients is in doubt and the results have not been included in subsequent chapters. Reasons for the poor reproducability of TBI may include poor or variable

timing of the cardiac cycle by the equipment due to the presence of left bundle branch block in some patients. The elevated catecholamine levels induced by infusions of dopamine, dobutamine and tyramine might in theory have interfered with calculation of ejection fraction as the equation used assumes that the PEP and /or VET do not change without a change in haemodynamics, and this may not be the case in states of adrenaline surges such as is found with acute myocardial infarction, which shorten both PEP and VET, (underestimates EF) or left bundle branch block (LBBB) which artificially prolongs the the PEP (overestimates EF). These theoretical considerations can only partly explain the consistent lack of significant change in stroke volume, ejection fraction and thoracic fluid index in each study performed, thus for whatever reason, the method proved unreliable in this patient population.

Table 2.2 Doppler and thoracic bioimpedance stroke volume

Table showing changes in stroke volume at baselin (B'LINE), during incremental dobutamine infusion and washout (W'OUT) measured by Doppler and by thoracic bioimpedance (TBI).

DOBUTAMINE µg/kg/min	B'LINE	2.5µg	5µg	10µg	W'OUT
TBI	64±7	67±8	67±8	62±7	73±9
DOPPLER	63±5	65±5	69±5	68±4	75±5

Table 2.3 Absolute and % change in stroke volume

Changes from baseline measured by Doppler and thoracic bioimpedance (TBI) during infusion of dobutamine 10μg/kg/min.

ABSOLUTE CHANGE IN STROKE VOLUME	TBI	9.3±3.5mls
	DOPPLER	12.1±6.0mls
% CHANGE IN STROKE VOLUME	TBI	14±5%
	DOPPLER	24±13%

Figure 2.9 Stroke volume measured by Doppler and Thoracic Bioimpedance during dobutamine infusion

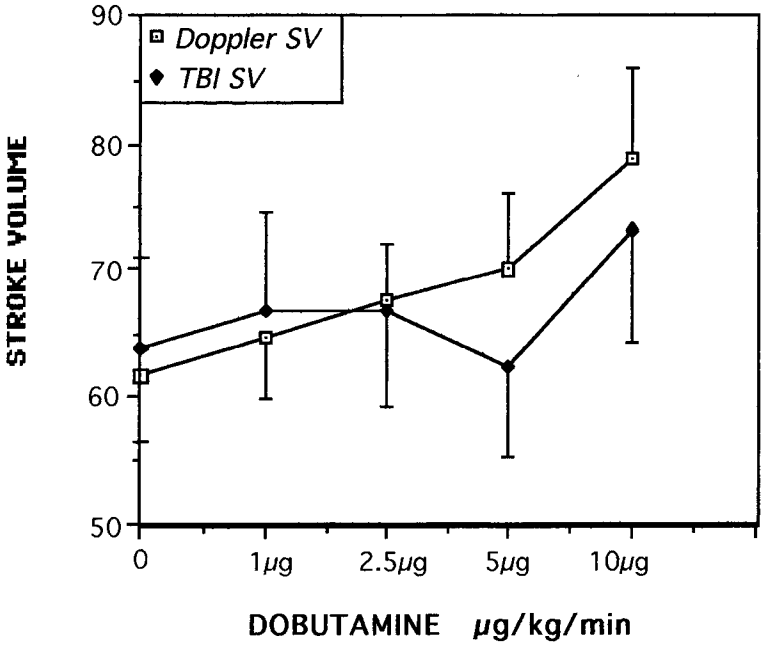


Table 2.4 Thoracic bioimpedance data for placebo, dobutamine and nitroprusside in 8 patients with chronic heart failure

Changes in peak flow index (PFI) ejection fraction (EF), end diastolic index (EDI), cardiac index (CI), stroke index (SI) and thoracic fluid index (TFI) during infusion of placebo (PLAC), dobutamine (DOBUT) and nitroprusside (SNP)

		START	0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
PFI	PLACEBO	205±16	209±17	215±17	217±16	215±16
	DOBUTAMINE	281±42	282±45	289±53	288±44	262±34
	SNP	232±19	235±28	223±27	230±25	228±23
EF	PLACEBO	51±4	50±5	50±5	51±5	51±5
	DOBUTAMINE	51±5	49±6	48±5	52±4	51±3
	SNP	48±4	48±3	46±3	44±4	50±3
EDI	PLACEBO	62±11	68±11	67±10	66±9	66±10
	DOBUTAMINE	75±9	84±12	82±12	78±12	64±8
	SNP	63±6	65±7	64±8	68±8	64±7
CI	PLACEBO	2.2±0.2	2.2±0.2	2.2±0.2	2.3±0.2	2.2±0.2
	DOBUTAMINE	2.8±0.4	3.0±0.5	3.0±0.6	3.5±0.8	2.7±0.4
	SNP	2.2±0.2	2.3±0.3	2.2±0.3	2.2±0.3	2.3±0.3
SI	PLACEBO	29±3	30±3	31±3	30±2	29±2
	DOBUTAMINE	37±6	38±6	39±8	38±7	34±5
	SNP	30±3	31±4	29±4	29±4	31±4
TFI	PLACEBO	36±3	35±3	35±2	35±2	34±2
	DOBUTAMINE	29±2	28±3	31±3	28±2	29±2
	SNP	32±4	31±4	34±4	30±4	32±3

Table 2.5 Thoracic bioimpedance data placebo and digoxin

Changes in peak flow index (PFI) ejection fraction (EF), end diastolic index (EDI), cardiac index (CI), stroke index (SI) and thoracic fluid index (TFI) at baseline and during infusion of placebo (PLAC) and digoxin (DIG).

		B'LINE	0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
PFI	DIGOXIN	225±14	236±20	236±20	245±19	244±18
	PLACEBO	216±18	214±16	224±17	223±15	222±16
EF	DIGOXIN	55±3	55±3	57±3	58±3	58±5
	PLACEBO	55±2	54±2	56±3	56±3	56±2
EDI	DIGOXIN	57±5	61±6	61±5	64±5	65±6
	PLACEBO	55±6	59±5	60±5	59±4	58±5
CI	DIGOXIN	2.3±0.1	2.4±0.2	2.4±0.2	2.5±0.2	2.5±0.2
	PLACEBO	2.3±0.1	2.2±0.2	2.3±0.2	2.3±0.2	2.3±0.2
SI	DIGOXIN	31±2	33±3	35±3	37±3	37±4
	PLACEBO	31±3	32±3	33±3	32±2	31±3
TFI	DIGOXIN	32±3	31±3	31±3	32±3	31±3
	PLACEBO	36±3	36±3	36±2	35±2	35±2

Table 2.6 Thoracic bioimpedance data: placebo, dopamine and dobutamine

Changes in peak flow index (PFI) ejection fraction (EF), end diastolic index (EDI), cardiac index (CI), stroke index (SI) and thoracic fluid index (TFI) at baseline and during infusion of placebo (PLAC), dopamine (DOPA) and dobutamine (DOBUT).

		B'LINE	0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
PFI	DOPAMINE	287±49	278±41	288±36	308±41	269±21
	DOBUTAMINE	281±42	282±45	289±53	288±44	263±34
	PLACEBO	290±67	291±65	288±56	283±51	294±58
EF	DOPAMINE	50±2	51±3	52±3	53±3	48±3
	DOBUTAMINE	51±5	49±6	48±5	52±4	51±3
	PLACEBO	52±2	52±2	52±3	53±2	54±2
EDI	DOPAMINE	74±11	75±10	77±8	80±9	74±5
	DOBUTAMINE	75±9	84±12	82±12	78±12	64±8
	PLACEBO	76±19	79±16	74±12	74±12	78±15
CI	DOPAMINE	2.9±0.4	2.8±0.4	2.9±0.4	3.2±0.5	2.7±0.3
	DOBUTAMINE	2.8±0.4	3±0.5	3±0.6	3.5±0.8	2.7±0.4
	PLACEBO	3.1±0.8	3.0±0.7	3.0±0.6	2.9±0.6	3.0±0.6
SI	DOPAMINE	38±6	39±6	41±6	42±6	36±3
	DOBUTAMINE	37±4	38±6	39±8	38±7	34±5
	PLACEBO	40±9	41±9	39±7	39±8	42±10
TFI	DOPAMINE	30±2	30±2	29±2	30±2	31±2
	DOBUTAMINE	29±2	28±3	31±3	28±2	29±2
	PLACEBO	33±3	33±3	33±3	32±3	32±3

Table 2.7 Thoracic bioimpedance data: placebo, ANP, tyramine and phentolamine

Changes in peak flow index (PFI) ejection fraction (EF), end diastolic index (EDI), cardiac index (CI), stroke index (SI) and thoracic fluid index (TFI) at baselin (B'LINE) and during infusion of placebo (PLAC), ANP, tyramine (TYR) and phentolamine (PHENT).

		B'LINE	1 HOUR	2 HOURS	3 HOURS	4 HOURS
SI	PLACEBO	37±3	36±3	38±3	37±3	38±3
	ANP	33±2	34±2	35±3	37±3	37±4
	TYRAMINE	36±3	38±3	39±4	40±3	39±3
	PHENTOLAMINE	36±3	37±2	37±3	36±3	36±3
CI	PLACEBO	2.6±0.1	2.5±0.2	2.6±0.2	2.5±0.2	2.5±0.2
	ANP	2.3±0.1	2.3±0.1	2.3±0.2	2.4±0.1	2.4±0.1
	TYRAMINE	2.6±0.2	2.6±0.2	2.7±0.3	2.7±0.2	2.7±0.3
	PHENTOLAMINE	2.5±0.1	2.5±0.1	2.7±0.2	2.9±0.1	2.7±0.1
EF	PLACEBO	57±2	56±2	58±3	58±3	57±3
	ANP	52±3	54±3	55±3	55±2	55±2
	TYRAMINE	56±2	57±2	58±2	60±3	58±3
	PHENTOLAMINE	52±4	55±5	56±3	56±3	54±4
PFI	PLACEBO	252±11	245±14	252±13	246±14	245±11
	ANP	239±11	232±9	239±13	246±13	256±18
	TYRAMINE	250±15	155±18	255±17	257±16	262±19
	PHENTOLAMINE	252±14	245±10	252±17	265±17	260±17
TFI	PLACEBO	38±1	37±1	37±1	37±1	37±1
	ANP	37±2	37±2	37±2	37±2	36±2
	TYRAMINE	34±3	34±3	34±3	34±3	34±3
	PHENTOLAMINE	35±3	35±3	35±3	35±3	35±2

Chapter 3 The effect of dietary sodium restriction on furosemide induced diuresis and natriuresis

3.1 Introduction

Dietary sodium restriction is traditionally advocated in patients with heart failure. The renal response to furosemide is known to be impaired in patients with heart failure, despite plasma and urinary concentrations of furosemide which are similar to those in normal subjects. Patients with heart failure controlled with diuretics have an excess of total bodily sodium, but still exhibit renal sodium avidity. It is not known whether sodium restriction will decrease sodium output in response to furosemide in heart failure or whether it will merely reduce weight and blood pressure. In normal subjects, sodium depletion with concomitant furosemide administration for three days was found to cause a fall in weight of approximately 2 kg and a fall in GFR but no change in blood pressure (Memoli, Libetta, Sabbatini, et al 1991). The present study was conducted to assess the effect of sodium restriction on the diuretic and natriuretic response to furosemide in patients with mild to moderate chronic heart failure.

3.2 Subjects and methods

Nine patients, of whom one was female, aged 57-73 years (mean age 62 years) with chronic heart failure requiring regular diuretics were studied on two occasions, not less than one week apart. Subjects with severe failure were excluded because of the risk of precipitating acute cardiac decompensation by increasing salt intake. The mean maintenance dose of furosemide was 62mg (40-120mg)/day. 7 patients were assigned to NYHA class II and 2 patients to NYHA class III.

Table 3.1 Patient characteristics

Table showing individual patients' age, sex, New York Heart Association classification (NYHA), daily diuretic requirements in milligrammes (furosemide-F), type of ACE-inhibitor and daily dosage (captopril-CAPT) and aetiology of heart failure (ischaemic heart disease-IHD, dilated cardiomyopathy-DCM).

PATIENT	AGE	SEX	NYHA	DIURETIC	ACE-I (mg)	AETIOLOGY
1	64	M	IIB	F 40	-	IHD
2	68	M	IIA	F 40	-	IHD
3	73	M	III	F 80	CAPT 37.5	IHD
4	69	F	IIB	F 40	-	IHD
5	58	M	IIB	F 40	CAPT 37.5	IHD
6	65	M	III	F 120	-	IHD
7	64	M	IIB	F 40	-	IHD
8	51	M	III	F 120	CAPT 75	IHD
9	66	M	IIA	F 40	-	DCM

Patients adhered to a diet containing 20mmol of sodium and 40mmol of potassium per day for three days (sodium restriction) or 150 mmol of sodium and 40mmol of potassium per day for three days (high salt) in random, crossover fashion. Furosemide was infused at a predetermined rate throughout the study to maintain a moderate, constant diuresis and urinary and insensible losses were replaced orally. PAH was infused for estimation of renal blood flow. Blood was drawn at 0, 2 and 4 hours from the start of the study for measurement of electrolytes, plasma hormones (AVP, plasma renin, ANP, adrenaline and noradrenaline) and PAH. Urine was measured and retained for measurement of electrolytes and PAH. Heart rate was counted from the apex and arterial pressure was measured with a mercury sphygmomanometer (See chapter 2 for further details of methodology).

3.3 Statistics

The Friedman test for analysis of variance was used to test significance of any differences and where indicated, differences were compared using appropriate t-tests. Figures denote mean ± one standard error of the mean.

3.4 Results

Mean weight was lower on the low salt diets (76.7 and 77.3kg on low and normal salt diets respectively) but the differences did not reach significance. One patient on a calorie-restricted diet lost 2kg in the 2 week period between studies. When this patient was excluded, mean weight fell by 1kg on sodium restriction.

Table 3.2: Individual weights on normal and low salt diets

PATIENTS	LOW SALT (Kg)	NORMAL SALT (Kg)
1	64.5	65.5
2	70.0	70.5
3	78.5	80.0
4	63.3	65.2
5	86.0	86.0
6	76.6	77.0
7	66.0	65.5
8	68.6	70.7
9	106.0	104.0

Cumulative urine volume over 4 hours decreased (1175±302ml to 956±459ml, $p<0.05$) during salt restriction (Figure 3.1) although differences between individual hourly collections did not reach statistical significance. Excretion of sodium ($p=0.07$, Figure 3.2) and chloride ($p<0.07$, Figure 3.3) showed a trend to fall during the first hour of the study with salt restriction. Cumulative sodium and chloride excretion over 4 hours did not differ significantly on the two study days. Excretion of potassium fell during the first hour of the study with salt restriction but cumulative excretion over 4 hours was similar to that during salt repletion. Fractional excretion of electrolytes did not change (Table 3.3) but concentrations of urea and creatinine were higher at baseline and at 2 hours during salt restriction.

Figure 3.1 Hourly urine volume.

The differences in hourly volume did not reach statistical significance although cumulative urine output over the four hour period fell during salt restriction ($p<0.05$).

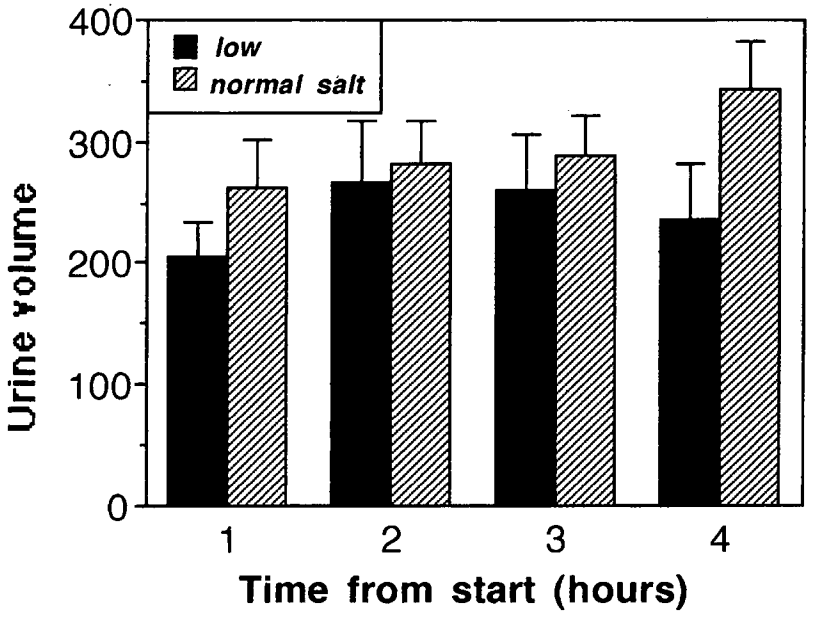


Figure 3.2 Hourly sodium excretion after salt restriction and normal salt intake.

Sodium excretion tended to fall during the first two hours of the study but this did not reach significance.

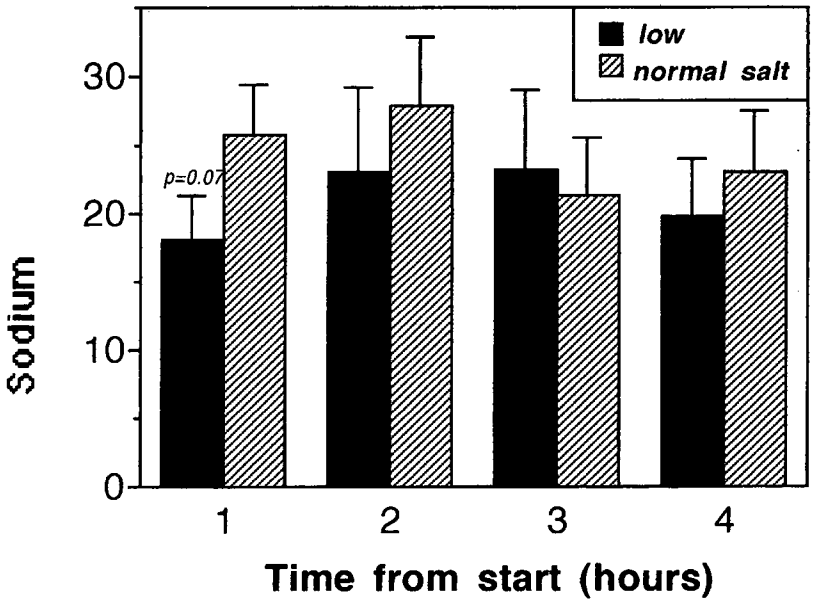


Figure 3.3 shows changes in hourly chloride excretion after salt restriction and normal salt intake. The trend towards a reduction in chloride excretion during the first hour of the study did not reach statistical significance.

Figure 3.3: Hourly chloride excretion

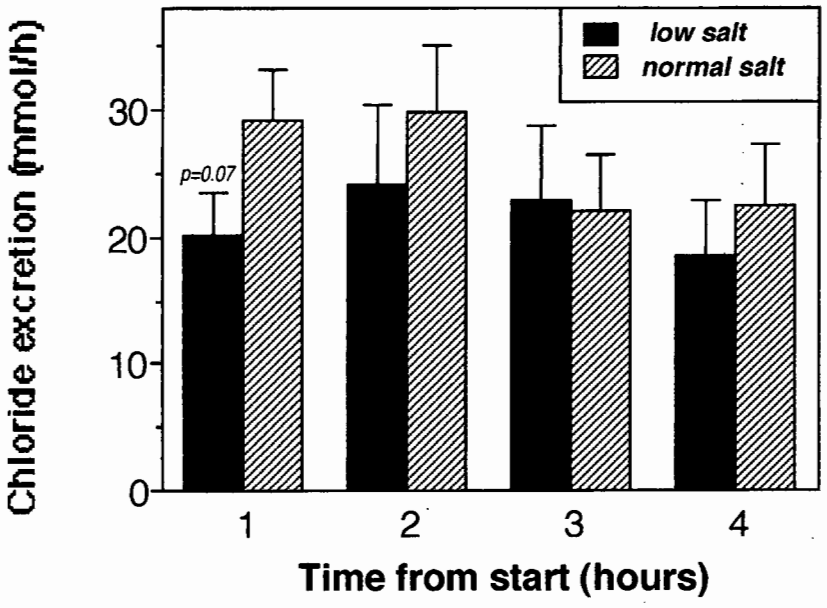


Figure 3.4: Potassium excretion

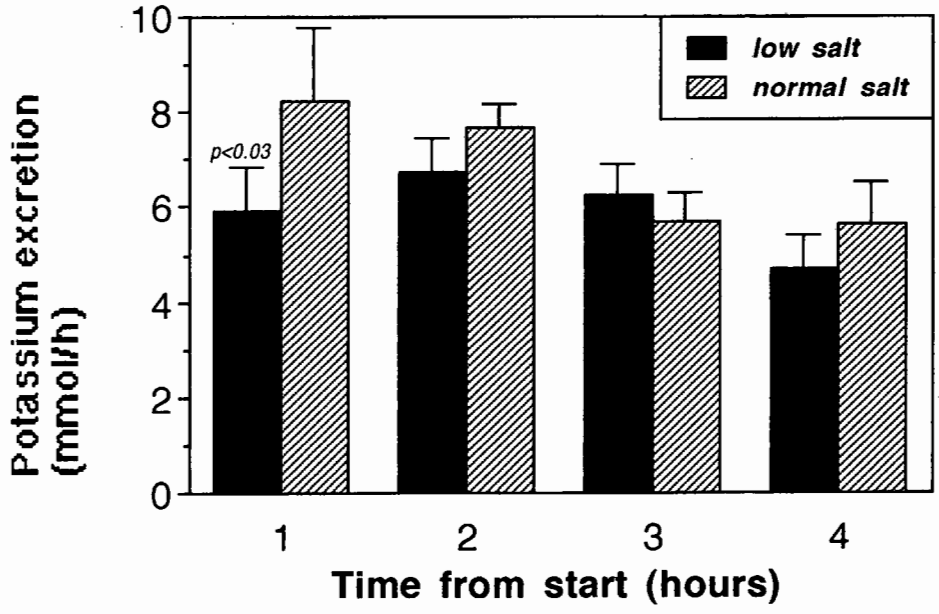


TABLE 3.3 Excretion of urea and creatinine and fractional excretion of electrolytes

There was no significant difference between fractional excretion of sodium (Na), potassium (K), urea (U), chloride (Cl), magnesium (Mg) or phosphate (Ph) to creatinine (Creat, Cr) after high or low salt diets.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
Urea	HIGH SALT	23±2	22±1	18±2	17±1
mmol/hr	LOW SALT	19±2	24±2	21±1	17±2
Creat	HIGH SALT	0.52±0.06	0.51±0.04	0.40±0.06	0.38±0.06
mmol/hr	LOW SALT	0.44±0.08	0.54±0.09	0.47±0.06	0.34±0.04
Na/Cr	HIGH SALT	57±12	55±11	71±16	70±16
mM/mM	LOW SALT	45±8	51±14	49±13	75±35
K/Cr	HIGH SALT	13±2	16±3	16±2	15±2
mM/mM	LOW SALT	16±4	15±2	14±2	16±3
U/Cr	HIGH SALT	42±7	48±4	48±5	49±5
mM/mM	LOW SALT	49±4	47±5	49±4	54±6
Cl/Cr	HIGH SALT	47±9	57±10	64±18	67±21
mM/mM	LOW SALT	55±17	55±17	55±18	69±29
Mg/Cr	HIGH SALT	0.6±0.1	0.7±0.1	0.8±0.1	0.8±0.1
mM/mM	LOW SALT	0.8±0.1	1.1±0.2	0.8±0.1	0.9±0.2
Ph/Cr	HIGH SALT	1.7±0.4	2.1±1.4	2.7±0.5	3.1±0.5
mM/mM	LOW SALT	2.3±0.3	2.3±1.1	2.6±0.3	3.4±0.5

Table 3.4 Plasma electrolyte concentrations

Changes in plasma sodium (Na), potassium (K), urea, creatinine (Creat), chloride (Cl), magnesium (Mg) and phosphate (Phos) concentrations. * denotes p<at least 0.05.

		START	2 HOURS	4 HOURS
Na	HIGH SALT	139±1	138±1	138±1
mmol	LOW SALT	138±1	138±1	137±1
K	HIGH SALT	3.8±0.2	3.7±0.1	3.5±0.1
mmol	LOW SALT	3.6±0.2	3.5±0.1	3.4±0.1
Urea	HIGH SALT	7.9±0.8	7.4±0.7	7.0±0.7
mmol	LOW SALT	8.5±0.8	8.2±0.8*	7.7±0.7
Creat	HIGH SALT	99±7	96±7	95±6
μmol	LOW SALT	106±8*	101±8*	100±9
Cl	HIGH SALT	104±1	102±1	101±1
mmol	LOW SALT	102±1	102±2	100±2
Mg	HIGH SALT	0.82±0.03	0.83±0.01	0.82±0.02
mmol	LOW SALT	0.84±0.02	0.84±0.02	0.81±0.02
Phos	HIGH SALT	0.85±0.07	0.86±0.06	0.98±0.05
mmol	LOW SALT	0.82±0.08	0.88±0.07	1.00±0.05

Hormones Plasma ANP levels were lower at the start of the study and at 2 hours after salt restriction ($p<0.03$, Figure 3.6), while plasma renin showed a trend to rise after salt restriction, reaching statistical significance after 4 hours ($p<0.05$, figure 3.5). Adrenaline ($p<0.05$) and noradrenaline ($p=0.05$) rose. AVP levels were not affected (Figures 3.7 & 3.8).

Figure 3.5 Plasma active renin concentration
Renin concentrations tended to rise after salt restriction, significant only at 4 hours ($p<0.05$).

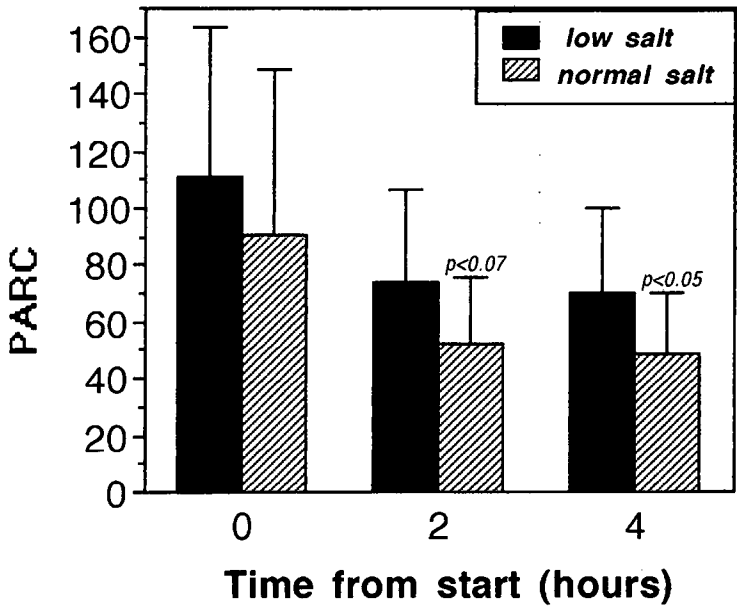


Figure 3.6 Plasma atrial natriuretic peptide

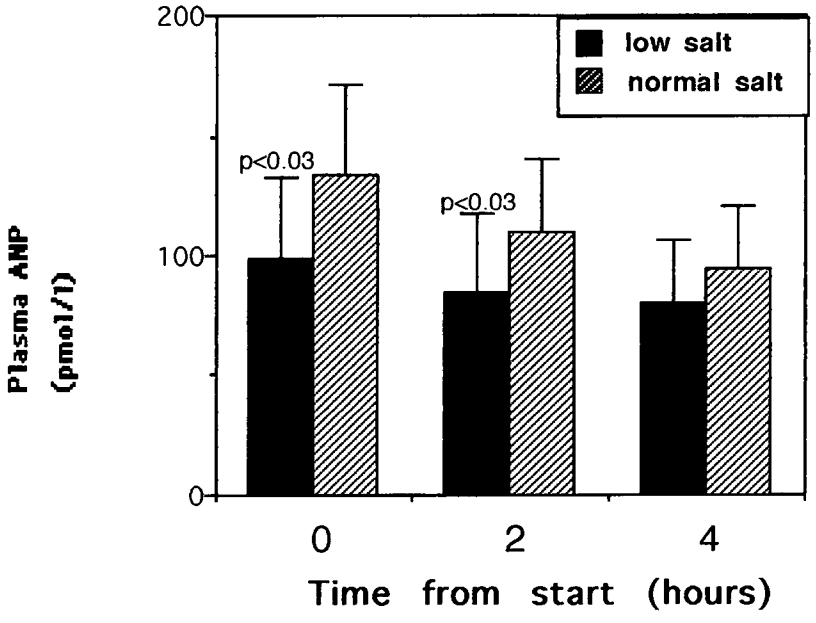


Figure 3.7 Plasma adrenaline concentration

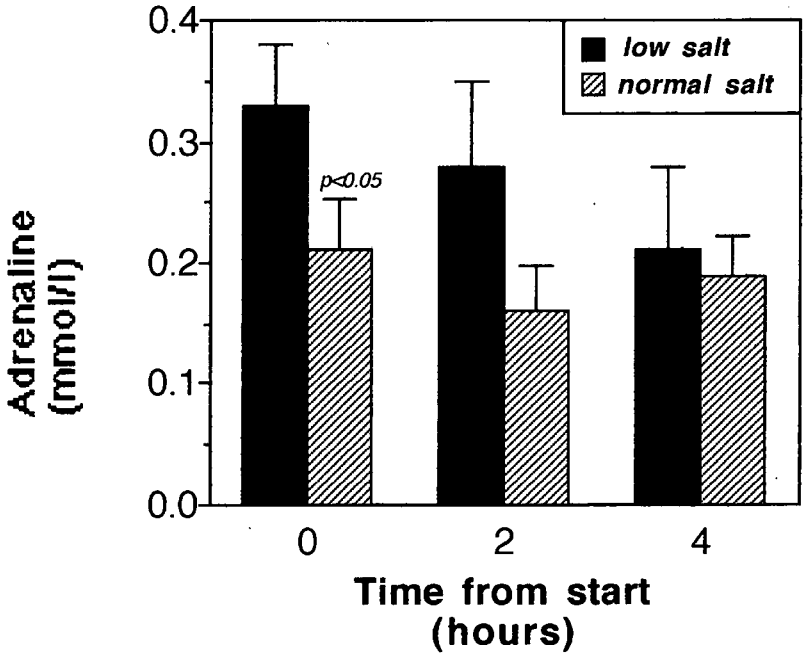
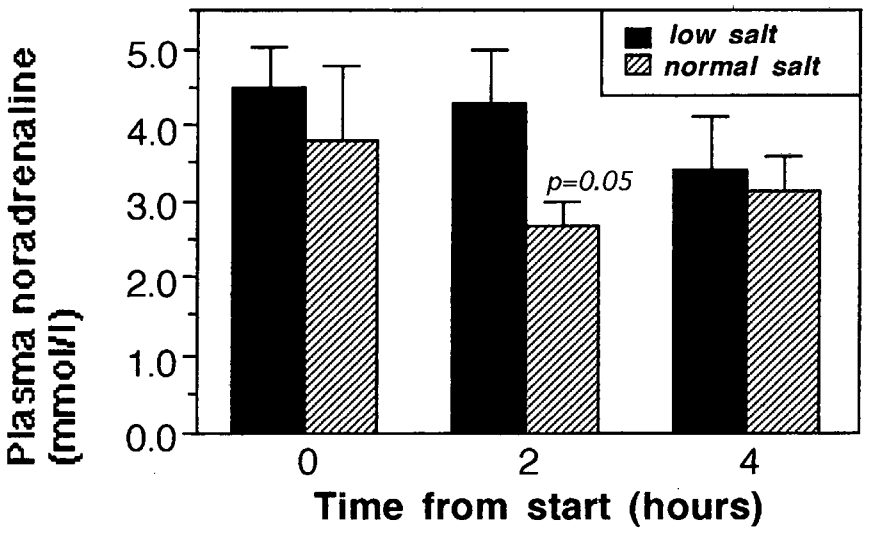


Figure 3.8 Plasma noradrenaline concentration



Haemodynamic variables

Heart rate did not change between study days. Systolic blood pressure at baseline was lower during salt restriction (112 vs 119mmHg, $p<0.01$: figure 3.9).

ERPF was lower for the first 3 hours after salt restriction ($p<0.005$: figure 3.10, table 3.5). Creatinine clearance was not significantly different on salt restriction compared to normal sodium intake (table 3.5).

Table 3.5 Haemodynamic variables

Changes in heart rate (HR), systolic and diastolic blood pressure (SBP, DBP), glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) following usual salt intake (HIGH) and salt restriction (LOW). * denotes significance at at least $p<0.05$.

		START	0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
HR	HIGH	74±4	71±5	69±4	71±4	69±4
bpm	LOW	72±4	70±4	69±4	69±4	70±4
SBP	HIGH	119±7	111±7	117±8	116±7	115±7
mmHg	LOW	112±7*	109±7	110±7	115±7	113±7
DBP	HIGH	75±5	72±5	77±5	76±5	74±5
mmHg	LOW	71±6	72±5	73±6	73±5	71±5
GFR	HIGH	-	90±13	106±20	72±12	72±11
mls/min	LOW	-	70±14	81±10	83±13	54±7
ERPF	HIGH	-	267±31	298±31	300±33	308±36
mls/min	LOW	-	250±32*	252±32*	244±31*	230±29

Figure 3.9 Systolic blood pressure

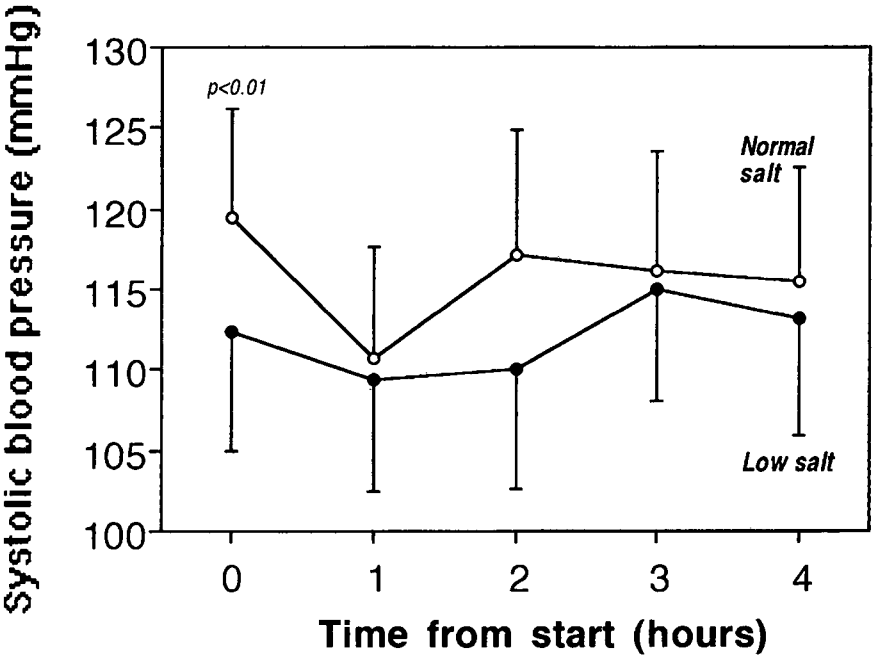
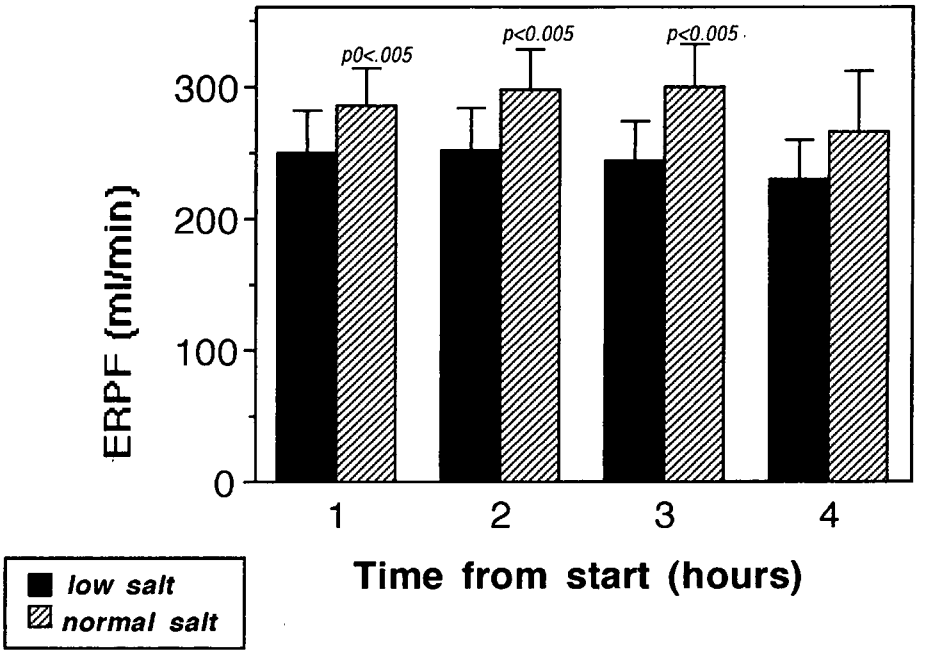


Figure 3.10 Effective renal plasma flow



3.5 Discussion

In the present study, sodium restriction was associated with further activation of the renin-angiotensin system and sympathetic nervous system and a fall in ANP. Systolic blood pressure and body weight were lower at baseline during salt restriction and prolonged furosemide infusion was associated with a fall in ERPF, a decline in the cumulative furosemide induced diuresis over 4 hours and a non-significant fall in natriuresis ($p=0.07$) during the first hour of the study.

The braking phenomenon

Quite clearly compensatory mechanisms come into play to prevent progressive dehydration with regular diuretic therapy. Wilcox, Mitch, Kelly, et al (1983) showed that normal subjects develop resistance to the effects of furosemide when sodium is restricted, with attendant falls in GFR, but also demonstrated that compensatory renal sodium retention could only be prevented after furosemide by salt restriction. The overall sodium balance thus depends not only on the initial diuresis but the subsequent compensatory sodium retention. It is likely that many of the mechanisms involved in the braking phenomenon are the same as those resulting in furosemide resistance in heart failure, although in heart failure the mechanisms co-exist with total bodily sodium excess.

The mechanisms for the decreased furosemide induced natriuresis are various. There is evidence that diminished delivery of sodium to the loop of Henle as a consequence of alteration in glomerular filtration rate impairs the action of furosemide. Honrath, Chong, Wilson, et al (1994) found that chronic salt deprivation effectively prevented the diuretic and natriuretic actions of ANP in rats. Salt deprivation caused a fall in GFR and marked

elevation in tubular reabsorption of delivered loads of fluid and electrolytes in the medullary collecting duct. This blunting could be rapidly reversed by intravenous infusion of isotonic sodium chloride, although interestingly, the effect was more complete in the collecting duct and only partially corrected in the more proximal nephron segments. The authors speculate that at least two salt-conserving mechanisms exist, one quickly reversible, operating upstream as well as in the duct and a second, which is not acutely reversible, operating upstream only.

Sodium intake may modulate the responsiveness of the tubular epithelium to furosemide. Reduced amounts of sodium in the ascending loop of Henle may adversely affect the renal action of furosemide (Nomura, Yasuda, Minami, et al 1981). (The furosemide assay only became available after this study was completed, hence furosemide excretion was only measured in subsequent Chapters). Micropuncture studies in rats suggest that salt intake modulates the responsiveness of tubular epithelium to furosemide (Gutshe, Müller-Suur, Hegel, et al 1980) but the role this plays in patients with heart failure is not known.

Falls in blood pressure modulate renal sodium handling (Schrier, De Wardener 1971) and falls in arterial pressure can blunt the natriuretic and diuretic effects of furosemide in animals (Haeusler, Gerold 1979). A statistically significant fall in systolic blood pressure was observed in the present study during salt restriction, thus this mechanism may also have contributed to the blunted natriuresis and diuresis observed. Although GFR did not change significantly in the present study, a fall cannot be excluded because of the wide scatter. Reduced renal plasma flow has been shown to increase reabsorption of sodium at the proximal tubule in patients with

advanced heart failure.

Activation of the RAAS by salt restriction was previously thought to be responsible for the reduced responsiveness to furosemide, but elegant studies by Wilcox, Guzman, Mitch, et al (1987) and Kelly, Wilcox, Mitch, et al (1983) showed that elevated concentrations of A-II were not a prerequisite for furosemide resistance. In addition Wilcox, Guzman, Mitch, et al (1987) showed that resistance to the renal effects of furosemide persisted when both the α1-adrenoceptor and formation of A-II were blocked by prazosin and captopril respectively. Whether elevation of catecholamines by salt restriction (as was the case in the present study) further reduces renal responsiveness to furosemide remains speculative. Cody, Covit, Schaer, et al (1986b) described similar neuroendocrine activation and impaired natriuresis with salt depletion in patients with heart failure but did not measure plasma ANP concentrations.

Urine creatinine excretion did not change significantly after salt restriction in the present study, although creatinine clearance is a less reliable estimate of GFR than inulin clearance (it tends to overestimate GFR because of tubular secretion of creatinine in addition to filtration). Further error could be introduced by administration of furosemide, although the doses were identical for individual patients, who acted as their own controls.

There were 3 patients taking ACE-inhibitors which could have influenced the results of the study. All were converted to captopril two weeks prior to study and 3 doses omitted to allow washout, and patients acted as their own controls. These measures would minimise the error introduced.

Limitations of the study

It is always difficult to be sure that apparently statistically significant results are valid or that the non-significant results are truly negative in small clinical studies (for example the GFR measurements). Wide variations in individual response occur and the possibility that some findings were merely the result of chance cannot be excluded. Using two-tailed t-tests reduced the bias but decreased the sensitivity of the tests to show significance.

24 hour sodium excretion was not measured in all patients to confirm that the diet had been strictly adhered to, although changes in weight and neuroendocrine activation supported adherence to the appropriate salt intake. Although differences in sodium excretion did not reach significance, the mean hourly sodium excretion after three days of salt restriction was 20mMol and after normal sodium intake, 25mMol. If these results were extrapolated to 24 hours, the difference (120mMol) was very similar to the difference in daily sodium intake (150mMol vs 20mMol). Patients were not catheterised to ensure complete bladder emptying but any patients with a history suggestive of outflow obstruction were excluded from the study and it was not felt to be ethical to catheterise the patients repeatedly. This may have introduced error in urine collections but the patients acted as their own controls, stood to micturate and furosemide infusion was maintained to ensure a constant but modest diuresis in order to minimise the error.

The plasma hormone samples were saved and assayed as a batch. Individual patient samples for both days were assayed in the same assays to reduce interassay variability inaccuracies. This may have resulted in loss of some ANP activity (Nelesen, Dimsdale, Ziegler 1992) although a recent in-house study of variability of ANP concentrations in samples sent from

patients under different storage conditions and stored for different periods of time prior to assay showed good agreement between results of samples assayed within 3 days of sampling and those saved for 6 weeks. Individual patients had samples collected within 14 days of one another so any loss of hormone activity was likely to have affected both sets of samples, thus minimising error. There was little baseline variability in plasma hormone concentrations in the patients although inter-patient variability was wide.

Conclusion

Appropriate renal sodium retaining mechanisms come into play when dietary sodium is severely restricted or urinary sodium loss is greatly enhanced by diuretic therapy in normal subjects. In patients with heart failure, similar compensatory responses may reduce diuretic efficacy despite total body sodium excess. Sodium restriction is also associated with activation of sympathetic nervous system and renin-angiotensin system and decreased circulating concentrations of ANP. Effective renal plasma flow also falls. These neuroendocrine and haemodynamic effects are not dissimilar to those achieved by a further increase in the dose of loop diuretics. Although only furosemide was used in the present study, and the neuroendocrine response to incremental dosing was not tested, other authors (Nicholls, Espiner, Hughes, et al 1976; Turini, Brunner, Ferguson, et al 1978; Levine, Francis, Goldsmith, et al 1982; Schaer, Covit, Laragh, et al 1983) have demonstrated neuroendocrine activation with increasing doses of all diuretic agents, the least marked with thiazide diuretics. While sodium restriction is essential to achieve diuresis in patients with refractory congestive heart failure, its role in <u>controlled</u> heart failure appears to be no more physiological or beneficial than the loop diuretic agents. It is difficult to motivate patients to restrict dietary sodium to less than 40mMol per day.

When symptoms and signs of heart failure are controlled on a loop diuretic such as furosemide, routine dietary sodium restriction appears to be of limited additional value and adds to the misery of patients.

Chapter 4 The effect of suppression of angiotensin II on a furosemide induced diuresis and natriuresis in patients on chronic angiotensin converting enzyme inhibitor therapy

4.1 Introduction

Suppression of A-II in the circulation and in renal tissues should increase sodium loss, either by withdrawing a direct sodium retaining effect of A-II on the renal tubule or by reducing plasma concentrations of aldosterone (Cleland, Dargie 1987b; Cleland 1991). In practice, most patients with heart failure not only fail to reduce their diuretic requirement but tend to retain fluid and sodium in the days following initiation of an ACE inhibitor (Fitzpatrick, Nicholls, Ikram 1983; Cleland, Dargie, Ball, et al 1985a; Cleland, Dargie, East, et al 1995b; Cleland, Gillen, Dargie 1988; Flapan, Davies, Waugh 1991b; Motwani, Fenwick, Morton, et al 1992b). Although the direct renal effects of suppression of A-II and aldosterone predict that a natriuresis should occur, ACE inhibitors also reduce arterial pressure, plasma natriuretic peptides and GFR. All these effects favour salt and water retention (Cleland 1990b).

The measurement of weight and total body sodium suggest that ACE inhibitors have a neutral effect on water and sodium balance over 6-8 weeks (Cleland, Dargie, East, et al 1985b; Cleland, Dargie, Gillen, et al 1986), indicating reversal of the initial trend with chronic use. The interaction of diuretic agents and ACE inhibitors during long-term combined therapy has not been addressed. Intense ACE inhibition may be associated with more pronounced effects on arterial pressure and GFR than with partial ACE inhibition which would tend to attenuate the diuretic effects of furosemide. However intense inhibition would lead to greater reductions in aldosterone

and A-II which would favour a greater diuresis than with partial suppression with lower doses of captopril.

The aim of the present study was to compare the effects of furosemide during periods of intense A-II suppression with a period when circulating A-II was not suppressed. Monitoring and collections of blood and urine were continued as plasma A-II concentrations began to rise again towards the end of the study period.

4.2 Subjects

Eight patients with chronic heart failure due to left ventricular dysfunction were studied. All had a left ventricular ejection fraction of less than 30% on radionuclide ventriculography or a fractional shortening of less than 20% and impaired left ventricular systolic function on echocardiography. All had been taking ACE inhibitors for at least 3 months and had changed to captopril 12.5mg tid two weeks prior to being included in the study.

Table 4.1 Patient characteristics

Table showing individual patients' age, sex, New York Heart Association classification (NYHA), daily diuretic requirements in milligrams (furosemide-F, metolazone-MET), type and daily dosage of ACE-inhibitor (captopril-CAP) and aetiology of heart failure (ischaemic heart disease-IHD).

PATIENT	AGE	SEX	NYHA	DIURETIC	ACE-I (mg)	AETIOLOGY
1	60	M	IIB	F 80	CAPT 37.5	IHD
2	74	M	III	F 80	CAPT 37.5	IHD
3	66	M	IIB	F 120	CAPT 37.5	IHD
4	64	M	III	F 40, MET 5	CAPT 37.5	IHD
5	48	M	IIB	F 120	CAPT 37.5	IHD
6	63	F	III	F 80	CAPT 37.5	IHD
7	58	M	IIB	F 80	CAPT 37.5	IHD
8	73	M	III	F 80	CAPT 37.5	IHD

4.3 Study design

Patients adhered to a diet containing 40mM of potassium and 100mM of sodium/day for 3 days prior to each study. Each patient was studied twice, not less than 48 hours apart, in random fashion. Usual medications and caffeine-containing beverages were omitted on the study days. On arrival, cannulae were inserted into antecubital veins bilaterally and boluses of inulin 50mg/kg and PAH 800mg were administered. Infusions were continued for the duration of the study at rates of 32.5mg/min and 30mg/min respectively. Heart rate and arterial pressure were measured at half hourly intervals.

A bolus of furosemide 5mg was given and patients rested supine for 30 minutes before passing urine. Subsequent hourly boluses of furosemide were given in identical fashion to maintain a steady, moderate diuresis (3-5mg/hour, depending on usual daily dose of furosemide). Urinary and insensible losses were replaced hourly with water. Blood was drawn hourly for estimation of plasma hormones, electrolytes, PAH and inulin. Urine was collected hourly for estimation of electrolytes, PAH, inulin and cGMP. At the end of the first hour, blood and urine samples were collected then 12.5mg of captopril or matching placebo was given in random single-blind fashion.

Heart rate and arterial pressure were measured at half hourly intervals. Blood samples were collected at 1, 2, 3 and 4 hours from the start of the studies and saved for analysis of plasma hormones (ANP, active renin concentration and aldosterone), electrolytes, PAH and inulin. Urine was collected hourly (at 1,2,3 and 4 hours) and saved for measurement of electrolytes, PAH, inulin, cGMP and furosemide (see Chapter 2).

4.4 Results

Urine volume increased during the first and second hours after captopril compared to placebo. Both sodium and chloride excretion increased during the first and second hours after captopril compared with placebo (figure 4.2 & 4.3). Urinary concentrations of cGMP and furosemide were found not to differ significantly after captopril or placebo.

Table 4.2 Changes in urine volume and excretion of electrolytes, furosemide and cGMP

Changes in hourly excretion of urine, sodium (Na), potassium (K), urea, creatinine (Creat), chloride (Cl), magnesium (Mg), phosphate (Phos), furosemide (F'MIDE) and cGMP. * denotes p< at least 0.05. Results are expressed as mean ± one standard error of the mean

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
UV	PLACEBO	245±39	238±32	245±27	262±30
mls/hr	CAPTOPRIL	273±38	303±35*	312±33*	256±23
Na	PLACEBO	25±4	22±3	22±3	23±3
mmol/hr	CAPTOPRIL	28±5	26±4*	28±4*	22±3
K	PLACEBO	6.6±1.2	5.5±1.0	5.8±0.8	5.2±0.7
mmol/hr	CAPTOPRIL	7.1±1.3	5.9±1.1	6.4±0.6	5.2±1.1
Urea	PLACEBO	19±2	17±2	17±2	17±3
Mmol/hr	CAPTOPRIL	21±5	16±2	18±2	15±2
Creat	PLACEBO	0.44±0.06	0.36±0.04	0.37±0.04	0.38±0.05
Mmol/hr	CAPTOPRIL	0.48±0.10	0.33±0.03	0.40±0.04	0.33±0.04
Cl	PLACEBO	23±4	20±3	20±2	20±3
Mmol/hr	CAPTOPRIL	27±5	24±4*	25±4*	19±3
Mg	PLACEBO	0.37±0.06	0.33±0.05	0.32±0.03	0.30±0.04
Mmol/hr	CAPTOPRIL	0.38±0.05	0.33±0.06	0.34±0.04	0.27±0.05
Phos	PLACEBO	0.97±0.19	0.92±0.1	1.1±0.1	1.2±0.12
Mmol/hr	CAPTOPRIL	0.93±0.16	0.82±0.12	1.08±0.13	0.88±0.08
F'MIDE	PLACEBO	1.7±0.4	1.5±0.2	1.7±0.3	1.8±0.3
mg/hour	CAPTOPRIL	1.8±0.4	1.9±0.3	1.9±0.3	1.7±0.3
C GMP	PLACEBO	1.7±0.4	2.4±0.7	1.9±0.4	1.5±0.4
ng/min	CAPTOPRIL	2.6±0.6	2.8±0.9	2.7±0.8	1.4±0.4

Figure 4.1 Changes in hourly urine volume after placebo and captopril. Bars denote one standard error

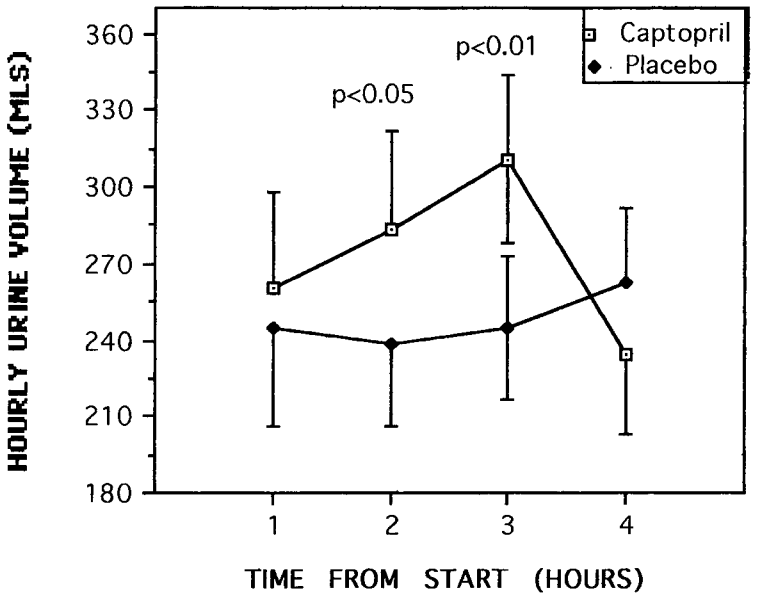


Figure 4.2 Changes in hourly sodium excretion after placebo and captipril. Bars denote one standard error

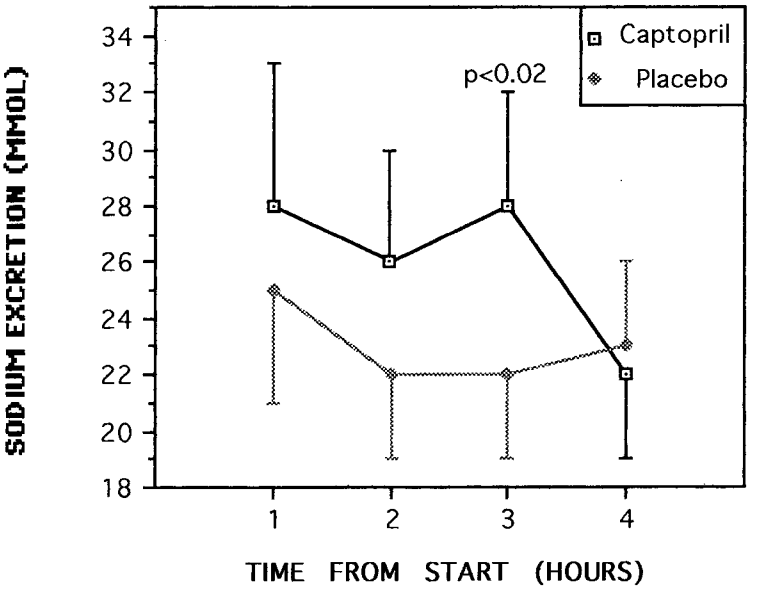
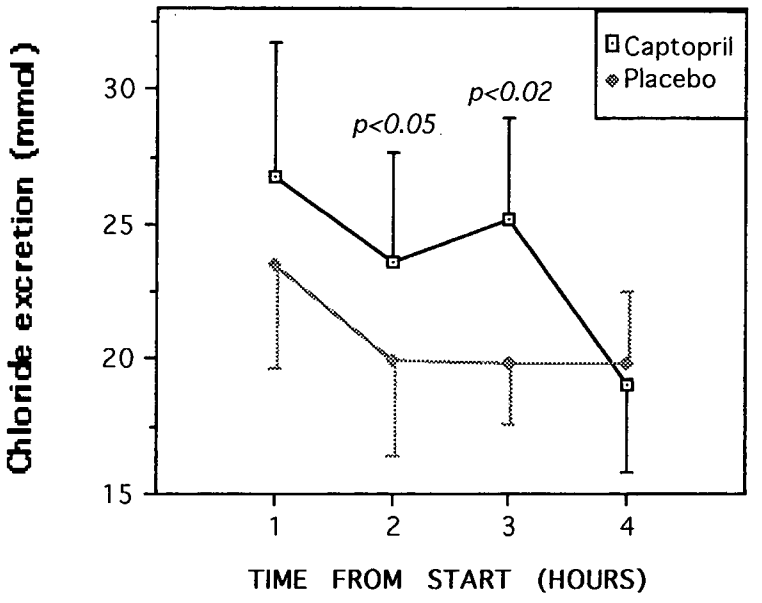


Figure 4.3 Changes in hourly chloride excretion after placebo and captopril. Bars denote one standard error



Fractional excretion of sodium (sodium/creatinine) increased (figure 4.4), as did excretion of sodium/phosphate (20±3 to 27±4, $p<0.05$ at 2 hours post dosing) and excretion of sodium/potassium (figure 4.5).

Figure 4.4 Changes in hourly fractional excretion of sodium after placebo and captopril

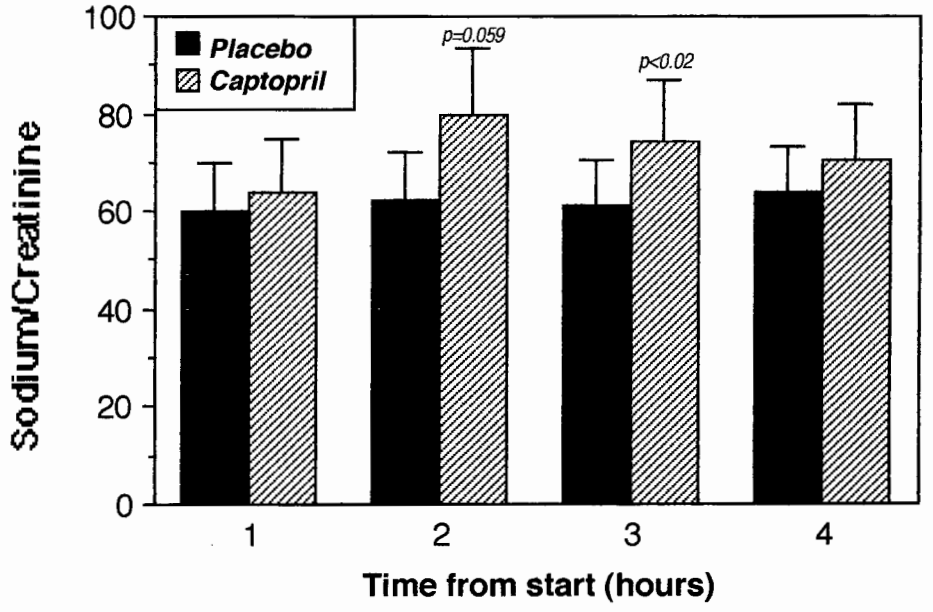
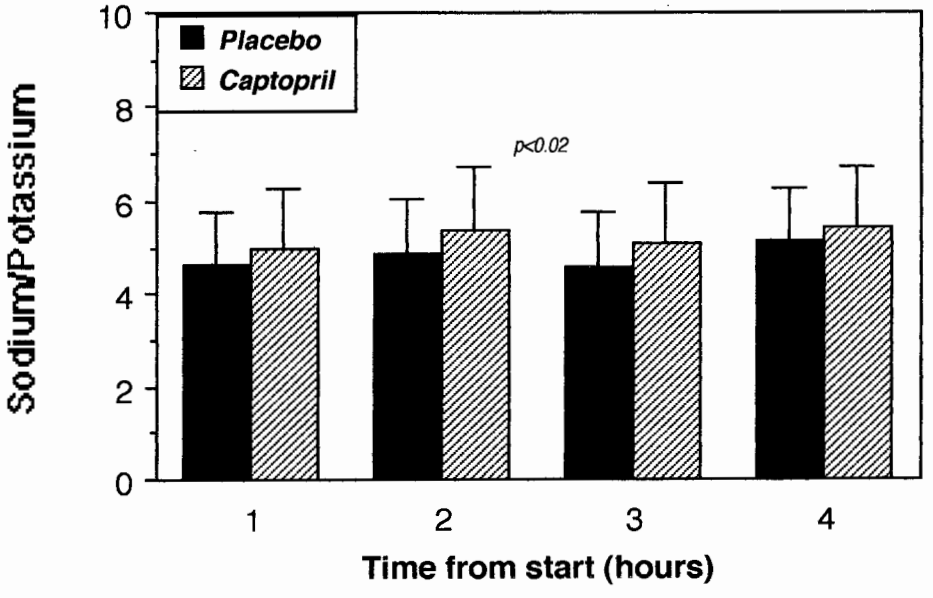


Figure 4.5 Changes in hourly excretion of sodium /potassium after placebo and captopril



Fractional excretion of furosemide and cGMP was not significantly different after captopril and placebo (table 4.3).

Table 4.3 Fractional excretion of furosemide (F'MIDE) **and cGMP**

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
F'MIDE/Cr	PLACEBO	3.6±0.4	3.8±0.3	4.4±0.4	4.4±0.5
mg/μmol	CAPTOPRIL	4.2±0.4	4.7±0.7	4.6±0.6	4.3±0.6
cGMP/Cr	PLACEBO	4.5±1.1	6.8±1.9	5.3±1.2	4.5±1.4
ng/μmol	CAPTOPRIL	6.3±1.3	7.6±2.3	7.7±2.5	5.0±2.5

Plasma electrolyte concentrations and haematocrit did not differ significantly after captopril and placebo (table 4.4).

Table 4.4 Plasma electrolyte concentrations and haematocrit

Changes in hourly haematocrit (HCT), plasma sodium (Na), potassium (K), urea, creatinine (Cr), chloride (Cl), magnesium (Mg) and phosphate after captopril (CAPT) and placebo (PLAC).

		START	1 HOUR	2 HOURS	3 HOURS	4 HOURS
HCT	PLAC	41±1	42±3	41±3	40±1	40±1
%	CAPT	40±2	40±1	39±2	40±2	39±2
Na	PLAC	139±1	139±1	138±1	138±1	138±1
mmol	CAPT	140±1	139±1	138±1	139±1	138±2
K	PLAC	3.8±0.2	3.8±0.2	3.8±0.3	3.7±0.3	3.6±0.2
mmol	CAPT	4.1±0.1	4.1±0.2	4.0±0.2	3.8±0.1	3.5±0.1
Urea	PLAC	10.7±1.4	10.5±1.4	10.2±1.3	10.1±1.3	10.0±1.3
mmol	CAPT	9.9±1.7	9.5±1.7	9.2±1.7	9.0±1.7	8.8±1.7
Cr	PLAC	127±4	123±5	120±4	119±4	120±5
μmol	CAPT	119±12	114±10	117±10	113±11	112±10
Cl	PLAC	104±1	105±2	102±3	102±3	103±2
mmol	CAPT	103±3	104±3	104±3	103±2	103±3
Mg	PLAC	0.85±0.02	0.85±0.01	0.86±0.03	0.84±0.03	0.83±0.03
mmol	CAPT	0.86±0.02	0.87±0.02	0.87±0.03	0.84±0.03	0.76±0.03
Phos	PLAC	0.96±0.05	0.96±0.07	0.98±0.07	1.09±0.07	1.16±0.07
mmol	CAPT	1.05±0.08	1.00±0.09	1.07±0.09	1.16±0.10	1.20±0.11

Plasma hormones

Plasma concentrations of A-II were maximally supressed one hour after captopril had been administered (22 to <5, $p<0.02$) and gradually increased thereafter, although baseline values were not achieved during the time course of the study (figure 4.6). Plasma concentrations of aldosterone fell throughout the study in both groups but were consistently lower in the captopril treated group, reaching significance one hour after dosing (82 to52ng/100ml, $p<0.03$: figure 4.7). plasma renin rose one hour after captopril and remained elevated throughout the study (figure 4.8).

Figure 4.6 Plasma concentrations of angiotensin II after placebo and captopril

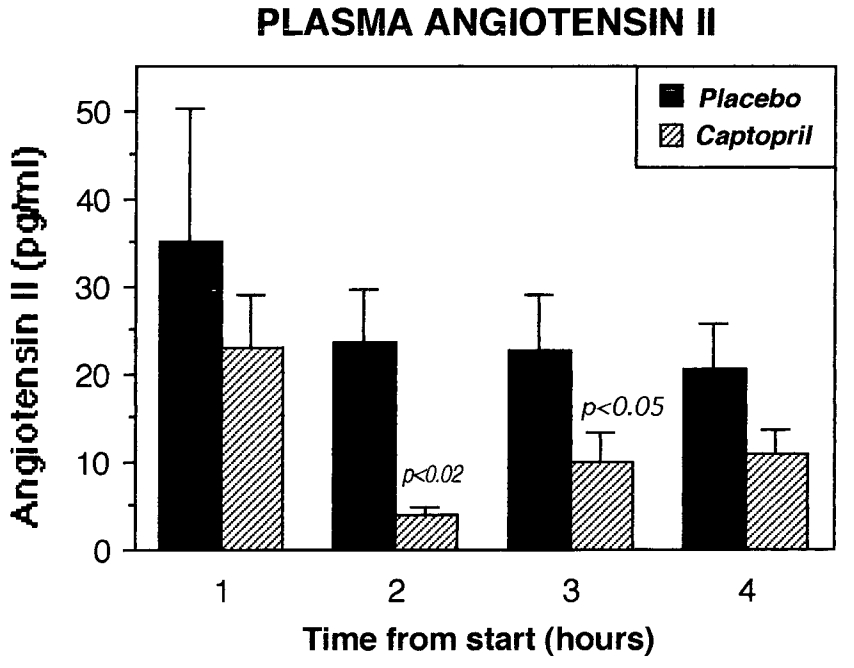


Figure 4.7 Plasma concentrations of aldosterone after placebo and captopril

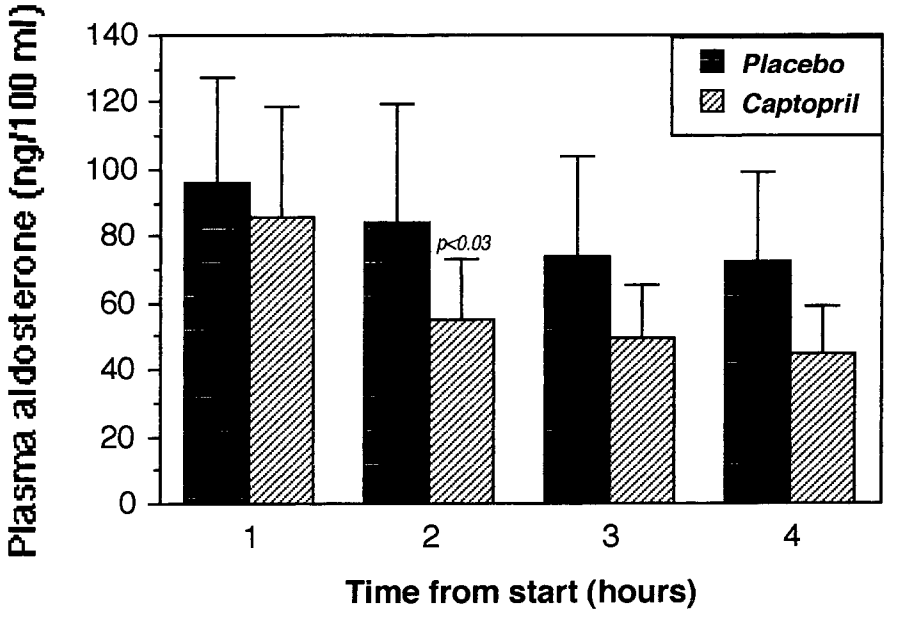
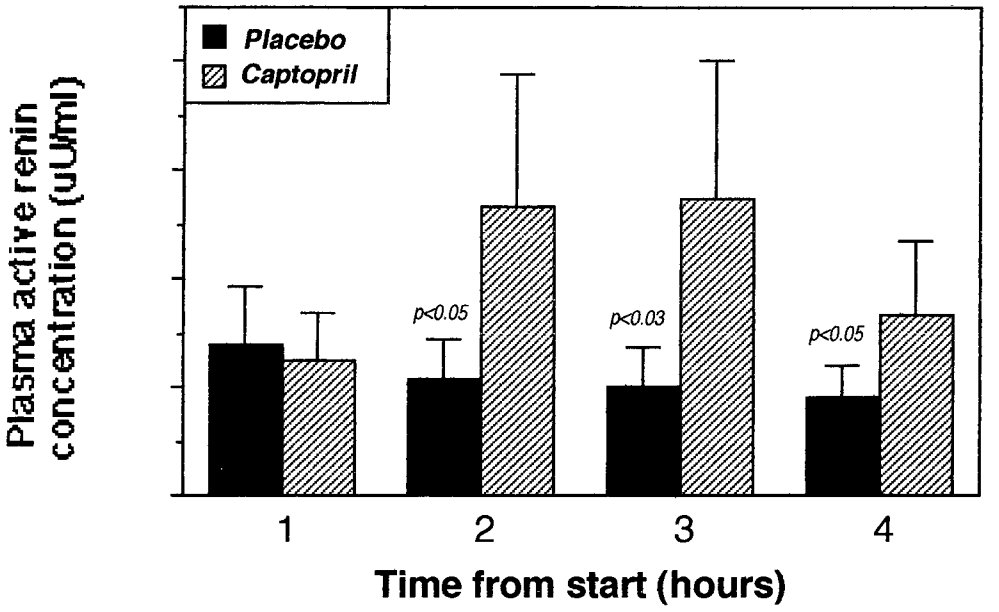


Figure 4.8 Plasma active renin concentration after placebo and captopril



Haemodynamic variables

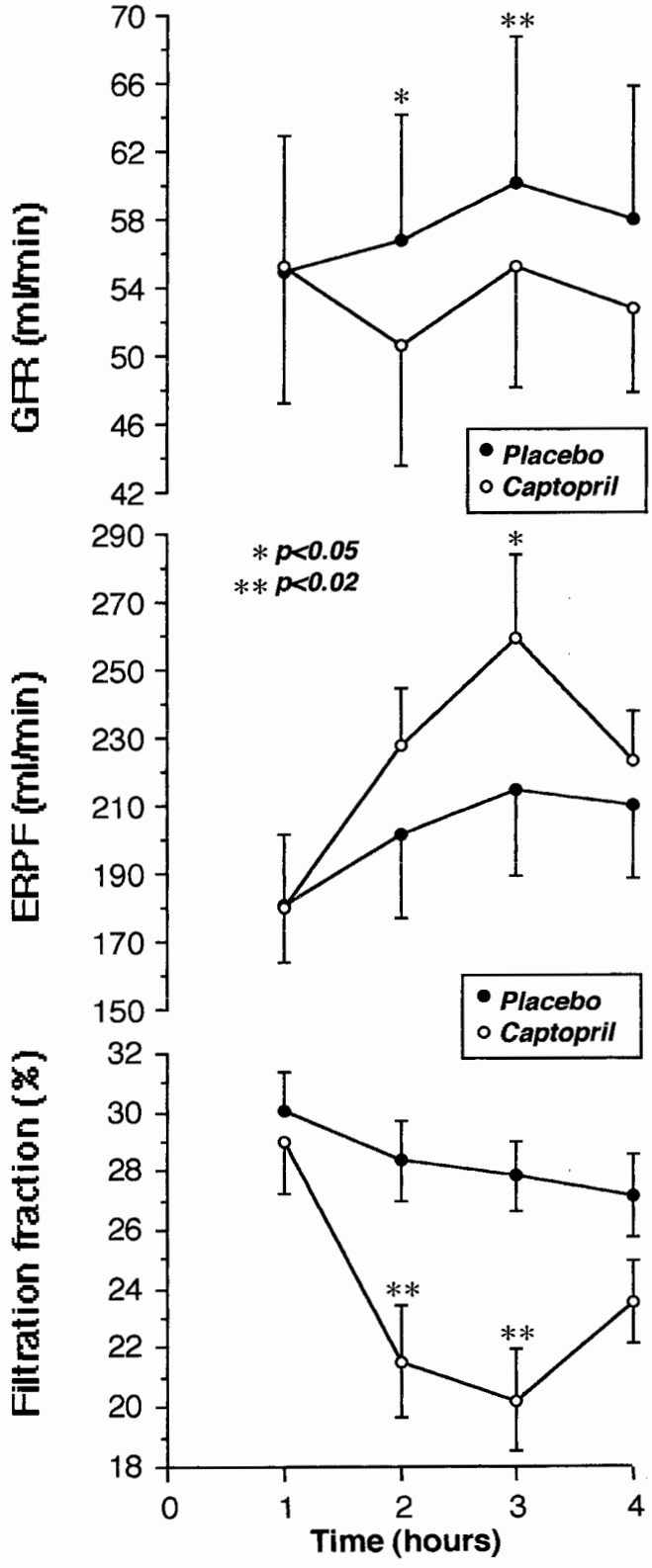
There was no change in heart rate after captopril, but both systolic and diastolic blood pressure fell one and two hours after captopril (table 4.5). There was a fall in GFR 1 and 2 hours after captopril while ERPF rose after captopril, achieving significance two hours after dosing. There was a sharp fall in filtration fraction for the entire three hour period following captopril with the most marked change two hours after dosing ($p<0.003$).

Table 4.5 Haemodynamic variables

Changes in heart rate (HR), systolic and diastolic blood pressure (SBP, DBP), GFR and ERPF. $p< 0.02$ for systolic blood pressure at 1-2 and 2-3 hours from the start. $p<0.05$ for diastolic blood pressure at 1-2 and 2-3 hours from the start.

		START	0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
HR	PLAC	69±5	68±3	67±4	66±3	69±3
bpm	CAPT	69±4	67±3	66±3	67±2	66±3
SBP	PLAC	127±11	130±10	128±11	129±10	130±9
mmHg	CAPT	127±10	129±9	120±10*	121±10*	127±9
DBP	PLAC	69±5	71±4	73±5	73±4	73±5
mmHg	CAPT	72±4	72±4	67±4*	68±3*	73±4
GFR	PLAC	-	55±8	57±7	60±9	58±8
ml/min	CAPT	-	55±8	50±7*	55±8*	53±5
ERPF	PLAC	-	180±21	201±24	214±25	210±21
ml/min	CAPT	-	180±17	223±13	259±24*	223±15

Figure 4.9 Changes in GFR, ERPF and filtration fraction after placebo and captopril



4.5 Discussion

The present study demonstrated that, in patients with heart failure treated chronically with ACE-inhibitors, salt and water excretion in response to furosemide was enhanced by supression of A-II. This contrasted with the findings during initiation of ACE inhibitor therapy in patients with heart failure, where the diuretic effect of furosemide was usually reduced by ACE inhibition (Fitzpatrick, Nicholls, Ikram, et al 1983; Cleland, Dargie, Hodsman, et al 1984; Di Nicolantonio, Morgan 1987; Flapan, Davies, Waugh, et al 1991; Motwani, Fenwick, Morton, et al 1992b). It is possible that angiotensin II receptors could have been up-regulated during chronic ACE-inhibition and hence have been more responsive to acute changes in angiotensin II during the studies.

The present study gives some insight into the site of the renal effects of captopril on sodium handling. The increased natriuresis induced by captopril in the present study was due to reduced tubular reabsorption of sodium, since GFR fell and the ratio of sodium to creatinine increased. Most phosphate reabsorption occurs in the proximal tubule. Excretion of sodium relative to phosphate did not change after captopril, suggesting that a fall in sodium reabsorption at this site contributed to the natriuresis (Bijovet, Morgan, Fourman 1969), however uncoupling of proximal tubular handling of sodium and phosphate after a loop diuretic has been described (Noormohamed, Lant 1991). The ratio of sodium to potassium excretion increased, suggesting that distal sodium reabsorption decreased, associated with the fall in plasma concentrations of aldosterone, contributing to the natriuresis.

Increased delivery of furosemide to its receptor site in the ascending limb of

the Loop of Henle following renal vasodilatation might account for the potentiation of the diuresis by captopril. Furthermore, captopril has been reported to impair the renal tubular secretion of furosemide (Toussaint, Masselink, Gentges, et al 1989). However, we observed no increase in the urinary excretion of furosemide after administering captopril.

ACE inhibitors have effects on both parasympathetic and sympathetic tone and alter baro-receptor function (Poleur, Rosseau, Oakley, et al 1991). Arterial pressure is a powerful determinant of renal salt and water handling and each individual appears to operate around a "set point" relating these variables. It is possible that chronic but not acute ACE inhibition alters the relationship between pressure and natriuresis accounting for the reversal of the initial tendency for fluid retention.

Angiotensin II exerts powerful vasoconstrictor effects on the renal vasculature; the efferent arteriole is particularly sensitive to its effects. Reduction of blood flow by angiotensin II in the vasa recta that descend into the renal medulla may increase salt and water reabsorption and captopril could reverse this (Cleland, Dargie 1987b; Cleland 1991), diverting global flow to the more superficial cortical nephrons.

Captopril enhances intra-renal production of prostaglandins, either directly or mediated through increases in bradykinin (Usberti, Minno, Ungaro, et al 1986), that may be important in preventing more profound falls in glomerular filtration than those actually observed after ACE inhibition. This protective effect on glomerular filtration is presumably due to changes in afferent arteriolar tone or glomerular function itself (Packer, Lee, Medina, et al 1987b). Renal prostaglandins may also increase salt and water excretion by

enhancing renal tubular sodium transport (Iino, Imai 1978) or by opposing the actions of anti-diuretic hormone in the collecting duct (Berl, Raz, Wald, et al 1977). Furosemide also stimulates renal synthesis of prostaglandin E2 either directly or by enhancing production of bradykinin (Katayama, Attallah, Stahl, et al 1984; Mackay, Nath, Cumming, et al 1985; Fujimura, Ebihara 1988). Administration of agents such as indomethacin impair glomerular filtration rate and promote sodium retention in patients with heart failure (Packer 1988b), supposedly by inhibiting renal cyclo-oxygenase and prostaglandin production, an effect that is more pronounced in patients with heart failure treated with diuretics and ACE inhibitors. Therefore, increased renal prostaglandin production, possibly in synergy with furosemide, could be responsible for the effects of captopril on salt and water excretion.

A decline in filtration fraction is one of the most consistent haemodynamic responses to an ACE inhibitor (Cleland, Dargie 1987b). Effective renal plasma flow was increased following administration of captopril; GFR and hence filtration fraction was reduced. The time course of the effects of captopril on plasma concentrations of angiotensin II and on filtration fraction were remarkably similar. This suggests either that efferent arteriolar tone, a major determinant of filtration fraction, is regulated mainly by the circulating renin-angiotensin system or that changes in tissue and circulating renin-angiotensin systems in response to captopril are closely related.

Implications for the Optimal Therapeutic Dose of ACE Inhibitors

The dose of captopril used in our study was chosen to achieve powerful supression of angiotensin II concentrations for some hours, but allowing the concentrations to recover towards the end of the study period. A larger dose of captopril would be unlikely to produce more intense suppression of

circulating angiotensin II, although the duration of suppression of angiotensin II would have been longer. Although recent studies support the use of larger doses of ACE inhibitors in patients with heart failure (Pacher, Globits, Bergler-Klein, et al 1993; Pacher, Stanek, Globits, et al 1996), in clinical practice, many patients are maintained on doses of this magnitude.

These data suggest that intense ACE inhibition either by frequent administration or by larger doses of ACE inhibitor may enhance a furosemide induced diuresis as well as prevent diuretic induced increases in angiotensin II. This may explain preliminary evidence that suggests that high-dose ACE inhibition is more effective than low-dose ACE inhibition in heart failure, in terms of symptoms and possibly prognosis (Poleur, Rosseau, Oakley, et al 1991; Vagelos, Yee, Boyle, et al 1992; Pacher, Globits, Bergler-Klein, et al 1993; Pacher, Stanek, Globits, et al 1996). An enhanced diuresis observed with high-dose ACE inhibition, by causing a degree of pre-renal uraemia, may also contribute to the greater increase in plasma urea observed with long-acting ACE inhibitors (Packer, Lee, Yushak, et al 1986b).

Limitations

Atrial natriuretic peptide (ANP) and bradykinin were not measured. Rouleau, Bichet and Kortas (1988) suggested that the relationship between plasma concentrations of ANP and atrial pressure are altered by ACE inhibition such that for a given pressure a higher plasma concentration of ANP exists. However, this resetting of the relationship between ANP and atrial pressure appears related to long- rather than short-term ACE inhibitor effects and it is not clear that increases in ANP concentrations will enhance a furosemide induced diuresis in patients with heart failure (Molina, Fowler, McCrory, et al 1988). Intense ACE inhibition may potentiate the effects of bradykinin on

renal medullary blood flow and thus promote a diuresis. In addition, ACE inhibitor therapy has been shown to result in delayed reduction in circulating argenine vasopressin concentrations which would favour diuresis and natriuresis (Cleland, Dargie, Ball, et al 1985a; Cleland, Gillen, Dargie 1988) but we did not measure it in this study. This study is not directly applicable to clinical practice as furosemide was given intravenously and captopril was used in a dose that would be considered sub-therapeutic by many. However, for the purposes of looking at the mechanisms of ACE-inhibitor effect, the use of intravenous furosemide to overcome variable oral absorption appears preferable. Lower than recommended doses of captopril were used so that patients could be studied at times when ACE-inhibition was no longer effective in suppressing angiotensin II, however it should be noted that less than a third of all prescriptions for captopril are for 12.5mg tid or less.

Conclusions

Short-term inhibition of ACE in patients with heart failure has been shown to reduce the diuretic efficacy of furosemide. In contrast in patients treated with ACE inhibitors chronically, the natriuretic and diuretic responses to furosemide are enhanced by intense supression of angiotensin II. The precise renal mechanism for this effect remains to be disclosed, but altered baroreceptor function with a change in the "set point" for sodium is possible. The implications of these findings as to the optimal dosing regime for ACE inhibitors should be resolved by ongoing studies.

Chapter 5 Investigation of the effects of agents which increase cardiac output

5.1 Introduction

Increasing cardiac output in patients with heart failure might be expected to potentiate a furosemide-induced diuresis, but little is known about the precise mechanism, nor which the most appropriate agent would be. Sodium nitroprusside increases cardiac output by balanced arterial and venous dilatation, associated with a fall in arterial pressure and in peripheral vascular resistance (Berkowitz, McKeever, Croke, et al 1977; Franciosa, Silverstein 1982a; Young, Leon, Pratt, et al 1988; Ferrari, Ceconi, De Guili, et al 1992). Dobutamine has both inotropic and vasodilator effects, usually causes arterial pressure to rise and has been shown to improve urine output in uncontrolled studies of patients with cardiac failure both alone and with concomitant furosemide administration (Berkowitz McKeever, Croke, et al 1977, Leier, Webel, Bush 1977; Leier, Heban, Huss, et al 1978). It is not clear whether any beneficial effect on diuresis is due to an increase in cardiac output or blood pressure or due to improved renal perfusion pressure.

The aim of the present study was to determine whether dobutamine or sodium nitroprusside augmented the renal effects of furosemide in patients with chronic heart failure and, if so, to establish by which mechanism this occurs:

a) Improved systemic and renal perfusion pressure

b) Increased renal blood flow

c) Altered delivery of furosemide to the loop of Henle.

Patients and methods

Eight patients aged 51-67 (mean age 62) years with chronic stable heart failure requiring diuretics to control symptoms were studied on three occasions in single blind fashion. Diuretic requirements ranged from 40mg-160mg furosemide per day (mean 100mg/day).

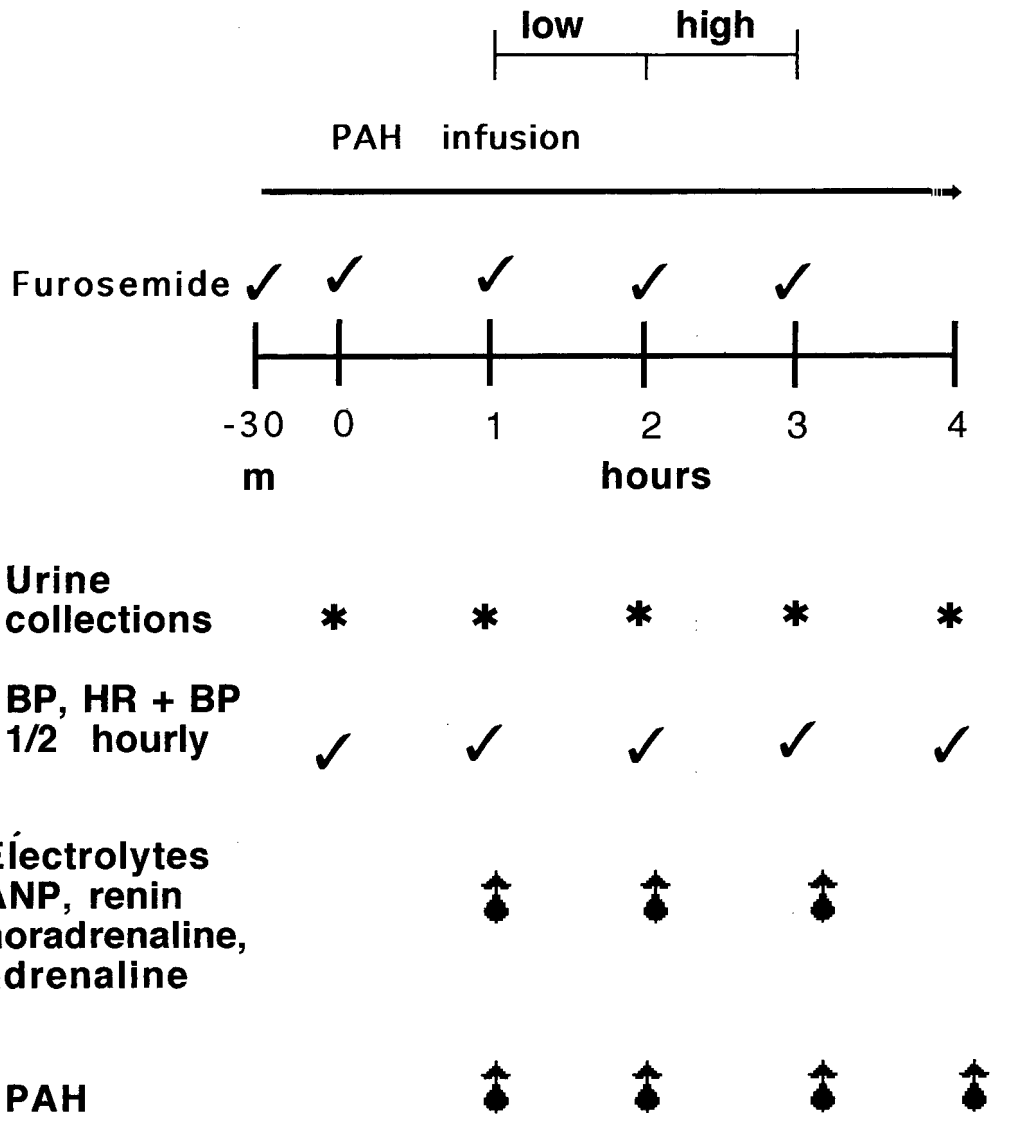
Table 5.1 Patient characteristics

Table showing individual patients' age, sex, New York Heart Association classification (NYHA), daily diuretic requirements in milligrams (furosemide-F, metolazone-MET), type of ACE-inhibitor and daily dosage (captopril-CAP, enalapril-ENAL, lisinopril-LISIN) and aetiology of heart failure (ischaemic heart disease-IHD, dilated cardiomyopathy-DCM).

PATIENT	AGE	SEX	NYHA	DIURETIC	ACE-I (mg)	AETIOLOGY
1	64	M	IIB	F 80	CAP 75	IHD
2	67	M	IIB	F 120	CAP 75	IHD
3	65	M	IIB	F 120	-	IHD
4	55	M	III	F 120	ENAL 20	IHD
5	62	M	III	F 40, MET 2.5	LISIN 10	IHD
6	63	M	III	F 120	ENAL 15	DCM
7	62	M	IIB	F 80	ENAL 10	IHD
8	51	M	III	F 160	ENAL 20	DCM

After the run-in period of one hour, sodium nitroprusside, dobutamine or matching placebo was infused as set out in the methods section. Dobutamine and sodium nitroprusside were infused at low dose for the second hour and at high dose for the third hour then discontinued for the final hour. Hourly urine and blood samples were collected for electrolytes, PAH, urinary cGMP and plasma ANP and active renin. Adrenaline and noradrenaline were measured during placebo and nitroprusside infusion only. Heart rate and blood pressure were measured at 15 minute intervals (figure 5.1).

Figure 5.1. Study design (Dobutamine, nitroprusside)



Results:

Urine output was not significantly different from placebo after nitroprusside although there was a significant rise in urine volume during the hour after high dose dobutamine infusion (figure 5.2).

Electrolyte excretion: There was no change in excretion of any of the electrolytes measured after sodium nitroprusside infusion (table 5.2). Natriuresis was potentiated by low and high-dose dobutamine infusion ($p<0.02$ and $p<0.04$ respectively) with a trend still evident an hour after the infusion was terminated ($p=0.08$); Figure 5.3. (Of note, urinary sodium excretion fell progressively during the placebo arm of the study examining the effect of captopril, suggesting that preservation of a constant natriuresis over 4 hours is an improvement). Chloride excretion increased after dobutamine, reaching significance only during low dose infusion (13±4 to 20±4mmol, $p<0.02$; table 5.2). Phosphate excretion tended to fall during dobutamine infusion. There was no change in renal excretion of furosemide or urinary cyclic GMP with either intervention (table 5.2).

Figure 5.2 Hourly urine output during placebo, dobutamine and sodium nitroprusside infusions.

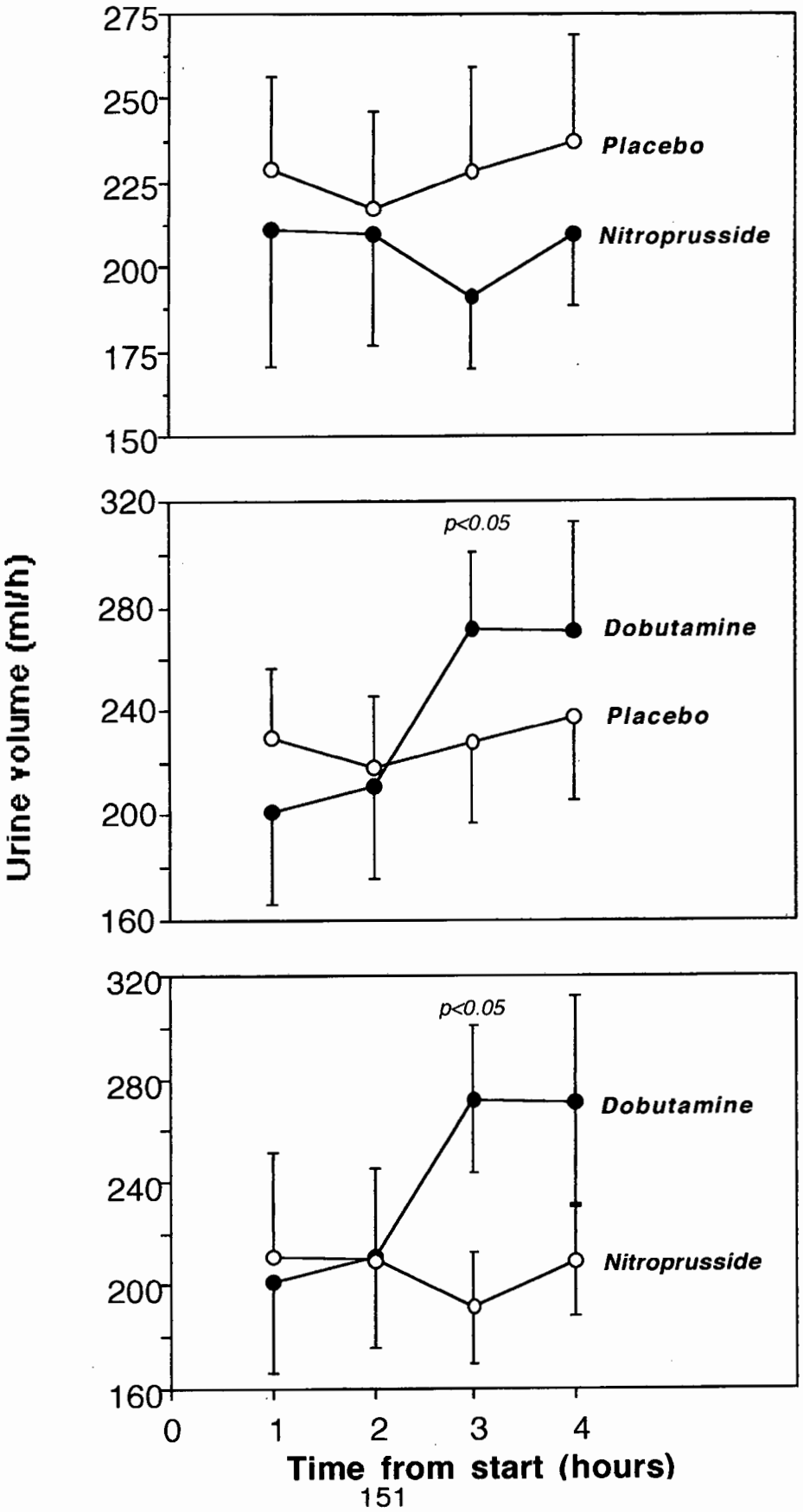


Figure 5.3: Hourly sodium excretion during infusion of placebo, dobutamine and sodium nitroprusside.

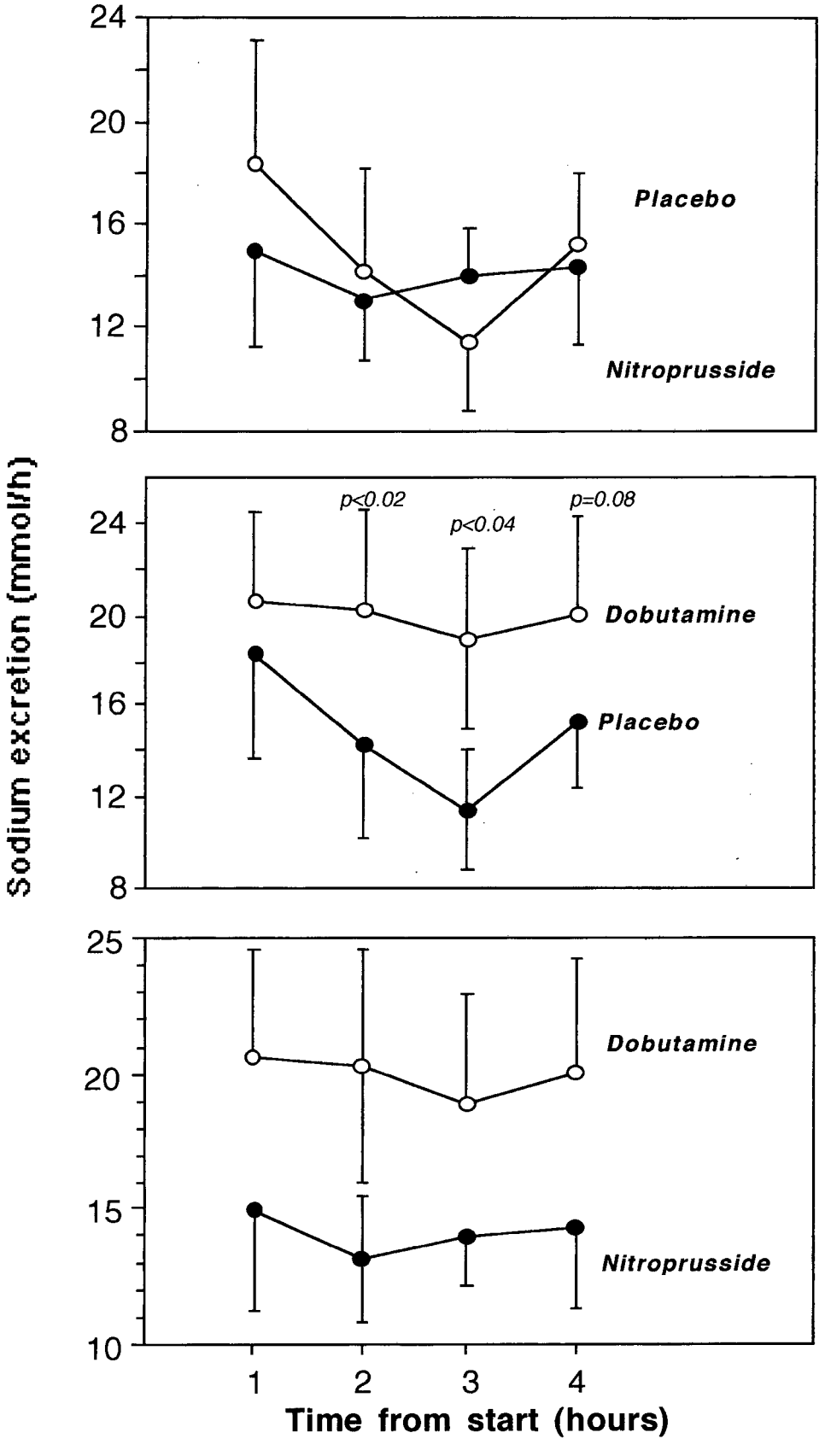


Table 5.2 Urine parameters during infusion of placebo, dobutamine and SNP

Changes in urine volume and excretion of sodium (Na), potassium (K), urea, creatinine (Cr), chloride (Cl), magnesium (Mg), phosphate (Phos), furosemide (F'SEMIDE) and cyclic GMP (cGMP). * denotes significance at $p<0.05$.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
VOLUME	PLACEBO	229±28	218±28	228±31	237±32
MLS	DOBUTAMINE	201±35	211±35	272±28*	271±41
	NITRO	211±40	210±33	191±21	210±21
Na	PLACEBO	18±5	14±4	11±3	15±3
mmol	DOBUTAMINE	21±4	20±4*	19±4*	20±4
	NITRO	15±4	13±2	14±2	14±3
K	PLACEBO	6.9±0.8	6.7±0.4	6.1±0.7	6.3±0.6
mmol	DOBUTAMINE	7.1±1.3	6.8±1.2	5.6±1.1	7.4±1.3
	NITRO	5.8±1.1	5.8±1.1	6.6±1.3	6.8±1.6
Urea	PLACEBO	21±3	18±2	16±2	16±1
mmol	DOBUTAMINE	24±3	20±3	16±2	20±2
	NITRO	18±2	16±1	15±2	15±3
Cr	PLACEBO	0.59±0.06	0.48±0.04	0.41±0.04	0.43±0.02
mmol	DOBUTAMINE	0.62±0.09	0.49±0.09	0.38±0.06	0.48±0.05
	NITRO	0.56±0.06	0.44±0.05	0.45±0.08	0.44±0.09
Cl	PLACEBO	17±4	13±4	13±2	13±2
mmol	DOBUTAMINE	20±3	20±4*	18±4	19±3
	NITRO	15±4	13±3	12±2	14±3
Mg	PLACEBO	0.41±0.05	0.3203±0.02	0.30±0.03	0.30±0.02
mmol	DOBUTAMINE	0.40±0.06	0.34±0.06	0.27±0.05	0.32±0.05
	NITRO	0.34±0.06	0.32±0.04	0.30±0.05	0.30±0.0
Phos	PLACEBO	1.17±0.21	0.92±0.12	1.02±0.08	1.12±0.07
mmol	DOBUTAMINE	1.18±0.14	1.07±0.12	0.88±0.10	0.93±0.1
	NITRO	0.88±0.11	0.84±0.08	0.91±0.15	1.12±0.18
F'SEMIDE	PLACEBO	1.99±0.26	1.93±0.15	1.81±0.2	1.86±0.22
mg	DOBUTAMINE	1.51±0.2	2.04±0.51	2.86±0.79	2.74±0.6
	NITRO	1.66±0.26	2.25±0.32	1.92±0.22	2.40±0.35
cGMP	PLACEBO	2.98±0.94	2.52±0.68	2.67±0.61	2.21±0.69
nmol/min	DOBUTAMINE	2.64±0.46	2.79±0.70	3.27±0.81	3.11±1.01
	NITRO	3.36±1.01	2.90±0.87	2.23±0.43	2.71±0.70

Fractional excretion of all urinary indices were calculated to eliminate the effect of inaccuracies of urine measurement. There was no significant change in fractional excretion of any of the electrolytes measured during nitroprusside infusion but fractional excretion of sodium and chloride

increased during low-dose dobutamine infusion ($p<0.03$)

Table 5.3 Fractional excretion of electrolytes, furosemide and cyclic GMP

Table of fractional excretion of sodium (Na), potassium (K), urea (U), chloride (Cl), magnesium (Mg), phosphate (Phos), furosemide (FRUS) and cyclic GMP (cGMP). * $p<0.05$.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
Na/Cr	PLACEBO	32±7	31±9	36±8	35±6
	DOBUTAMINE	36±8	44±8*	51±6	45±9
	NITRO	28±7	34±9	35±7	37±6
K/Cr	PLACEBO	12±2	14±1	15±1	14±1
	DOBUTAMINE	12±2	15±2	15±2	15±2
	NITRO	10±1	13±1	14±1	15±1
U/Cr	PLACEBO	35±2	38±2	39±2	37±2
	DOBUTAMINE	40±2	43±2	44±2	41±2
	NITRO	34±4	38±3	36±3	37±3
Cl/Cr	PLACEBO	30±6	29±8	34±7	31±4
	DOBUTAMINE	34±6	42±6*	46±6	42±7
	NITRO	28±8	32±8	28±4	36±7
Mg/Cr	PLACEBO	0.70±0.07	0.70±0.06	0.75±0.07	0.68±0.05
	DOBUTAMINE	0.65±0.08	0.72±0.08	0.72±0.07	0.66±0.07
	NITRO	0.62±0.10	0.74±0.07	0.69±0.08	0.71±0.06
Phos/Cr	PLACEBO	1.9±0.2	2.0±0.3	2.7±0.3	2.6±0.2
	DOBUTAMINE	2.1±0.3	2.5±0.4	2.6±0.4	2.1±0.4
	NITRO	1.7±0.7	2.0±0.1	2.0±0.2	2.7±0.2
FRUS/Cr	PLACEBO	3.7±0.4	4.5±0.5	5.1±0.4	4.9±0.5
	DOBUTAMINE	2.7±0.4	6.1±2.7	11.2±4.9*	6.5±2.2
	NITRO	3.5±0.9	5.8±1.3	5.0±1.0	8.4±3.6*
cGMP/Cr	PLACEBO	5.1±1.8	5.2±1.3	6.8±1.8	4.9±1.5
	DOBUTAMINE	4.8±1.2	7.5±3.0	11.9±4.4	6.9±2.1
	NITRO	6.4±2.0	8.1±3.0	6.2±1.5	9.8±3.3

Plasma electrolyte concentrations were not significantly different after placebo, dobutamine and nitroprusside infusions with the exception of phosphate, which fell during high-dose dobutamine infusion (Table 5.4).

Table 5.4 Plasma electrolyte concentrations

Changes in plasma concentrations of sodium (Na), potassium (K), urea, creatinine (Cr), chloride (Cl), magnesium (Mg) and phosphate (Phos) one, two and three hours from the start of each study.

* $p<0.05$.

		1 HOUR	2 HOURS	3 HOURS
Na	PLACEBO	137±1	136±1	137±1
mmol	DOBUTAMINE	137±1	137±1	137±1
	NITROPRUSSIDE	136±2	136±2	136±2
K	PLACEBO	4.1±0.2	4.0±0.1	3.9±0.1
mmol	DOBUTAMINE	3.9±0.2	3.8±0.1	3.6±0.1
	NITROPRUSSIDE	3.8±0.1	3.7±0.1	3.8±0.1
Urea	PLACEBO	8.0±0.7	7.9±0.7	7.7±0.7
mmol	DOBUTAMINE	8.1±0.7	8.0±0.7	7.8±0.7
	NITROPRUSSIDE	9.0±1.0	8.8±1.0	8.8±1.1
Cr	PLACEBO	114±9	112±9	109±9
mmol	DOBUTAMINE	112±10	110±10	109±9
	NITROPRUSSIDE	123±10	120±10	120±10
Cl	PLACEBO	99±1	99±1	100±1
mmol	DOBUTAMINE	100±2	101±1	101±1
	NITROPRUSSIDE	99±2	99±2	98±2
Mg	PLACEBO	0.82±0.03	0.81±0.03	0.80±0.03
mmol	DOBUTAMINE	0.79±0.03	0.79±0.03	0.78±0.03
	NITROPRUSSIDE	0.81±0.04	0.79±0.04	0.79±0.04
Phos	PLACEBO	1.06±0.07	1.07±0.05	1.13±0.05
mmol	DOBUTAMINE	0.92±0.05	0.90±0.05	0.87±0.04*
	NITROPRUSSIDE	0.96±0.04	0.98±0.04	1.06±0.03

Neuroendocrine effects

After high dose infusion of sodium nitroprusside, ANP concentrations decreased (100 to 70 pg/ml; $p<0.03$, figure 5.4) and noradrenaline and

adrenaline concentrations rose (3.8±0.62 and 0.25±0.08 to 6.0±1.3 and 0.39±0.12, $p<0.03$ for noradrenaline and adrenaline respectively, figure 5.6). Plasma active renin concentrations did not change (figure 5.5, table 5.5). Catecholamines were not measured after dobutamine infusion as it was not possible to distinguish endogenous secretion from exogenous administration. ANP concentrations were significantly higher at baseline prior to infusion of dobutamine than prior to placebo but concentrations did not differ from placebo after low or high dose dobutamine infusion (table 5.5). However plasma active renin concentrations increased significantly after high dose dobutamine infusion (figure 5.5).

Table 5.5 Plasma hormone concentrations

Changes in plasma concentrations of atrial natriuretic peptide (ANP), plasma renin (PARC), noradrenaline (N ADREN) and adrenaline (ADREN) at one, two and three hours from the start of the studies. * $p<0.05$.

		1 HR	2 HRS	3HRS
ANP	PLACEBO	101±43	117±49	100±31
pg/ml	DOBUTAMINE	143±52	117±42	121±53
	NITROPRUSSIDE	140±75	112±49	70±26*
PARC	PLACEBO	352±144	303±143	285±132
μU/ml	DOBUTAMINE	245±112	242±108	499±184*
	NITROPRUSSIDE	239±75	228±67	314±105
N ADREN	PLACEBO	4.4±0.93	4.4±0.91	3.8±0.62
nmol/l	DOBUTAMINE	-	-	-
	NITROPRUSSIDE	5.0±1.2	5.8±1.6	6.0±1.3*
ADREN	PLACEBO	0.27±0.09	0.22±0.07	0.25±0.08
nmol/l	DOBUTAMINE	-	-	-
	NITROPRUSSIDE	0.25±0.05	0.26±0.06	0.39±0.12*

Figure 5.4: Changes in plasma concentrations of ANP during infusion of placebo, dobutamine and nitroprusside.

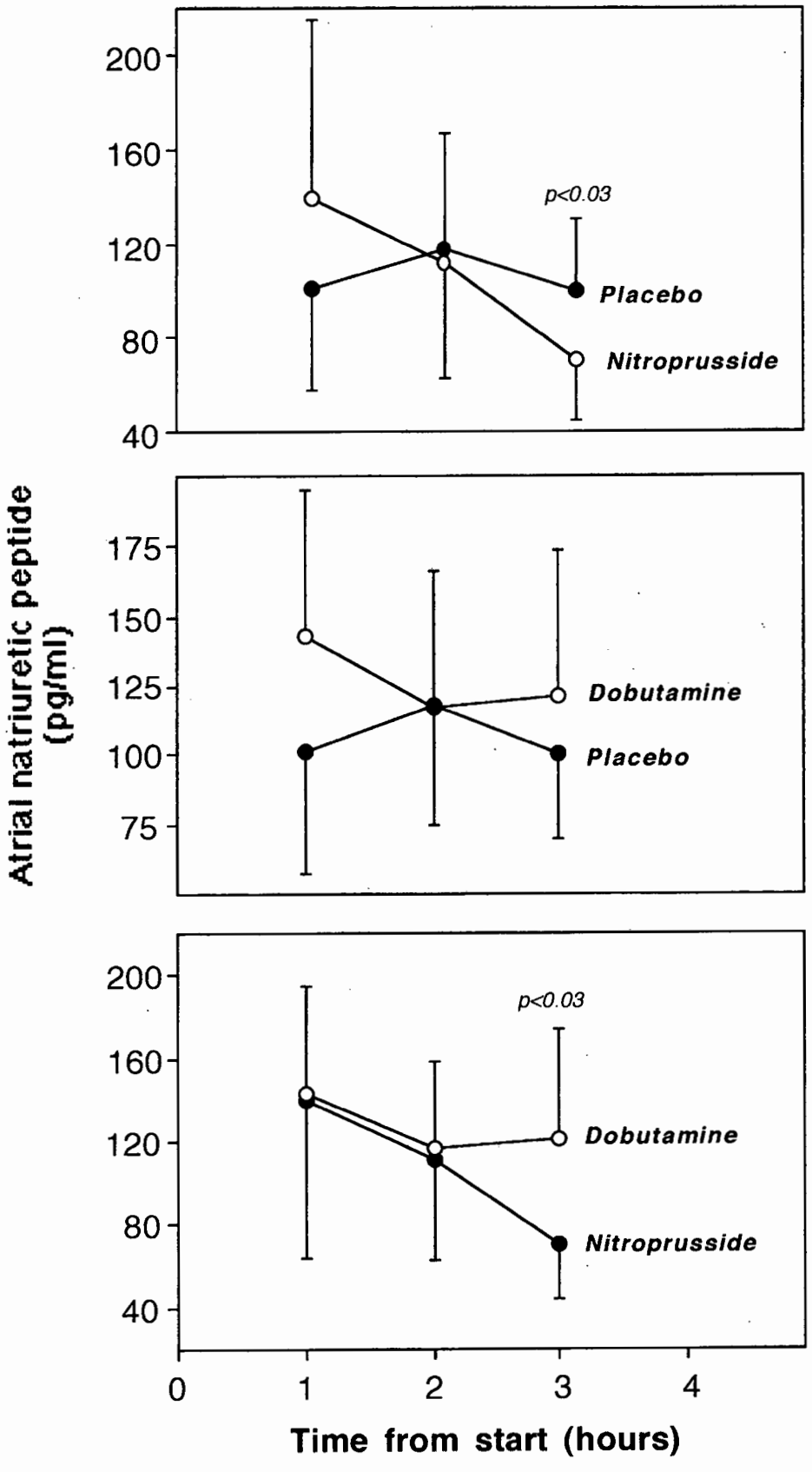


Figure 5.5 Changes in plasma active renin concentration during infusion of placebo, dobutamine and sodium nitroprusside

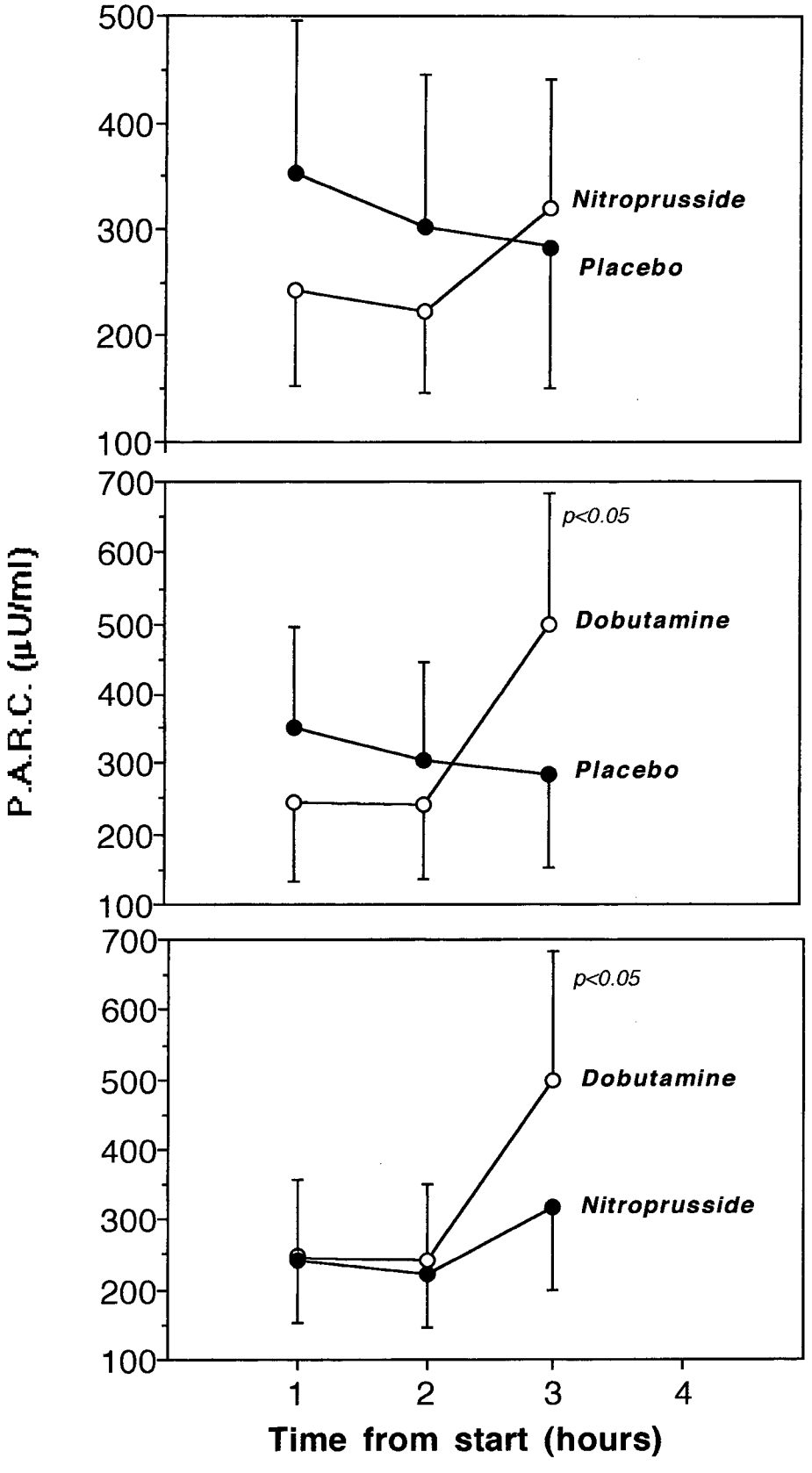
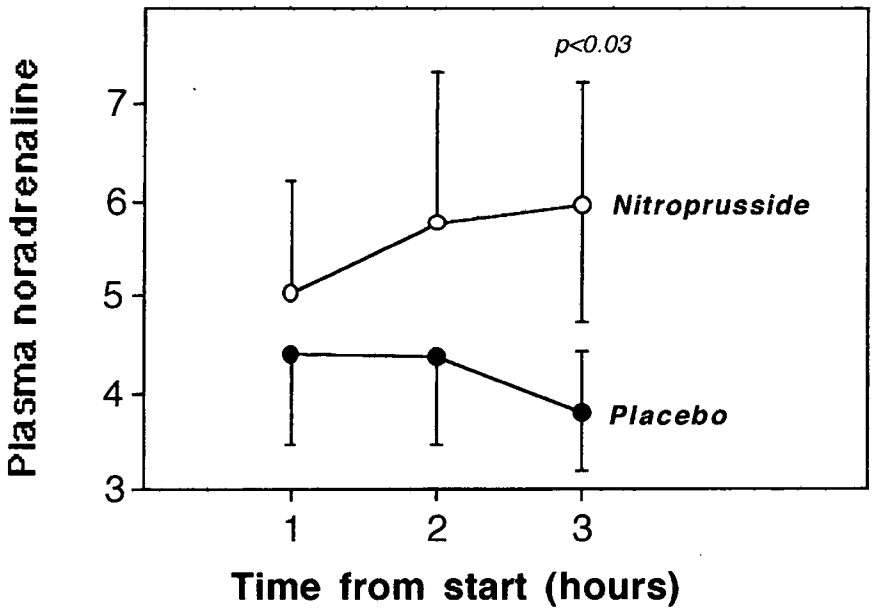


Figure 5.6 Changes in plasma noradrenaline concentrations during infusion of placebo and sodium nitroprusside



Haemodynamic effects

Heart rate rose during low-dose ($p<0.05$) and during high-dose dobutamine infusion ($p<0.02$), figure 5.7). Heart rate did not change during nitroprusside infusion, despite relative hypotension during high dose infusion (74±2 to 79±5bpm, p=NS).

Mean arterial pressure fell during high dose nitroprusside infusion ($p=0.018$) but was unchanged by dobutamine (figure 5.8).

Renal blood flow increased during low-dose nitroprusside infusion but not high-dose, and during high-dose dobutamine infusion but not low dose infusion (figure 5.9).

Figure 5.7 Changes in heart rate during infusion of placebo, dobutamine and sodium nitroprusside

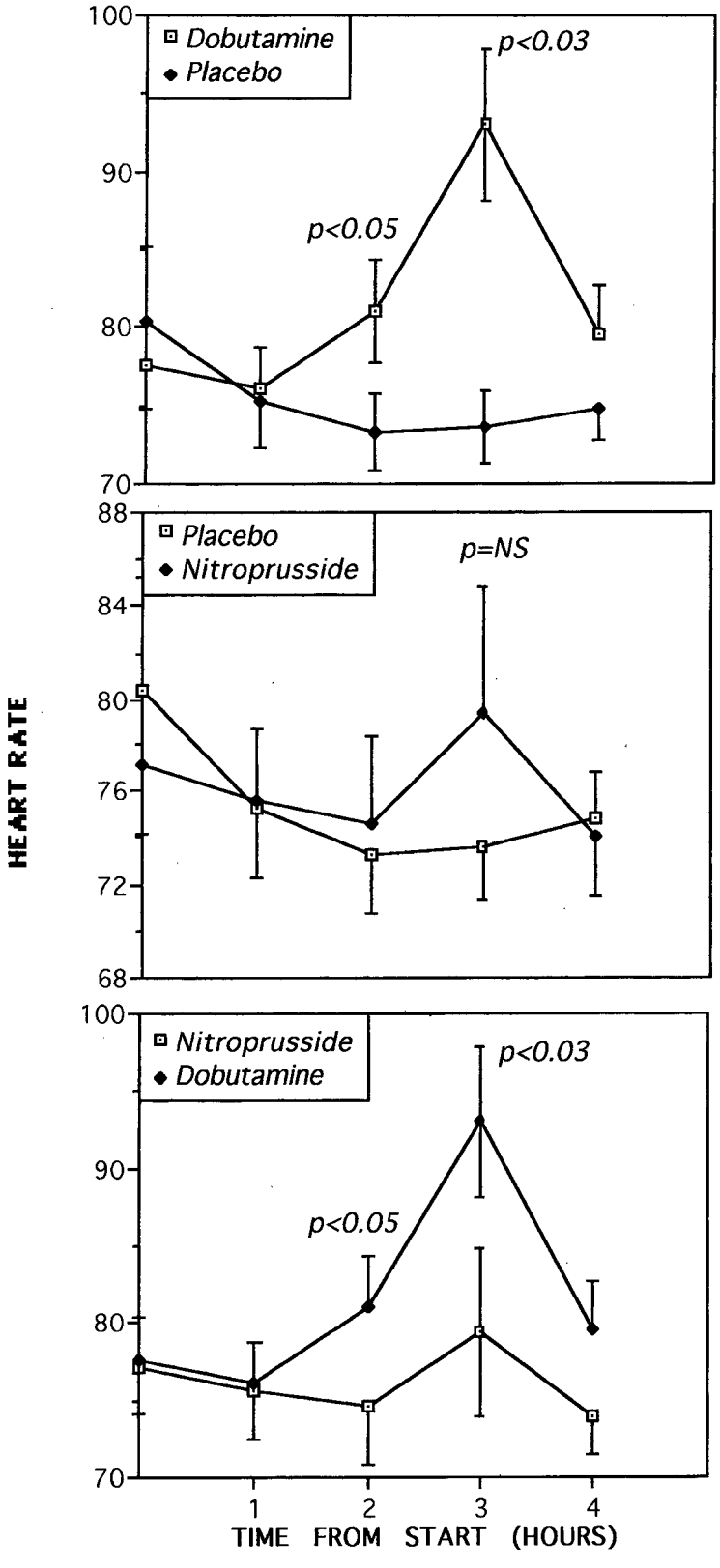
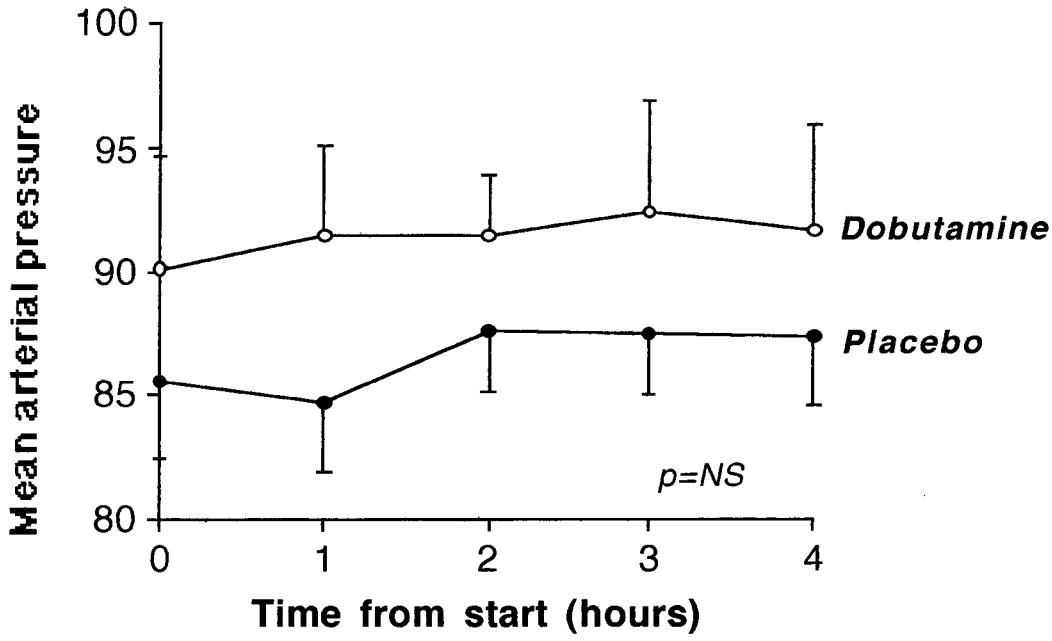


Figure 5.8 Changes in mean arterial pressure during placebo, dobutamine and nitroprusside infusion
Differences did not reach significance (NS)



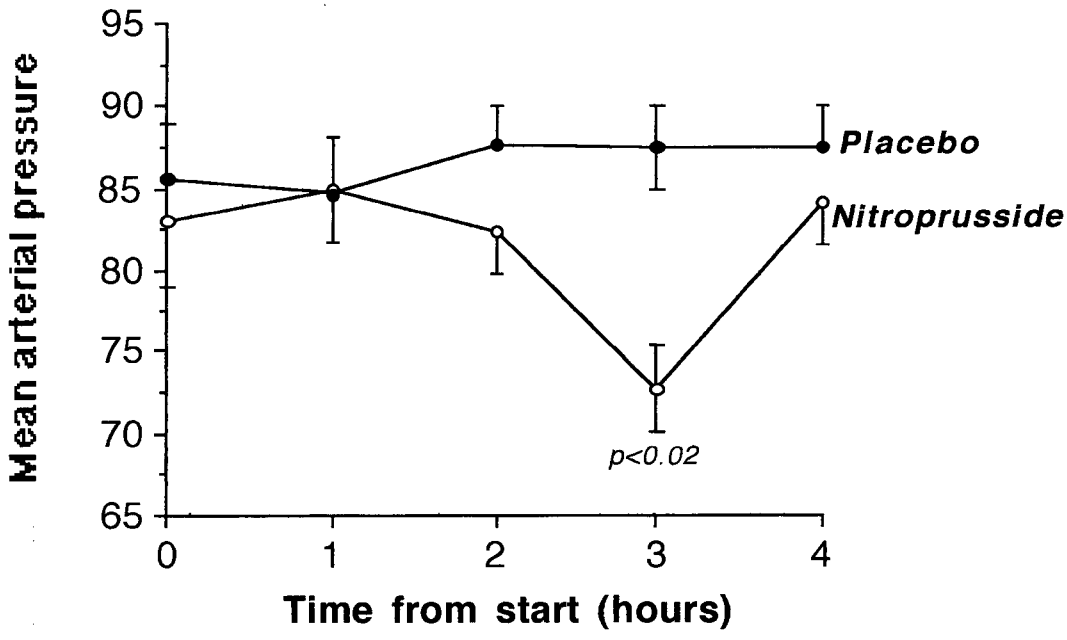
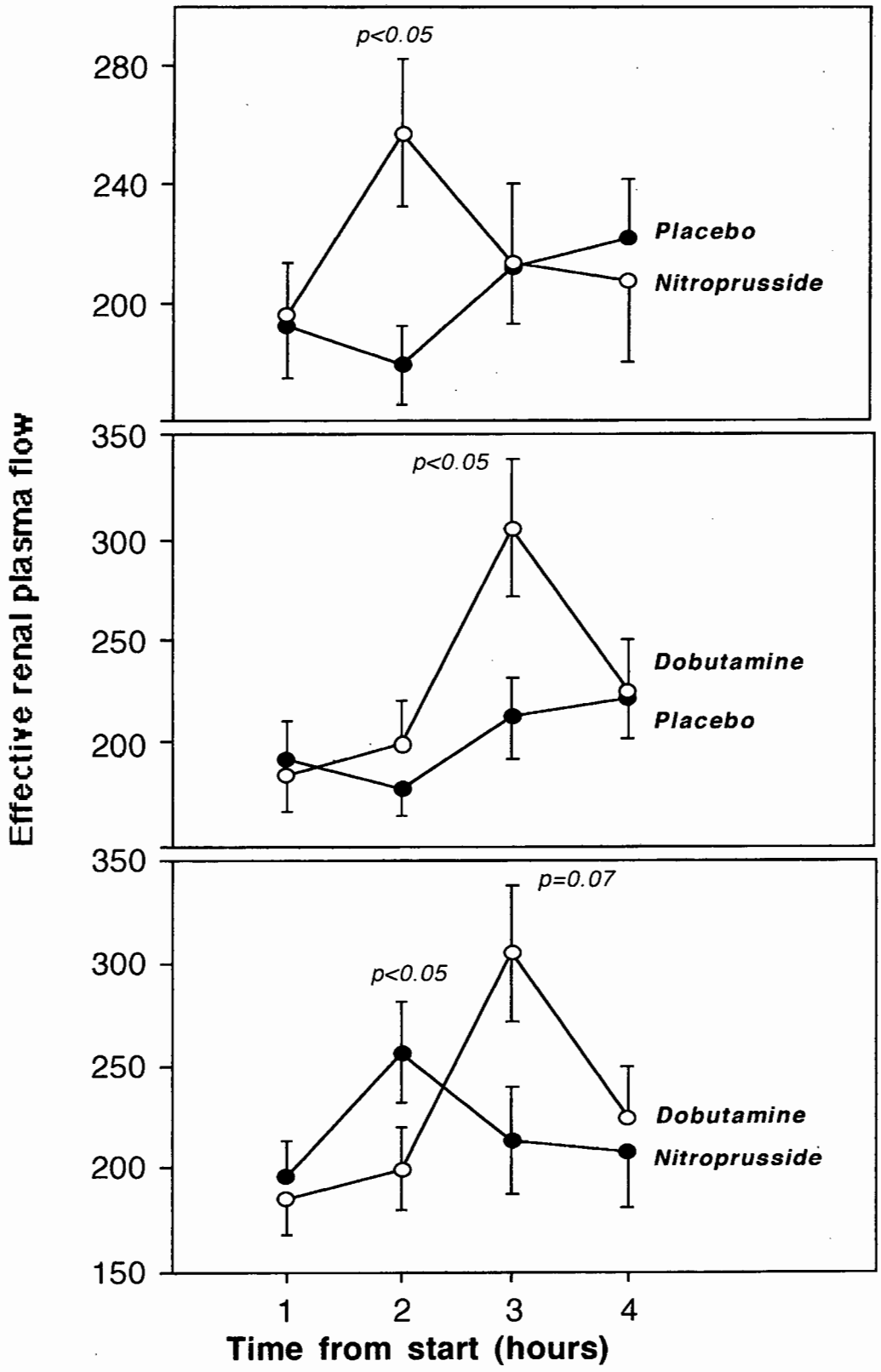


Figure 5.9 Changes in ERPF during infusion of placebo, dobutamine and sodium nitroprusside.



Creatinine clearance tended to rise after high dose dobutamine infusion was terminated although this did not reach statistical significance.

Table 5.6 Renal haemodynamics

Changes in glomerular filtration rate (GFR) measured by creatinine clearance (CR. CLEAR), changes in effective renal plasma flow (ERPF) measured by hippurate clearance (PAH CLEAR). *Denotes $p<0.05$

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
GFR (CR.CLEAR.)	PLACEBO	92±13	79±10	67±11	70±6
	DOBUTAMINE	97±17	76±15	58±9	76±9
	NITRO	80±12	63±7	64±10	62±12
ERPF (PAH CLEAR.)	PLACEBO	193±18	179±13	212±19	222±20
	DOBUTAMINE	185±18	200±20	305±33*	225±25
	NITRO	196±18	257±25*	214±26	208±28

5.4 Discussion

This study set out to explore the relative ability of nitroprusside and dobutamine to potentiate a furosemide induced diuresis by improving cardiac output. Indices of improved haemodynamics included changes in heart rate, arterial pressure and renal plasma flow. Unfortunately it proved impossible to measure changes in cardiac output using the Bomed, however nitroprusside induced a significant fall in arterial pressure and increase in renal plasma flow, compatible with improved cardiac output (Cogan, Humphreys, Carlson, et al 1980; Miller, Fennell, Young, et al 1982; Franciosa, Silverstein 1982a; Olivari, Levine, Cohn 1986; Young, Leon, Pratt, et al 1988).

Dobutamine improved the furosemide induced natriuresis and diuresis in the present study although the diuretic effect only reached statistical significance during the hour after high dose dobutamine infusion. Leier, Heban, Huss, et al (1978) compared the effects of dopamine and dobutamine infused over 24 hours in patients with severe congestive heart failure without coronary disease (12 of the 13 patients were assigned to NYHA class IV) in whom maintenance oral diuretics were continued. They demonstrated diuresis, natriuresis and increased fractional excretion of sodium with dobutamine infusion but as these findings were not compared with a control infusion, changes from baseline may have been partly due to an effect of circadian variation.

There are several mechanisms by which dobutamine could enhance a furosemide-induced diuresis and natriuresis:

a) enhanced furosemide delivery (Nomura, Yasuda, Minami, et al, 1981) in

patients with heart failure and in normal subjects (Fujimura, Ebihara 1988), where natriuresis and renal furosemide excretion were correlated. The natriuresis associated with dobutamine infusion was not accompanied by increases in renal furosemide excretion. To compensate for the potential inaccuracies of urine collections, fractional excretion of furosemide was examined. As this increased during high-dose dobutamine infusion, it is possible that the natriuresis was mediated by increased delivery of furosemide to its site of action in the proximal tubule, associated with the improvement in ERPF. However fractional excretion of furosemide also increased in the hour following high-dose nitroprusside infusion, which was not associated with natriuresis.

b) An alternative explanation for the diuresis and natriuresis could be that of an enhanced effect on tubular responsiveness to furosemide, however there was no data to support this from the present study.

c) By increasing ERPF. The increase in renal plasma flow with dobutamine coincided with natriuresis in the present study although Leier, Heban, Huss, et al (1978) showed that diuresis and natriuresis occurred without changes in renal blood flow during sustained infusion of dobutamine at similar doses. By contrast, ERPF was enhanced during low-dose nitroprusside infusion without concomitant potentiation of diuresis or natriuresis. It is possible that the kidney is more sensitive to changes in arterial pressure than changes in ERPF.

d) By an effect on blood pressure. There was no significant increase in arterial pressure during dobutamine infusion but most patients were not able to tolerate the maximum dose of dobutamine (10μg/kg/min) for the third hour.

Leier, Webel and Bush (1977) did not induce a significant rise in blood pressure at dobutamine doses of less than 10μg/kg/min. This relative dobutamine intolerance may have been due to underlying ischaemic heart disease in some of the patients although Leier's patients, none of whom had ischaemic heart disease, developed significant ventricular extrasystoles with dobutamine at doses of 10μg/kg/min. Nitroprusside infusion caused hypotension in all patients and failed to improve the furosemide induced diuresis or natriuresis. Thus it seems that while a fall in blood pressure is detrimental to the renal effects of nitroprusside, the beneficial renal actions of dobutamine are not dependent on a rise in arterial pressure.

e) Via a local renal sympathetic effect. Adrenoceptor agonists such as dobutamine affect the renal tubule via activation of receptors influencing cAMP production. α1-adrenoceptor agonists mimic the effect of renal nerve stimulation, resulting in vasoconstriction and increased renal vascular resistance. They have been implicated in the decrease in renal blood flow and alteration in intrarenal perfusion found in cardiac failure (Schrier, De Wardener 1971). Catecholamines release renin from juxtaglomerular cells through activation of intrarenal β–receptors, mediated by a rise in cAMP, calcium flux or renal prostaglandins. Thus sympathetic stimulation should tend to decrease salt and water excretion. However selective α2-adrenoceptor stimulation causes diuresis in vivo, probably due to modulation of the action of vasopressin on the collecting tubule (Garg 1992). A direct renal sympathetic effect is thus unlikely to be responsible for the natriuretic and diuretic effects observed with dobutamine infusion, although this mechanism cannot be entirely ruled out.

f) By enhancing creatinine clearance. Creatinine clearance did not change during either infusion, despite the rise in ERPF, suggesting a degree of glomerulo-tubular imbalance. Garg (1992) proposed that the failure of adrenergic drugs to increase renal papillary flow (as opposed to renal plasma flow) was responsible for the lack of change in GFR. Creatinine clearance is not the most accurate method of measuring glomerular filtration rate in patients with impaired cardiac function (Motwani, Fenwick, Struthers 1992c), tending to overestimate GFR. As patients acted as their own controls and changes in clearance were assessed rather than absolute values, it is unlikely that changes in GFR were responsible for the enhanced diuresis and natriuresis caused by dobutamine infusion.

g) Increased cardiac output. The renal haemodynamic changes support an improvement in cardiac output with low dose nitroprusside infusion and high-dose dobutamine infusion. In studies comparing similar groups of patients during nitroprusside infusion, these changes have been accompanied by increased total cation excretion, natriuresis or diuresis, in different studies (Cogan, Humphreys, Carlson, et al 1980; Miller, Fennell, Young, et al 1982; Young, Leon, Pratt, et al 1988). They differ from the present study in that the effect of nitroprusside alone was assessed, not how nitroprusside modified the diuretic or natriuretic effects of furosemide. Patients were also generally in a lower functional class with decompensated heart failure. The powerful diuretic actions of furosemide may have masked smaller changes in salt excretion in the present study.

h) A rise in cGMP. There was no change in urinary cGMP excretion or fractional excretion during nitroprusside infusion or dobutamine infusion although nitroprusside is itself a producer of cyclic GMP in the papillary

collecting tubule. Chiu, Vemulapalli and Sybertz (1991) compared the vascular and renal excretory effects of atrial natriuretic peptide, sodium nitroprusside and bromium labelled cGMP infused into spontaneously hypertensive rats and showed that the natriuretic effects of ANP could not be reproduced by infusion of exogenous cGMP or by generation of cGMP from soluble guanylate cyclase (the mechanism of action of sodium nitroprusside). These findings are in agreement with the present study, but do not explain by what mechanism diuresis and natriuresis occurred with nitroprusside in other trials.

The relatively modest diuretic effect of dobutamine demonstrated in the present study may have been due to relative salt depletion, but the 3 day period with controlled sodium intake of 100mmol/day should have been sufficient to correct more gross salt depletion. There is some evidence from animal studies that the action of many if not all drugs may be modulated by interstitial osmolality in the renal medulla, which itself depends on the state of hydration of the subject at the time (Garg 1992). The average ANP concentrations for the present group were well above normal, suggesting raised atrial pressure, not hypovolaemia (Lewis, Makhoul, Dakak, et al 1992). It is possible that the patients with decompensated heart failure who benefited the most from dobutamine infusion in the acute studies reported (Leier, Webel, Bush 1977; Leier, Heban, Huss, et al 1978; Applefeld, Newman, Sutton, et al 1987) had a greater improvement in haemodynamic variables and thus a greater diuresis and natriuresis than those in the present study who had compensated, moderate heart failure. In addition, in most of the studies describing diuresis with dobutamine infusion, furosemide was not administered concurrently.

Although ANP concentrations fell during dobutamine infusion in the present study, baseline concentrations were significantly different from placebo. Concentrations during infusion did not differ significantly from placebo at the same time-point. Stangl, Baumann, Gerzer, et al (1991) described a fall in ANP and cGMP during dobutamine infusion at comparable dosage, with prompt return to baseline concentrations when the infusion was terminated. The reasons for the discrepancy are unclear.

Limitations of the study

The study population was small. Clinically significant changes in blood pressure were apparent during nitroprusside infusion and thus it was assumed that clinically significant changes in water or electrolyte excretion too would be evident if present. Small changes could have been overlooked because of the small sample size but would have been of less clinical relevance.

The study was single-blind, introducing observer error in the measurement of urine volume, although heart rate and blood pressure were measured by an automated device. In addition error may have been introduced into urine volume measurements by incomplete bladder emptying in some of our patients as urinary catheters were not used to collect samples. However all assays were performed blind, by independent departments. Between-patient variability was wide which may have been accentuated by the inclusion criteria, with heart failure due to ischaemic heart disease, previously corrected valvular heart disease and dilated cardiomyopathy all included. It is possible that the aetiology of heart failure influences the response to diuretics in some cases however patients were their own controls.

Patients may not have adhered strictly to the prescribed diet as 24 hour urinary collections were not analysed for electrolyte excretion in all patients, however the effect of marked variation in sodium intake on diuresis and natriuresis (chapter 3) was modest.

As the measure of changes in cardiac output or stroke volume used in the present study proved unreliable, it was not possible to determine whether changes in these variables had actually taken place with acute interventions. However there was a clear change in ERPF. While several studies have documented the haemodynamic response to dobutamine and nitroprusside infusions in patients with decompensated heart failure (Cogan, Humphreys, Carlson, et al 1980; Bendersky, Chatterjee, Parmley, et al 1981; Young, Leon, Pratt, et al 1988) there is a lack of data on the haemodynamic response to dobutamine and nitroprusside in well-diuresed stable patients.

Conclusion: Both dobutamine and sodium nitroprusside have been reported to produce diuresis and natriuresis in with patients with heart failure. The renal effects of intravenous dobutamine and nitroprusside combined with furosemide, as they would usually be administered in clinical practice, were far less impressive in the present study. Dobutamine furosemide induced natriuresis at high dose, but had only a modest effect on diuresis, while sodium nitroprusside tended to impair both diuresis and natriuresis. These results could not be accounted for by changes in renal plasma flow, which was augmented by both agents. Nitroprusside infusion was distinguished from dobutamine infusion by the dramatic fall in blood pressure seen during high dose nitroprusside infusion. While dobutamine had no effect on blood pressure, preservation of blood pressure seems to have been important in augmenting the diuretic and natriuretic effects of

furosemide and possibly exerts a permissive effect on the enhancement of sodium excretion by increasing ERPF. Enhanced renal excretion of furosemide may have been partly responsible for the natriuresis and diuresis, as fractional excretion of furosemide increased. In compensated heart failure treated with diuretics, both dobutamine and nitroprusside were poorly tolerated, even at modest doses.

Chapter 6 The effect of digoxin on a furosemide induced diuresis

6.1 Introduction

Digoxin is a cardiac glycoside, one of a family of substances synthesized by plants and animals. The therapeutic benefits of extracts from digitalis purpurea (the foxglove) were described by William Withering (1785), but the extracts had been used in topical preparations as far back as 1250 (Greeff, Schadewaldt 1981a; Skou 1986). Similar products include the bufadienolides from the bulb of the sea squill (used to induce diuresis in Roman times: Greeff, Wirth 1981b; Movitt 1949) and the skin of toads (used by the Chinese and Japanese for the treatment of dropsy: Fieser, Fieser 1959), and ouabain from the bark and roots of the Ouabaio tree and the seeds of Strophanthus gratus. Despite this, the role of digoxin in heart failure and the mechanism by which it produces its effect remain controversial. Withering alluded to several mechanisms by which the beneficial effects of digitalis glycosides might be mediated, including a reduction in heart rate, a "strengthening" of the pulse and a diuresis. The opening paper in the first edition of the British Heart Journal (Gavey, Parkinson 1939) was on the subject of the benefit of using digoxin on patients with heart failure in sinus rhythm. The authors reported a marked diuresis in response to digoxin in many patients. Although ouabain extracted from plants has been used extensively as a research tool and binding sites or receptors had been discovered in nearly all cells in higher animals, it is only recently that it has been identified as the endogenous cardiac glycoside in animals (Hamlyn, Blaustein, Bova, et al 1991). Ouabain is an adrenal cortical hormone (Hamlyn, Blaustein, Bova, et al 1991; Ludens, Clark, Robinson, et al 1992) but the identity of the cells in the adrenal cortex that synthesize and store it remain unknown. Ouabain, unlike

digoxin, is highly soluble in aqueous media and thus poorly absorbed from the gastro-intestinal tract (Greeff, Wirth 1991b). A high salt diet causes plasma ouabain levels to rise (Manunta, Rogowski, Hamilton, et al 1992a) and concentrations are elevated in patients with congestive heart failure (Gottlieb, Rogowski, Weinberg, et al 1992b).

The molecular basis for the actions of cardiac glycosides is their selective and potent inhibition of the Na^+ pump (Schatzmann 1953). The Na-K-ATPase is associated with the Na^+ pump and is specifically inhibited by the cardiotonic steroids (Skou 1986). This enzyme, found on all eukaryotic cells, represents the major pathway for sodium-potassium exchange across the cell membrane. It is found on the basolateral aspect of renal tubular epithelial cells and may promote the renal tubular reabsorption of sodium.

Low concentrations of ouabain interact with and inhibit the Na^+ pump, causing intracellular concentrations of sodium to rise and potasium to fall. This results in a change in the resting membrane potential, which can influence calcium permeability in smooth muscle (Nelson, Standen, Brayden, et al 1988; Nelson, Patlak, Worley, et al 1990; Brayden, Nelson 1992). In addition the impairment of sodium transport can affect neurotransmitter release and re-uptake as many are co-transported with sodium (Johnson, Carty, Scarpa 1985; Kanner, Shuldiner 1987). Cytosolic calcium ions are major intracelular second messengers and regulate a variety of enzyme systems, membrane excitability, contraction, secretion, mitochondrial metabolism and cell division. Control of intracellular calcium is via the ATP-driven calcium pump and the Na^+-Ca^{2+}-exchanger. Most of the calcium is buffered by cytoplasmic proteins or sequestered in the

sarcoplasmic reticulum. The Na^{+}-Ca^{2+} exchanger plays a dominant role in extrusion of calcium from the cell when calcium concentrations are high. When the Na^{+} pump is slightly inhibited, a small rise in resting cytoplasmic calcium concentration results in a vast increase in the cytosolic to endoplasmic reticulum calcium ratio and in the case of cardiac muscle, enhanced contraction.

In vascular smooth muscle, ouabain modulates vascular contractility, probably also by increasing the stores of calcium in the sarcoplasmic reticulum (Blaustein 1993). Increased dietary salt intake causes plasma ouabain levels to rise (Manunta, Rogowski, Hamilton, et al 1992b) and chronic ouabain infusion in normal rats causes chronic hypertension (Doursout, Chelly, Liang, et al 1992; Manunta, Rogowski, Hamilton, et al 1992a; Yuan, Manunta, Chen, et al 1992). These findings have lent support to the theory of pressure natriuresis in patients with low-renin-hypertension. The primary problem is felt to be the inability of the kidney to excrete a salt load at a normal blood pressure. This leads to volume expansion and promotion of ouabain secretion. This results in a rise in blood pressure and pressure-natriuresis (Blaustein, Hamlyn 1991; Guyton 1991). However Luft, Aronoff, Fineberg, et al (1989) failed to demonstrate a natriuretic effect of digoxin in 14 normal volunteers during conditions of salt loading. Interestingly, elevated concentrations of ouabain in patients with heart failure correlate not with plasma volume but inversely with cardiac index (Gottlieb, Rogowski, Weinberg, et al 1992b). Perhaps ouabain helps support the blood pressure at the expense of plasma volume in heart failure.

Uncontrolled studies by Starr & Luchi (1969); Dall (1970) and Hull &

Mackintosh (1977) suggested that digoxin could be safely withdrawn from many patients with stable heart failure in sinus rhythm. This lead to concern about the efficacy of digoxin (Gavey & Parkinson 1979), however several subsequent controlled trials (Lee, Johnson, Bingham, et al 1982; Guyatt, Sullivan, Fallen, et al 1988; The Captopril-Digoxin Multicentre Research Group 1988; DiBianco, Shabetai, Kostuk, et al 1989) have shown that digoxin improves symptoms and exercise tolerance in patients with heart failure in sinus rhythm. Packer, Gheorghiade, Young, et al (1993) showed that withdrawal of digoxin from patients with chronic heart failure treated with ACE inhibitors resulted in worsening heart failure and in decreased exercise tolerance. The present study sought to determine in a controlled fashion whether digoxin potentiated the renal effects of furosemide in 8 patients receiving what is currently optimal therapy for the treatment of heart failure.

6.2 Patients and methods

8 patients with chronic stable heart failure requiring maintenance diuretics to control symptoms of heart failure were studied on two occasions in random, single blind fashion. All patients were in sinus rhythm and no patients were taking digoxin. Seven patients were taking ACE inhibitors.

Table 6.1 Patient characteristics

Individual patient characteristics: age, sex, New York Heart Association classification NYHA), usual diuretic requirements (furosemide-F, metolazone-MET, bumetanide-BUMET), ACE-inhibitor (captopril-CAP, enalapril-ENAL, lisinopril-LISIN, all expressed as daily dose) and aetiology of heart failure (ischaemic heart disease-IHD, dilated cardiomyopathy-DCM).

PATIENT	AGE	SEX	NYHA	DIURETIC	ACE-I (mg)	AETIOLOGY
1	64	M	IIB	F 80	CAP 75	IHD
2	67	M	IIB	F 120	CAP 75	IHD
3	65	M	IIB	F 120	-	IHD
4	55	M	III	F 120	ENAL 20	IHD
5	62	M	III	F 40, MET 2.5	LISIN 10	IHD
6	63	M	III	F 120	ENAL 15	DCM
7	62	M	IIB	F 80	ENAL 10	IHD
8	69	M	III	BUMET 4	ENAL 10	IHD

After the run-in period of one hour, digoxin 500μg or matching placebo (saline) was infused. Digoxin was infused as a single bolus over 30 minutes at the start of the 2nd hour, as set out in chapter 2, because of its long half-life. Hourly urine and blood samples were collected for electrolytes, urinary cGMP, PAH and plasma ANP, plasma renin, adrenaline and noradrenaline. Heart rate and blood pressure were measured at 15 minute intervals.

6.3 Results

Urine volume did not change although sodium and chloride excretion were greater during the 3 hours following digoxin administration ($p<0.03$). Excretion of potassium, urea, magnesium, phosphate, creatinine, furosemide and cyclic GMP did not change.

Table 6.2 Urine volume and excretion of electrolytes, furosemide and cGMP

Changes in urine volume, excretion of electrolytes (sodium-Na, potassium-K, urea, creatinine-Cr, magnesium-Mg, chloride-Cl and phosphate-Ph), cGMP and furosemide (F'MIDE).

		0-1 hrs	1-2 hrs	2-3 hrs	3-4 hrs
U VOL	PLACEBO	229±28	216±29	240±28	247±27
mls	DIGOXIN	227±32	227±28	247±26	258±31
Na	PLACEBO	19±5	14±4	15±3	17±3
mmol	DIGOXIN	19±4	18±3*	19±2*	20±2*
K	PLACEBO	6.7±0.9	6.2±0.6	6.1±0.7	6.1±0.7
mmol	DIGOXIN	5.7±0.9	6.2±0.8	6.5±0.9	6.4±0.6
Urea	PLACEBO	20±3	16±2	16±2	16±1
mmol	DIGOXIN	17±1	16±2	14±2	16±1
Cr	PLACEBO	0.56±0.07	0.43±0.05	0.40±0.04	0.42±0.02
mmol	DIGOXIN	0.43±0.03	0.41±0.04	0.41±0.04	0.43±0.02
Mg	PLACEBO	0.39±0.05	0.31±0.03	0.31±0.04	0.29±0.02
mmol	DIGOXIN	0.36±0.05	0.34±0.05	0.33±0.04	0.30±0.03
Cl	PLACEBO	17±3	13±4	14±2	14±2
mmol	DIGOXIN	20±4	18±4*	19±3*	18±2*
Phos	PLACEBO	1.10±0.23	0.91±0.12	1.06±0.07	1.13±0.07
mmol	DIGOXIN	0.84±0.11	0.92±0.10	1.00±0.13	1.15±0.1
c GMP	PLACEBO	2.86±0.82	2.14±0.62	2.63±0.60	2.10±0.71
(nmol)	DIGOXIN	2.71±0.54	2.36±0.38	2.16±0.46	2.54±0.61
F'MIDE	PLACEBO	10.3±1.6	10.2±1.6	10.5±1.8	10.6±1.8
(mg)	DIGOXIN	10.6±1.5	11.0±1.6	9.6±1.5	10.1±1.6

Fractional excretion of chloride was higher during the 3 hours after digoxin compared with placebo ($p<0.05$) while fractional excretion of sodium showed a trend to increase which did not reach statistical significance. Fractional excretion of potassium, urea, magnesium, phosphate, cyclic GMP and furosemide did not change (Table 6.3, Figure 6.1 and 6.2).

Table 6.3 Fractional excretion of electrolytes, furosemide and cGMP

Changes in excretion of electrolytes (sodium-Na, potassium-K, urea, chloride-Cl, magnesium-Mg and phosphate-Phos), furosemide (F'MIDE) and cGMP /creatinine (Cr). *denotes significance at $p<0.05$.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
Na/Cr	PLACEBO	36±7	35±8	41±8	41±7
mM/mM	DIGOXIN	43±6	45±5	48±5	48±6
K/Cr	PLACEBO	12.4±1.5	14.7±1.2	15.5±1.2	14.5±1.2
mM/mM	DIGOXIN	13.1±1.6	14.9±1.4	15.8±1.3	15.0±1.4
U/Cr	PLACEBO	35±2	38±2	40±2	38±2
mM/mM	DIGOXIN	40±1	40±2	40±1	38±2
Cl/Cr	PLACEBO	33±6	31±7	37±6	35±4
mM/mM	DIGOXIN	45±7	44±6	47±6*	44±6*
Mg/Cr	PLACEBO	0.71±0.07	0.74±0.06	0.79±0.06	0.71±0.04
mM/mM	DIGOXIN	0.83±0.07	0.81±0.07	0.78±0.05	0.69±0.05
Phos/Cr	PLACEBO	1.86±0.26	2.13±0.21	2.78±0.21	2.77±0.18
mM/mM	DIGOXIN	2.0±0.24	2.27±0.19	2.45±0.20	2.65±0.18
F'MIDE/Cr	PLACEBO	4.2±0.6	4.8±0.5	5.2±0.4	5.6±0.9
μg/mM	DIGOXIN	4.9±0.5	5.3±0.6	6.0±0.9	5.9±0.8
cGMP/Cr	PLACEBO	5.2±1.5	5.1±1.3	6.9±1.8	4.8±1.5
ng/mM	DIGOXIN	6.5±1.4	5.8±0.8	5.2±1.0	6.0±1.6

Figure 6.1 Changes in hourly sodium excretion after placebo and digoxin

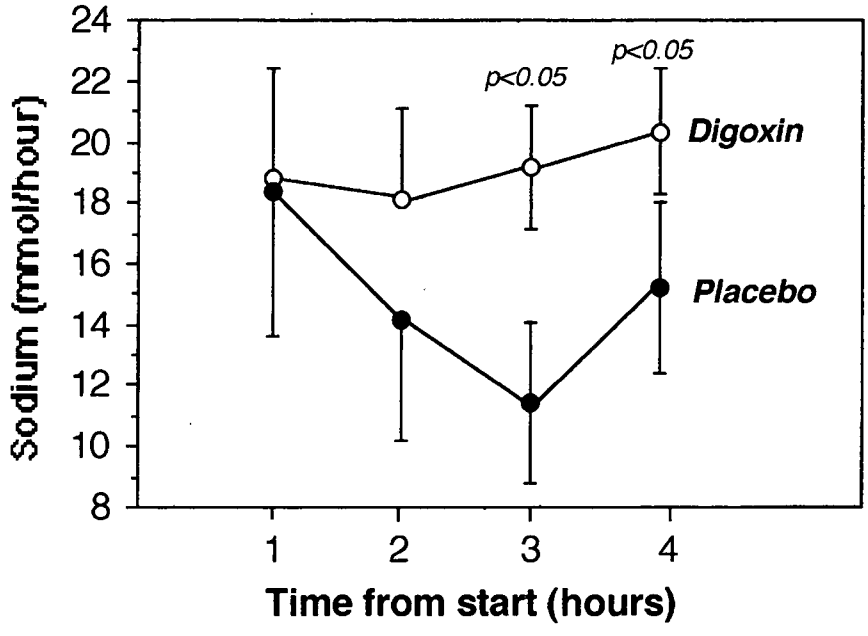
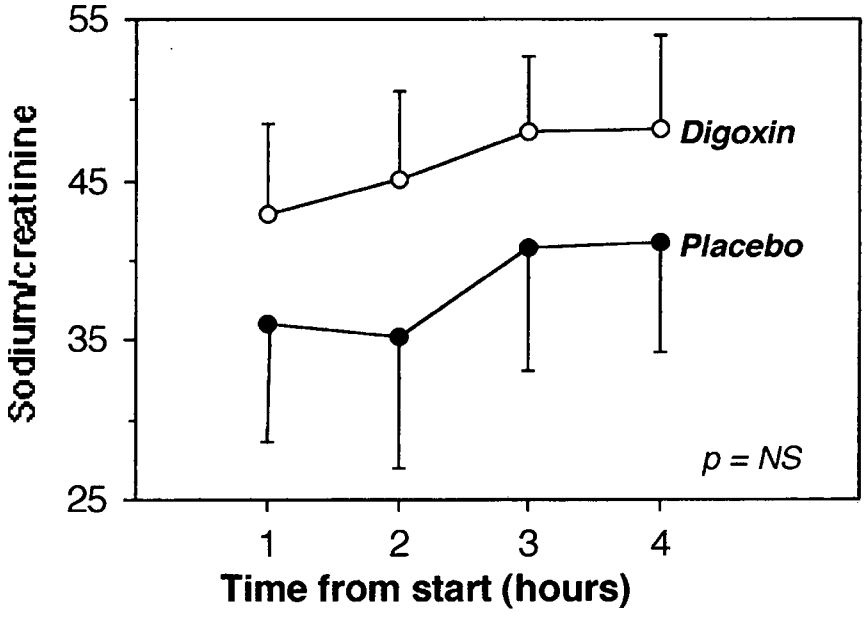


Figure 6.2 Changes in fractional excretion of sodium after placebo and digoxin. Changes were not significant(NS)



Although fractional excretion rose after digoxin, baseline levels were higher than placebo and the change from baseline was not significantly different after the 2 interventions.

Plasma electrolyte concentrations did not differ significantly.

Table 6.4 Plasma electrolyte concentrations

Changes in plasma concentrations of sodium-Na, potassium-K, urea, creatinine-Creat, chloride-Cl, magnesium-Mg and phosphate-Phos one, two and three hours from the start of the study.

		1 HOUR	2 HOURS	3 HOURS
Na	PLACEBO	139±1	138±1	139±1
mmol/l	DIGOXIN	138±1	138±1	138±1
K	PLACEBO	4.1±0.2	4.1±0.1	4.1±0.1
mmol/l	DIGOXIN	3.8±0.1	4.0±0.1	3.8±0.1
Urea	PLACEBO	8.2±0.6	8.1±0.6	7.9±0.6
mmol/l	DIGOXIN	7.9±0.5	7.7±0.5	7.5±0.5
Creat	PLACEBO	113±9	112±9	110±9
μm/l	DIGOXIN	109±10	105±10	106±9
Cl	PLACEBO	100±1	100±1	100±1
mmol/l	DIGOXIN	101±1	100±1	99±1
Mg	PLACEBO	0.84±0.04	0.83±0.03	0.83±0.03
mmol/l	DIGOXIN	0.80±0.03	0.80±0.04	0.77±0.04
Phos	PLACEBO	1.02±0.08	1.05±0.06	1.10±0.06
mmol/l	DIGOXIN	0.92±0.04	0.97±0.04	1.01±0.03

Plasma hormone concentrations

Plasma concentrations of ANP, catecholamines and plasma renin did not change after digoxin infusion with respect to placebo (table 6.5).

Table 6.5 Plasma hormone concentrations

Changes in plasma concentrations of ANP, plasma renin, noradrenaline (N ADREN) and adrenaline (ADREN).

		1 HR	2 HRS	3 HRS
ANP (pg/ml)	PLACEBO	105±44	107±48	106±33
	DIGOXIN	100±48	111±38	98±38
Plasma renin (μU/ml)	PLACEBO	240±107	174±69	173±65
	DIGOXIN	225±100	153±64	143±58
N ADREN (nmol/l)	PLACEBO	4.7±0.9	4.5±0.9	4.0±0.6
	DIGOXIN	5.1±1.1	4.3±0.7	3.9±0.7
ADREN (nmol/l)	PLACEBO	0.30±0.09	0.23±0.07	0.25±0.08
	DIGOXIN	0.32±0.09	0.25±0.05	0.26±0.06

Haemodynamic variables

Heart rate decreased during the 3 hours after digoxin compared with placebo but arterial pressure was unchanged. ERPF increased during the 3 hours after digoxin compared to placebo. Creatinine clearance did not change (table 6.6, figure 6.3).

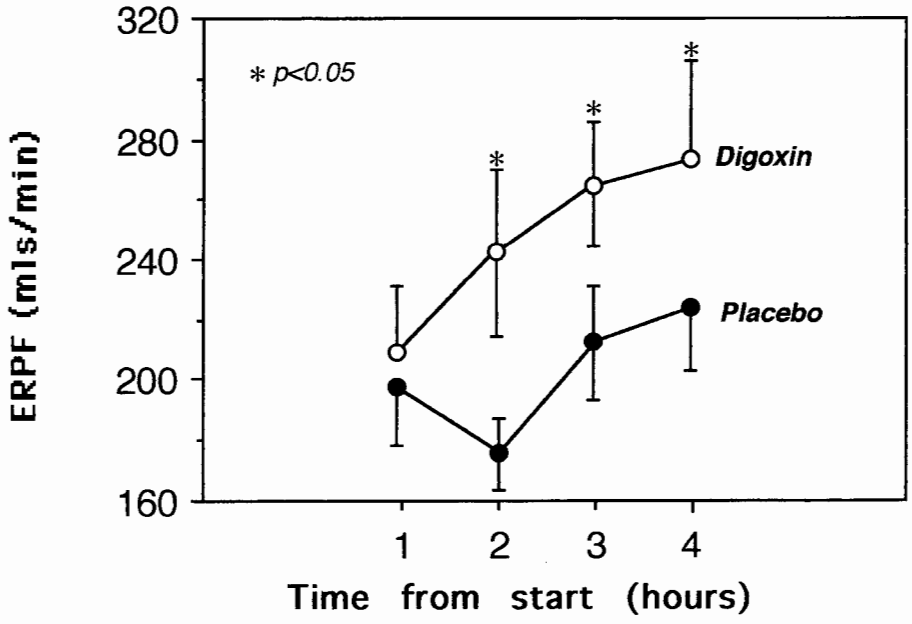
Table 6.6 Haemodynamic variables

Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), GFR and ERPF after digoxin and placebo.

* denotes p< at least 0.05.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
SBP	PLACEBO	111±5	113±3	112±5	111±4
mmHg	DIGOXIN	112±4	115±5	115±4	118±5
DBP	PLACEBO	73±3	74±3	74±2	74±2
mmHg	DIGOXIN	73±3	77±3	74±3	75±3
MAP	PLACEBO	86±3	88±2	88±3	87±3
mmHg	DIGOXIN	87±3	90±3	89±3	89±4
HR	PLACEBO	73±4	71±4	70±4	71±3
bpm	DIGOXIN	74±4	72±4*	67±3*	69±4*
GFR	PLACEBO	86±12	69±8	65±10	66±6
ml/min	DIGOXIN	71±8	70±8	68±7	73±6
ERPF	PLACEBO	198±19	175±12	212±19	224±21
ml/min	DIGOXIN	209±22	242±28*	265±21*	273±32*

Figure 6.3 Changes in ERPF after placebo and digoxin



6.4 Discussion

Bolus administration of digoxin enhanced the natriuresis but not the diuresis induced by furosemide in patients with moderately severe heart failure in sinus rhythm.

Previous authors have described an increase in urine volume in patients with heart failure after oral administration of digoxin or the need for increased diuretic therapy when digoxin has been withdrawn, both in patients in atrial fibrillation and in sinus rhythm (Withering 1785; Gavey & Parkinson 1939; Farber, Alexander, Pellegrino, et al 1961; Hull & Mackintosh 1977) although other authors have shown no significant diuretic effect in the setting of sinus rhythm (Starr & Luchi 1969). Packer, Gheorghiade, Young, et al (1993) showed that 20% of patients with left ventricular systolic dysfunction and an ejection fraction of less than 35% deteriorated when digoxin was withdrawn. The patient population (placebo N=93, digoxin N=85) were all in sinus rhythm and were stable on digoxin, diuretics and ACE inhibitors. All had the dose of digoxin adjusted to achieve plasma concentrations of 0.9-2ng/ml, resulting in a mean digoxin dose of 0.38mg, slightly higher than the dose of digoxin used in other studies. 11 patients in the placebo group required an increase in their diuretic dose compared with 4 in the digoxin group over a 12 week follow-up period. Lloyd, Sandberg and Edwards (1992) demonstrated that ouabain, a naturally ocurring cardiac glycoside, has diuretic and natriuretic actions in a dog model of heart failure.

The use of powerful diuretics which produce an intense anti-diuresis, may have obscured the modest diuretic properties of digoxin when given in the post-diuretic phase. Rader, Smith, Berger, et al (1966) found no further

diuresis when digoxin was added to full diuretic therapy. Another possibility is that the diuretic properties of repeated oral doses of digoxin may be greater than that of a single intravenous bolus.

There are several mechanisms by which digoxin could produce a natriuresis.

Local renal effects: Inhibition of renal Na^+-K^+-ATPase produces a diuresis and natriuresis in experimental models of heart failure by inhibiting fluid reabsorption in the proximal tubule (Hyman, Jaques, Hyman 1956) although Luft, Aronoff, Fineberg, et al (1989) failed to demonstrate natriuresis when infusing digoxin in normal volunteers. Ouabain is synthesised in the human body, is elevated in heart failure and hypertension, and has been proposed as an endogenous natriuretic factor (Bova, Blaustein, Ludens, et al 1991; Harris, Clark, Fisher, et al 1991; Mathews, Ducharme, Hamlyn, et al 1991; Blaustein 1993).

Cardiotonic effects: Digoxin exerts cardiac effects within 15 minutes of injection (Powell, Horowitz, Hasin, et al 1990). The effects of digoxin on cardiac output were not measured in the present study but heart rate fell within the first hour, and ERPF rose, possibly reflecting a cardiotonic effect on myocardial cells and a rise in cardiac output (Mason, Braunwald 1964; Heistad & Abboud 1980; Eisner & Smith 1992).

Vasoconstrictor effects and pressure natriuresis: Blaustein and Hamlyn (1991) suggest that ouabain is responsible, at least in part, for the hypertension in low-renin hypertension. They proposes that the primary problem of inability to excrete a sodium load results in ouabain secretion

and hypertension. The rise in blood pressure allows normal plasma volume to be maintained by causing pressure natriuresis. Ouabain concentrations are also elevated in heart failure and may be important in maintaining blood pressure. No change in blood pressure was detectable after digoxin infusion in the present study, but changes in systemic pressure might have been observed had a more prolonged infusion been administered. Despite the rise in ERPF in the present study, urinary creatinine excretion did not increase. SNP increases renal blood flow but fails to cause a diuresis. However digoxin does not reduce renal perfusion pressure or GFR and this may enhance diuresis.

Neuro-endocrine effects: Cardiopulmonary reflexes are impaired in heart failure (Eckberg, Drabinski, Braunwald 1971; Goldstein, Beiser, Stampfer, et al 1975; Bristow, Ginsburg, Minobe, et al 1982; Brodde 1991; 1991), with reduced sensitivity of atrial stretch receptors, leading to impairment of inhibitory influences on renin release, sympathetic activity and anti-diuretic hormone secretion (Francis 1985a). Other studies have indicated that acute administration of digoxin inhibits renin secretion either directly or mediated through changes in cardiac output or antagonism of sympathetic activity (Ribner, Plucinski, Hsieh, et al 1985; Covit, Schaer 1983). Similarly, digitalis glycosides exert direct sympatho-inhibitory effects in heart failure (Ribner, Plucinski, Hsieh, et al 1985; Alicandri, Fariello, Boni, et al 1987; Ferguson, Berg, Sanders, et al 1989; Gheorghiade, Hall, Lakier, et al 1989) possibly by restoring cardiac and aortic baroreceptor sensitivity and thus reducing the outflow of sympathetic impulses from the central nervous system and minimising their sodium retaining effects. Digoxin also suppreses renin release by virtue of its inhibitory action on the renal tubular

Na^+-K^+-ATPase (Churchill 1979; Covit, Schaer, Sealey, et al 1983; Ribner, Plucinski, Hsieh, et al 1985).

Yamamoto, Shouji, Kimura, et al (1988) reported that high dose ouabain increases circulating ANP which could in theory result in enhanced salt and water excretion. Although plasma concentrations of renin and noradrenaline fell after digoxin administration, similar falls, presumably the result of prolonged supine rest, were observed after placebo. ANP did not fall in either group. The failure of ANP to increase in-vivo may reflect a fall in atrial pressure balancing any stimulus to increased secretion.

Furosemide delivery: Digoxin could theoretically enhance delivery of furosemide to the renal tubules but this mechanism for natriuresis is unlikely to have been responsible in the present study as an increase in urinary furosemide excretion was not observed.

Limitations of the study

As with the previous studies, the major limitation was the size of the study sample. Reliable monitoring of changes in cardiac output or ejection fraction were not available, thus it was only possible to speculate on the haemodynamic response to digoxin infusion in the patients. Most invasive studies describing positive haemodynamic effects of digoxin infusion have been performed on patients with decompensated heart failure and the literature suggests that stable, well diuresed patients may not demonstrate improvement in stroke volume or ejection fraction with intravenous digoxin administration (Ribner, Plucinski, Hsieh, et al 1985; Gheorghiade, Hall, Lakier, et al 1989). The dose of digoxin infused may not have been adequate as other authors have used bolus doses of 1mg (Ribner, Plucinski,

Hsieh, et al 1985; Gheorghiade, Hall, Lakier, et al 1989) and 1-1.5 mg (Farber, Alexander, Pellegrino, et al 1961). Measurement of creatinine clearance as an index of GFR would have tended to overestimate GFR but changes in GFR were being assessed rather than absolute values so this was unlikely to have introduced significant error.

Conclusion

Digoxin modestly enhanced furosemide induced natriuresis but not diuresis. The present study suggests that the likely cause of the increase in sodium output was either from a direct effect on renal Na-K-ATPase or through an improvement in haemodynamics. This natriuretic effect may contribute to the beneficial effects of digoxin in heart failure.

Chapter 7 The effect of infusion of ANP on the renal response to furosemide

7.1 Introduction

ANP is one of a group of naturally occurring peptides with powerful diuretic, natriuretic and haemodynamic properties in animals, normal volunteers and hypertensive patients. Since the description by De Bold, Borenstein, Veress, et al (1981) of the diuretic and natriuretic properties of extracts of atrial tissue injected into rats and the subsequent identification and synthesis of ANP, a considerable volume of literature exists on the action of ANP in health and disease. ANP is a cardiac hormone that in health is secreted primarily by atrial myocytes in response to atrial stretch. Secretion may result in a fall in arterial pressure (De Bold, Borenstein, Veress, et al 1981; Maack, Marion, Camargo, et al 1984), augmentation of salt and water excretion (Camargo, Kleinert, Atlas, et al 1984; Maack, Marion, Camargo, et al 1984), facilitation of transudation of plasma water to the interstitium (Cody, Atlas, Laragh, et al 1986a) and inhibitory effects on the release or actions of a variety of hormones, including aldosterone, plasma renin, A-II, (Goetz 1988; Donckier, De Coste, Vanoverschelde, et al 1991) and AVP (Maack, Marion, Camargo, et al 1984; Sampson 1985; Cody, Atlas, Laragh, et al 1986a). The overall effects of ANP result in both acute and chronic reduction in systemic blood pressure and intravascular volume. The beneficial renal actions are mediated by cGMP, the second messenger for ANP (Appel, Dunn 1987; Brenner, Ballerman, Gunning, et al 1990; Wilkins, Settle, Needleman 1990).

Plasma concentrations of ANP are elevated in a number of pathological conditions characterised by volume overload or increased right atrial wall

tension such as renal failure, malignant hypertension and heart failure (Tikkanen, Fyhrquist, Metsarinne, et al 1985; Raine, Erne, Burgisser, et al 1986; Takemura, Fujiwara, Mukoyama, et al 1991). Not only do patients with heart failure demonstrate inappropriate salt and water retention in the face of significantly elevated plasma concentrations of ANP, but they also demonstrate a blunted renal response to infusion of ANP (Moe, Canepa-Anson, Armstrong 1992; Cody, Atlas, Laragh, et al 1986a). However beneficial changes in pulmonary wedge pressure, left atrial pressure and systemic vascular resistance still occur (Cody, Atlas, Laragh, et al 1986a).

In animal models of heart failure, sympathetic inhibition, either by sectioning the vagus nerve or by administration of a central α-adrenergic antagonist such as clonidine is able to restore renal responsiveness to ANP (Feng, Hedner, Hedner, et al 1990). Recent work suggests that the responsiveness to ANP can likewise be partially restored in patients and in animal models of heart failure when plasma concentrations of ANP are increased by inhibition of neutral endopeptidase, the enzyme responsible for degradation of ANP (Cavero, Margulies, Winaver, et al 1990; Trippodo, Gabel, Harvey, et al 1991; Good, Peters, Wilkins, et al 1995; Münzel, Kurz, Holtz, et al 1992). The interaction of ANP and furosemide is less well understood. 8 patients with stable heart failure on chronic diuretic therapy were studied to determine whether ANP had any influence on the natriuresis and diuresis induced by furosemide.

7.2 Patients and methods

Eight patients with chronic heart failure aged 63-69 years (mean 66 years) were studied on two occasions, not less than 48 hours apart, having adhered to a fixed salt intake for 3 days prior to each study, as set out in

Chapter 2. A constant, steady diuresis was maintained by hourly administration of furosemide and replacement of urinary and insensible losses with oral water. Mean daily furosemide dose was 90mg and intravenous dose for the 4 hour period of study was 12-20mg, mean 17mg. During the 2nd hour of study, ANP (0.01μg/kg/min) or normal saline was infused. During the 3rd hour, the dose of ANP was increased in incremental fashion at 5 minute intervals from 0.05μg/kg/min to 0.1μg/kg/min to 0.15μg/kg/min and maintained at this rate for the duration of the 3rd hour.

The 1st and 4th hour periods acted as internal control periods. Urine was collected at the end of each hour and retained for estimation of cGMP, furosemide, electrolytes and PAH. Plasma samples were taken at the end of the 1st, 2nd and 3rd hours for estimation of electrolytes, ANP, catecholamines and plasma renin in addition to PAH. Blood pressure and heart rate were measured at 15 minute intervals. At the end of the study, patients were examined and discharged.

Table 7.1 Patient characteristics

Table showing individual patients' age, sex, New York Heart Association classification (NYHA), daily diuretic requirements in milligrammes (furosemide-F, bumetanide-BUMET), type of ACE-inhibitor and daily dosage (captopril-CAP, enalapril-ENAL) and aetiology of heart failure (ischaemic heart disease-IHD).

PATIENT	AGE	SEX	NYHA	DIURETIC	ACE-I (mg)	AETIOLOGY
1	63	M	IIB	F 40	CAP 37.5	IHD
2	68	M	IIB	F 40	CAP 12.5	IHD
3	63	M	IIB	F 80	CAP 37.5	IHD
4	68	M	IIB	F 120	CAP 75	IHD
5	66	M	III	F 40	-	IHD
6	74	F	III	F 40	ENAL 5	IHD
7	69	M	III	BUMET 4	ENAL 10	IHD
8	65	M	III	F 120	-	IHD

7.3 Results

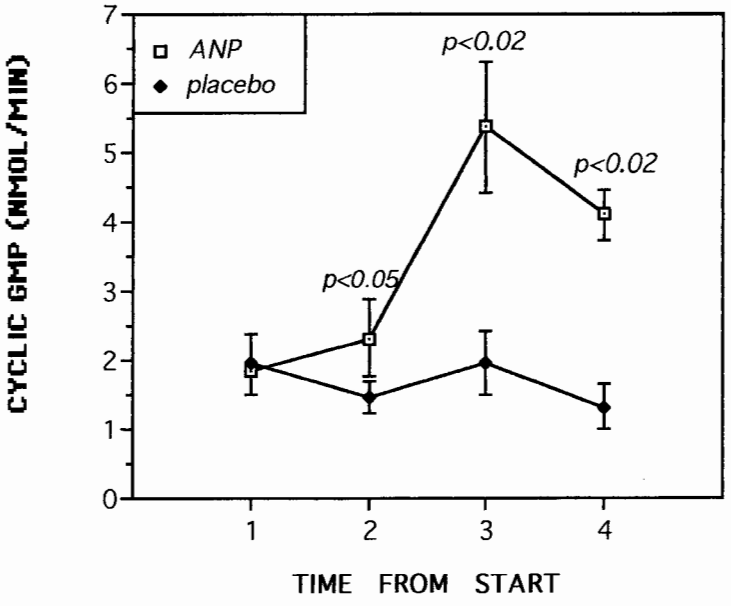
All results are expressed as the mean and one standard error of the mean. Urine volume showed a trend towards increasing during high dose infusion (not significant) and electrolyte excretion did not change significantly. cGMP rose after low and high dose ANP infusion with effects persisting 1 hour after discontinuation. Furosemide excretion did not change (table 7.2, figure 7.1).

Table 7.2 Hourly changes in urine volume and excretion of electrolytes, furosemide and cGMP

Changes in hourly urine volume (U.Vol), sodium (Na), potassium (K), urea, creatinine (Creat), chloride (Cl), magnesium (Mg), phosphate (Phos), cGMP and furosemide (F'MIDE). * denotes significance at p< at least 0.05.

		0-1 hrs	1-2 hrs	2-3 hrs	3-4 hrs
U.Vol	Placebo	231±26	204±17	234±16	238±17
mls	ANP	238±37	241±30	288±47	252±36
Na	Placebo	20.5±3.6	14.2±1.7	17.4±2.1	15.1±1.3
mM	ANP	18.1±3	14.0±2	17.7±2	18.1±2
K	Placebo	5.5±1.0	4.5±0.8	5.3±0.9	4.5±0.9
mM	ANP	5.0±0.7	4.7±0.8	4.9±0.7	4.4±0.8
Urea	Placebo	20.7±3.0	15.8±2.2	17.5±1.8	14.7±1.9
mM	ANP	18.1±3.6	16.6±2.9	17.7±2.1	16.6±2.3
Creat	Placebo	0.51±0.08	0.37±0.05	0.41±0.04	0.34±0.05
mM	ANP	0.44±0.05	0.36±0.06	0.40±0.05	0.39±0.05
Cl	Placebo	20.0±3.5	12.9±1.9	16.3±1.4	14.0±1.4
mM	ANP	17.8±4.1	13.8±3.0	15.4±2.5	15.4±2.7
Mg	Placebo	0.40±0.06	0.29±0.05	0.33±0.04	0.27±0.04
mM	ANP	0.36±0.08	0.32±0.07	0.34±0.04	0.35±0.06
Phos	Placebo	1.07±0.23	0.78±0.10	0.95±0.08	0.93±0.09
mM	ANP	0.88±0.15	0.90±0.11	0.99±0.09	1.08±0.11
cGMP	Placebo	2.0±0.4	1.5±0.2	2.0±0.5	1.3±0.3
nmol/min	ANP	1.9±0.4	2.3±0.5*	5.4±0.9*	4.1±0.4*
F'MIDE	Placebo	2075±276	1554±159	1792±277	1914±277
mg/hr	ANP	1609±223	1692±230	1772±378	1934±252

Figure 7.1 Changes in hourly cGMP excretion after placebo and ANP infusion



Fractional excretion of sodium increased during high dose infusion. Fractional excretion of cGMP increased during low and high-dose ANP infusion and for the hour afterwards. Fractional excretion of urea and furosemide did not change (Table 7.3).

Table 7.3 Fractional excretion of electrolytes, furosemide and cGMP

Changes in hourly excretion of sodium (Na), potassium (K), urea (U), chloride (Cl), magnesium (Mg), phosphate (Phos), furosemide (F'MIDE) and cGMP/creatinine (Cr).

* denotes p< at least 0.05.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
Na/Cr	PLACEBO	46±7	44±8	47±9	50±8
mM/mM	ANP	42±7	44±9	51±14*	48±8
K/Cr	PLACEBO	11.1±1.6	11.9±1.2	12.5±1.45	12.4±1.6
mM/mM	ANP	11.5±1.4	12.3±0.9	11.8±1.0	11.1±1.1
U/Cr	PLACEBO	42±3	43±1	43±1	43±1
mM/mM	ANP	45±2	45±1	44±1	43±1
Cl/Cr	PLACEBO	46±9	39±7	44±7	45±5
mM/mM	ANP	41±8	40±7	48±13	41±8
Mg/Cr	PLACEBO	0.87±0.14	0.81±0.10	0.84±0.08	0.82±0.08
mM/mM	ANP	0.90±0.16	0.62±0.12	0.91±0.11	0.92±0.09
Phos/Cr	PLACEBO	2.08±0.24	2.20±0.22	2.38±0.13	2.88±0.24
mM/mM	ANP	2.15±0.20	3.07±0.60	2.69±0.25	3.03±0.29
F'MIDE/Cr	PLACEBO	22±5	24±5	19±2	27±5
μg/mM	ANP	18±5	27±8	23±8	27±7
cGMP/Cr	PLACEBO	4.3±1.1	4.5±0.9	4.8±1.1	4.4±1.4
ng/mM	ANP	4.6±1.0	7.7±1.9*	14.2±1.7*	12.2±2.1*

Plasma electrolyte concentrations did not change (table 7.4)

Table 7.4 Plasma electrolyte concentrations

Changes in plasma concentrations of sodium (Na), potassium (K), urea, creatinine (Creat), chloride (Cl), magnesium (Mg) and phosphate (Phos).

		1 HOUR	2 HOURS	3 HOURS
Na	PLACEBO	140±1	140±1	140±1
mmol/l	ANP	138±1	138±1	139±1
K	PLACEBO	3.9±0.2	3.9±0.2	4.0±0.2
mmol/l	ANP	3.6±0.2	3.5±0.2	3.6±0.2
Urea	PLACEBO	7.2±0.5	7.0±0.6	6.9±0.6
mmol/l	ANP	8.2±1.4	8.4±1.4	8.2±1.4
Creat	PLACEBO	93±3	92±3	91±3
μmol/l	ANP	101±8	98±8	99±7
Cl	PLACEBO	103±2	103±2	103±1
mmol/l	ANP	104±1	104±1	105±1
Mg	PLACEBO	0.82±0.04	0.83±0.03	0.82±0.03
mmol/l	ANP	0.86±0.02	0.84±0.02	0.81±0.01
Phos	PLACEBO	0.87±0.1	0.89±0.08	0.94±0.07
mmol/l	ANP	0.83±0.06	0.89±0.05	0.93±0.04

Plasma hormone concentrations

ANP levels increased from 70±22 to 157±35pg/ml, $p<0.02$ (mean±one standard error) after low dose infusion and from 91±29 to 225±31pg/ml, $p<0.02$, (figure 7.2) after high dose infusion (normal range <50pg/ml). Plasma renin rose from 84±27 to 150±61μU/ml, $p<0.05$ after high dose ANP infusion compared with placebo (figure 7.3).

Plasma catecholamine concentrations did not change (table 7.5).

Table 7.5 Hormonal data during infusion of placebo and ANP

Changes in plasma concentrations of atrial natriuretic peptide (ANP), plasma renin (PARC), noradrenaline (NA) and adrenaline (ADREN). * denotes significance at p< at least 0.05.

		1 HOUR	2 HOURS	3 HOURS
ANP	PLACEBO	63±18	70±22	91±29
pg/ml	ANP	56±12	157±35*	225±31*
PARC	PLACEBO	119±44	86±25	84±27
μU/ml	ANP	107±40	99±36	150±61*
NA	PLACEBO	4.3±0.8	3.9±0.7	4.1±0.6
nmol/l	ANP	4.6±0.8	3.7±0.7	5.3±1.3
ADREN	PLACEBO	0.2±0.07	0.2±0.06	0.3±0.07
nmol/l	ANP	0.3±0.08	0.3±0.09	0.3±0.1

Figure 7.2 Changes in plasma concentrations of ANP during infusion of placebo and ANP

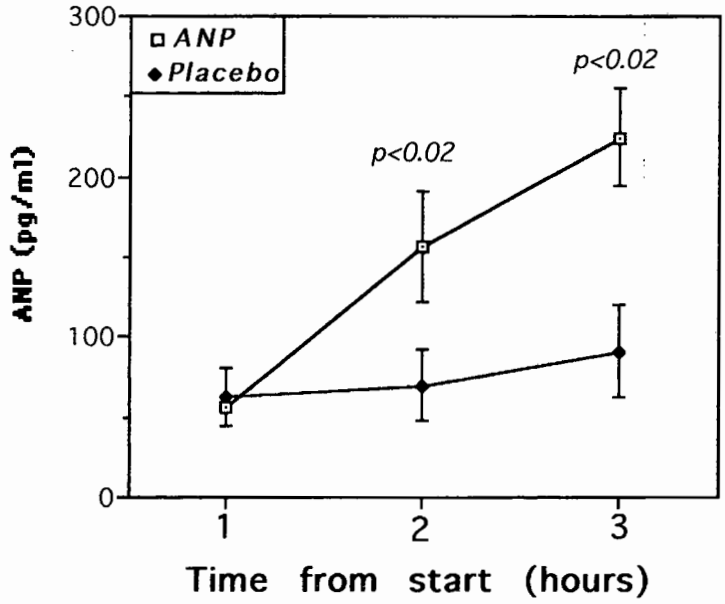
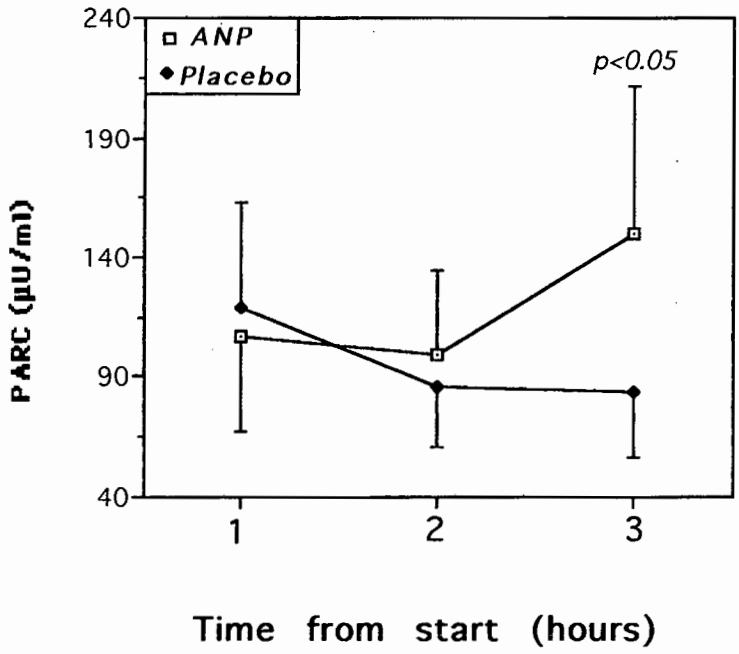


Figure 7.3 Changes in PARC during infusion of placebo and ANP



Haemodynamic variables

Heart rate was unchanged despite a fall in systolic blood pressure, diastolic pressure and mean arterial pressure during high dose infusion after placebo and ANP respectively. The hypotensive effect persisted for a further hour after the infusion was terminated (table 7.6, figure 7.4).

ERPF increased (225±35 to 254±31, p=0.036) during low dose ANP infusion but not during high dose (figure 7.5).

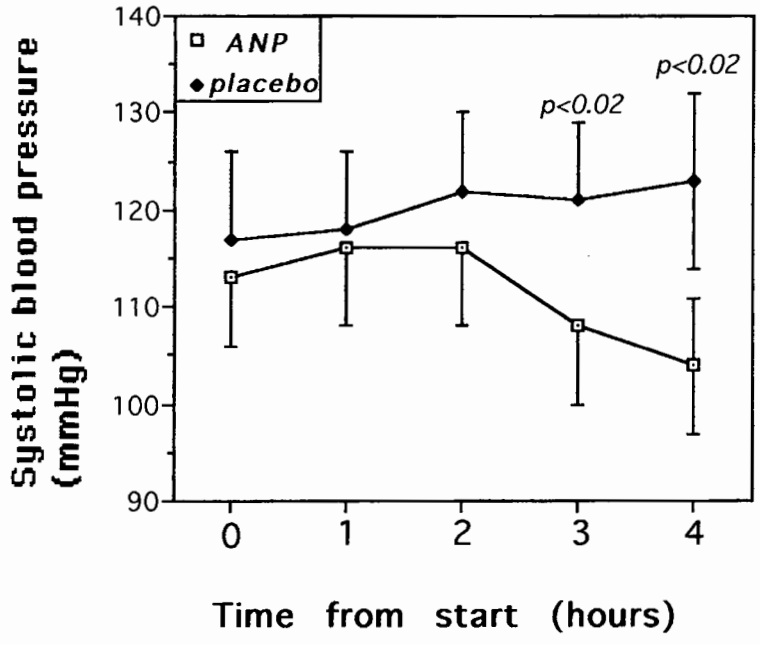
Creatinine clearance did not change significantly after ANP infusion (table 7.6).

Table 7.6 Haemodynamic variables during infusion of placebo and ANP

The table shows hourly changes in heart rate (HR), systolic and diastolic blood pressure (SBP, DBP), ERPF and creatinine clearance (CREAT C). * denotes significance at p< at least 0.05

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
HR	PLACEBO	69±4	70±5	69±5	69±5
	ANP	68±4	68±4	69±3	70±4
SBP	PLACEBO	118±8	122±8	121±8	123±9
(MM HG)	ANP	116±8	116±8	108±8*	104±7*
DBP	PLACEBO	74±3	76±4	76±3	79±4
(MM HG)	ANP	75±2	77±4	69±4*	66±3*
ERPF	PLACEBO	239±31	225±35	234±27	244±28
(MLS/MIN)	ANP	233±35	254±31*	226±25	259±32
CREAT C	PLACEBO	89±12	67±9	76±8	63±8
(MLS/MIN)	ANP	71±5	63±8	64±6	66±8

Figure 7.4 Changes in systolic and diastolic blood pressure during infusion of placebo and ANP



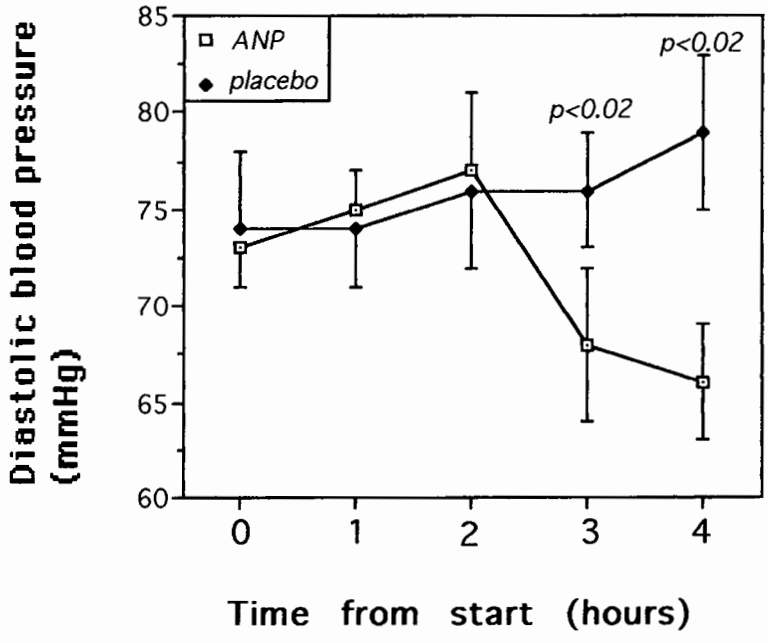
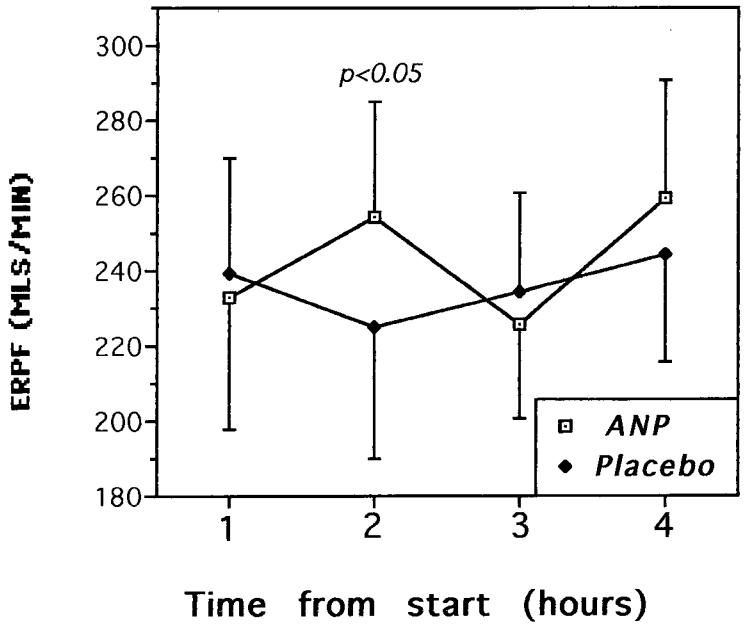


Figure 7.5 Changes in ERPF during infusion of placebo and ANP



7.4 Discussion

Exogenous ANP administration did not augment the diuretic response to furosemide. This finding was not unexpected as the response to endogenous or exogenous ANP administration is blunted in heart failure (Cody, Atlas, Laragh, et al 1986a; Molina, Fowler, McCrory, et al 1988). ANP infusion does however usually result in a fall in elevated systemic vascular resistance, pulmonary capillary wedge pressure and right atrial pressure (Cody, Atlas, Laragh, et al 1986a). Changes in cardiac output and stroke volume are more variable (Molina, Fowler, McCrory, et al 1988). A fall in blood pressure and in cardiac output has been demonstrated in animal models (Bie, Wang, Leadley, et al 1988; Ota, Kimura, Masura, et al 1992) during ANP infusion and could partly explain the failure of ANP infusion to augment a furosemide induced diuresis in the present study. A more probable explanation for the fall in blood pressure produced by the infusion of ANP is that of alteration of vascular resistance, redistribution of the intravascular volume to the extravascular space and decreased venous return or venous pooling, resulting in a fall in cardiac output (Cody, Atlas, Laragh, et al 1986a, Pegram, Trippodo, Natsume, et al 1986).

Cody, Atlas, Laragh, et al (1986a) has shown that ANP infusion in normal subjects results in suppression of the renin-angiotensin system and antagonism of the sympathetic nervous system. Molina, Fowler, McCrory, et al (1988) has reported suppression of aldosterone and noradrenaline in patients with heart failure after ANP infusion while Moe, Canepa-Anson and Armstrong (1992) showed no change in plasma renin activity or noradrenaline concentrations but a fall in aldosterone. Suppression of plasma renin activity was not seen in studies by Cody, Atlas, Laragh, et al (1986a); Riegger, Kromer and Kochsiek (1986a) and Saito, Ogihara,

Nakamaru, et al (1987). Aldosterone and A-II were not measured in the present study but plasma renin rose significantly after ANP infusion compared to placebo, without changes occurring in adrenaline or noradrenaline concentrations. There is in-vitro evidence for direct inhibition of renin release by ANP (Hiruma, Ikemoto, Yamamoto 1986) although the findings of Volpe, Odell, Kleinert, et al (1984 & 1985) and Sosa, Volpe, Marion, et al (1985) suggested that the effects of ANP on renin release are secondary to renal haemodynamic changes. It is also possible that the inhibitory effect of ANP on renin release is overridden by renal sympathetic nerve stimulation. There was improvement in ERPF during low dose ANP infusion in the present study, but this was not sustained during the high dose infusion, possibly due to the associated hypotension, although creatinine clearance did not fall. It is likely that the fall in blood pressure caused active renin concentrations to rise. In addition, most of these patients were taking long-acting ACE inhibitors which could interfere with basal renin production, although this does not explain the differences in plasma renin noted after ANP compared with placebo, as patients were their own controls.

ANP infusion did not affect the furosemide induced diuresis or natriuresis, despite a significant fall in arterial pressure during high dose ANP infusion which persisted for 1 hour after the infusion was stopped. ANP clearance is prolonged in older patients and those with heart failure (Ruskoaho 1992) and similar prolonged hypotension with ANP has been described in heart failure, without compensatory tachycardia (Molina, Fowler, McCrory, et al 1988). Feng, Hedner, Hedner, et al (1990) showed that ANP-induced diuresis can be restored in a rat model of heart failure by simultaneous infusion of clonidine, despite further lowering of arterial pressure, suggesting that presynaptic noradrenaline release plays a part in the blunted response.

There was no significant change in excretion of any of the urinary electrolytes measured. Recent work has shown that urinary excretion of sodium and chloride in response to BNP infusion was higher in heart failure patients than in control subjects, unlike the renal effects of ANP (Yoshimura, Yasue, Morita, et al 1991). This could be one of the mechanisms for improved diuresis and natriuresis observed with neutral endopeptidase (EC 24.11) inhibitors, which cause elevation of brain natriuretic peptide in addition to ANP (Vogt-Schaden, Gagelmann, Hock, et al 1989; Bourne, Kenny 1990; Vanneste, Pauwels, Lambotte, et al 1990; Lang, Motwani, Coutie, et al 1991).

ERPF increased only during low dose infusion of ANP, perhaps because blood pressure was maintained at low dose, but fell during high dose. Eiskjaer, Bagger, Danielsen, et al (1991) found a correlation between relative increase in natriuresis and renal plasma flow after ANP infusion in patients with heart failure, while Molina, Fowler, McCrory, et al (1988) demonstrated an insignificant rise in ERPF with higher doses of ANP but furosemide was not administered concurrently. Molina, Fowler, McCrory, et al (1988) also showed that ANP infusion increased GFR, although Awazu, Imada, Kon, et al (1989) found no significant change in GFR after anti-ANP antibody administration in rats with acute myocardial infarction, despite demonstrating anti-natriuresis. GFR failed to rise after administration of a neutral endopeptidase inhibitor in dogs with heart failure (Cavero, Margulies, Winaver, et al 1990). In Molina's study patients, striking beneficial haemodynamic responses were recorded, which may not have been the case in the well diuresed, stable patients in the present study.

Urinary cGMP increased significantly during ANP infusion in the present

study but neither diuresis nor natriuresis were potentiated. Moe, Canepa-Anson and Armstrong (1992), looking at the cGMP response to ANP in patients with heart failure and controls, found that plasma and urinary cGMP excretion was higher at baseline in heart failure compared with the normal controls; however after ANP infusion, proportional increases in plasma cGMP and cGMP excretion were less in patients than in controls. Thus the renal cGMP response was attenuated. This may have reflected the blunted augmentation of plasma cGMP by ANP infusion due to ANP receptor down-regulation or dissociation or a more generalised problem with attenuated cGMP generation. Molina, Fowler, McCrory, et al (1988) demonstrated doubling of urinary cGMP excretion after ANP infusion in their patients with severe congestive heart failure and an associated blunted diuretic response. Urinary excretion of cGMP did not to correlate with the degree of diuresis or natriuresis induced by candoxatrilat (a neutral endopeptidase inhibitor) in patients with heart failure (Good, Peters, Wilkins, et al 1995).

There are many possible causes for the reduced renal response to exogenous ANP in heart failure patients.

Arterial perfusion pressure: Firth, Raine and Ledingham (1988) demonstrated in isolated perfused rat kidneys that the response to infused ANP varies in response to changes in arterial perfusion pressure, with no natriuretic effect at lower perfusion pressures. This mechanism may also apply in heart failure, if the predominant nephron site of action of ANP is the collecting tubule. Decreased renal perfusion results in decreased delivery of sodium to the collecting tubule and thus little opportunity for ANP to influence sodium reabsorption at this site (Seifter, Brenner 1989) however furosemide should have corrected this.

Limitations of the study

The limitations noted in the previous chapters are applicable in the present study as the design, execution and analysis of the studies was similar.

Conclusion

ANP infusion did not augment the renal response to furosemide in patients with heart failure. The significant hypotensive effect of high dose ANP infusion was probably partly responsible, although competition from activation of the sympathetic nervous system, arginine vasopressin and the renin-angiotensin system may also have played a part.

Antagonism by noradrenaline: McMurray, Seidelin, Brown, et al (1989) showed that low dose noradrenaline was antinatriuretic in normal volunteers without inducing either changes in GFR or systemic blood pressure; the natriuretic effects of ANP were also antagonised by noradrenaline. Feng, Hedner, Hedner, et al (1990) showed that α 2-adrenergic agonism with clonidine reversed the blunted renal response to ANP infusion in a rat model of heart failure. Although an additional rise in noradrenaline levels was not demonstrated during ANP infusion in the present study, basal concentrations were elevated and could theoretically have impaired the natriuretic effects of ANP.

Neurohumoral activation by furosemide: Most neurohumoral systems which operate to retain salt and water in heart failure are further stimulated by furosemide, including the sympathetic nervous system, the RAAS (Anand, Kalra, Harris, et al 1991; Cleland, Gillen, Dargie 1988) and AVP (Yamane 1968). Further neurohumoral activation could prevent any augmentation of the renal effects of furosemide by ANP.

ANP receptor: The attenuated response to ANP may include attenuation of the activity of guanylate cyclase by other peptides, abnormalities in guanylate cyclase activation after binding of ANP or may be secondary to ANP receptor abnormalities (Molina, Fowler, McCrory, et al 1988). Antibody studies suggest that ANP is still active in heart failure as when circulating ANP is blocked by antibody administration, clinical deterioration occurs, in animal models (Awazu, Imada, Kon, et al 1989; Shepperson, Barclay, Bennett, et al 1991).

Chapter 8 The effect of infusion of tyramine and phentolamine on a furosemide induced diuresis

8.1 Introduction

Patients with heart failure have evidence of α and β-adrenergic activation with down-regulation of cardiac β-adrenoceptors and reduced sensitivity to endogenous or exogenously administered catecholamines (Chidsey, Braunwald 1966; Levine, Francis, Goldsmith 1982; Bristow, Ginsburg, Minobe et al 1982; Harden 1983; Bristow, Ginsburg, Umans, et al 1986; Brodde, Schuler, Kretsch, et al 1986; Bristow, Port, Hershberger, et al 1989). In addition, elevated concentrations of plasma catecholamines correlate inversely with prognosis in heart failure (Cohn, Levine, Olivari, et al 1984; Rector, Olivari, Levine, et al 1987). Excessive adrenergic drive leads to renal artery constriction with resultant sodium and water retention in animal models (Dell, Sciacca, Lieberman, et al 1973; Katz, Shear 1975), and infusion of phenylephrine or noradrenaline in normal subjects decreases sodium excretion (Lang, Rahman, Balfour, et al 1993a; Lang, Rahman, Balfour, et al 1993b). Conversely, administration of β-adrenergic antagonists to patients with moderate to severe heart failure often causes fluid retention or exacerbation of symptoms (Ikram, Fitzpatrick 1981; Binkley, Lewe, Lima, et al 1986; Shanes 1987) and ganglion blockade with guanethidine results in fluid retention in patients with heart failure (Gaffney, Braunwald 1962; Gill, Mason, Bartter, et al 1965). α– blockade with prazosin and doxazosin causes fluid retention (Bayliss, Noreel, Canepa-Anson, et al 1985; Riegger, Haeske, Kraus, et al 1987b). Lang, Rahman, Balfour, et al (1993a) have shown that infusions of noradrenaline cause antinatriuresis in normal volunteers but paradoxically, direct stimulation of

β1, β2 and α1-adrenergic receptors by dobutamine, a synthetic adrenergic agonist, may increase sodium excretion (Leier, Webel, Bush 1977; Leier, Heban, Huss, et al 1978) and indirect stimulation with tyramine, which mediates its sympathomimetic effects by displacement of nonvesicular noradrenaline from noradrenergic neurons, (Goodman, Gilman 1980) has also been associated with diuresis in normal volunteers (Lang, Rahman, Balfour, et al 1993b).

This study compares the effects of indirect sympathetic stimulation with tyramine with antagonism with phentolamine on diuresis.

8.2 Patients and methods

8 patients with chronic heart failure aged 63-69 years (mean 65 years) on chronic diuretic therapy for control of symptoms of heart failure were studied on 3 occasions, in random single blind fashion with tyramine, phentolamine and placebo. The usual dietary restrictions and study protocol were adhered to as set out in the methods section. Phentolamine and tyramine were infused at low dose during the second hour (1μg/kg/min) with incremental increases to the maximum dose during the third hour (10μg/kg/min). Hourly urine samples were saved for cGMP, PAH, electrolytes and furosemide. Urinary and insensible losses were replaced hourly with water. Plasma samples were drawn at the end of the 1st, 2nd and 3rd hours for analysis of catecholamines, renin, ANP, PAH and electrolytes. Heart rate and blood pressure were recorded at 15 minute intervals using an automated device (Dynamap Criticon).

Table 8.1 Individual patient characteristics

Individual patients' age, sex, New York Heart Association classification (NYHA), daily diuretic requirements (furosemide-F, metolazone-MET), dose of ACE-inhibitor per day (captopril-CAP, enalapril-ENAL, lisinopril-LISIN), aetiology of heart failure (ischaemic heart disease-IHD) and whether patients were taking digoxin.

PATIENT	AGE	SEX	NYHA	DIURETIC (MG)	ACE-I (MG/D)	AETIOLOGY	DIGOXIN
1	63	M	IIB	F 40	CAP 37.5	IHD	
2	68	M	IIB	F 40	CAP 12.5	IHD	
3	63	M	IIB	F 80	CAP 37.5	IHD	
4	68	M	IIB	F 120	CAP 75	IHD	YES
5	66	M	III	F 40	-	IHD	
6	74	F	III	F 40	ENAL 5	IHD	YES
7	62	M	III	F 40, MET 2.5	LISIN 10	IHD	
8	65	M	IIB	F 120	-	IHD	

8.3 Results

Results are expressed as the mean and one standard error of the mean.

Diuresis and natriuresis were not significantly increased by either phentolamine or tyramine.

Chloride excretion was preserved by low but not high dose phentolamine while it tended to fall during placebo and tyramine infusions ($p<0.05$, table 8.2).

There was no significant change in excretion of urea, creatinine, magnesium, potassium, phosphate, furosemide or cyclic GMP with either intervention (table 8.2).

Table 8.2 Changes in urine volume and excretion of electrolytes, cGMP and furosemide

Changes in hourly urine volume and excretion of sodium (Na), potassium (K), urea, creatinine (Creat), magnesium (Mg), phosphate (Phos), chloride (Cl), furosemide (F'MIDE) and cyclic GMP during infusion of placebo, tyramine and phentolamine (PHENTOL). Results are expressed as mean and one standard error of the mean. * $p<0.05$.

		0-1 hrs	1-2 hrs	2-3 hrs	3-4 hrs
VOLUME	PLACEBO	225±23	189±21	218±21	228±18
Mls/hr	TYRAMINE	265±33	215±20	234±29	245±33
	PHENTOL	242±35	253±34	240±47	222±35
Na	PLACEBO	20±4	13±2	15±2	14±1
mmol	TYRAMINE	20±3	15±3	15±3	17±3
	PHENTOL	18±3	17±3	18±4	16±3
K	PLACEBO	5.5±1.0	4.7±0.8	5.1±0.9	4.6±0.9
mmol	TYRAMINE	5.8±0.8	5.0±0.8	5.0±0.8	5.0±0.9
	PHENTOL	4.3±0.7	4.7±0.6	4.6±0.5	4.8±1.1
Urea	PLACEBO	21±3	16±2	17±2	15±2
mmol	TYRAMINE	20±2	16±2	15±2	16±2
	PHENTOL	18±2	18±2	17±1	16±3
Creat	PLACEBO	0.49±0.09	0.37±0.05	0.38±0.04	0.34±0.05
mmol	TYRAMINE	0.45±0.04	0.35±0.05	0.34±0.05	0.39±0.06
	PHENTOL	0.37±0.04	0.38±0.04	0.39±0.05	0.41±0.07
Mg	PLACEBO	0.41±0.06	0.29±0.05	0.31±0.04	0.28±0.04
mmol	TYRAMINE	0.42±0.08	0.31±0.06	0.30±0.05	0.33±0.05
	PHENTOL	0.36±0.07	0.35±0.05	0.33±0.04	0.31±0.04
Phos	PLACEBO	1.16±0.20	0.82±0.09	0.95±0.07	0.96±0.10
mmol	TYRAMINE	0.88±0.09	0.74±0.08	0.79±0.06	0.95±0.11
	PHENTOL	0.89±0.19	0.94±0.19	0.91±0.09	0.87±0.16
Cl	PLACEBO	21±3	13±2	15±2	14±1
mmol	TYRAMINE	22±3	16±3	15±3	16±3
	PHENTOL	19±3	18±3*	17±4	15±2
F'MIDE	PLACEBO	1.9±0.3	1.5±0.2	1.6±0.3	1.7±0.2
mg/ml	TYRAMINE	1.4±0.2	1.9±0.4	1.9±0.4	2.3±0.6
	PHENTOL	1.6±0.3	1.8±0.2	1.5±0.3	1.8±0.2
cGMP	PLACEBO	2.0±0.4	1.5±0.2	1.8±0.4	1.5±0.3
ng/min	TYRAMINE	2.0±0.3	1.5±0.2	1.7±0.5	1.8±0.3
	PHENTOL	1.7±0.4	2.0±0.5	1.5±0.4	1.4±0.5

Fractional excretion of furosemide increased during low and high dose tyramine infusion ($p<0.03$ and $p<0.2$ respectively) but fractional excretion of electrolytes and cyclic GMP did not differ significantly from placebo.

Table 8.3 Fractional excretion of electrolytes, cyclic GMP and furosemide with respect to creatinine

Sodium (Na), potassium (K), urea (U), magnesium (Mg), phosphate (Ph), chloride (Cl), furosemide (Frus) and cyclic GMP excretion divided by creatinine (Cr) excretion during infusion of placebo, tyramine and phentolamine (PHENTOL). * denotes significance at $p<0.05$.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4HRS
Na/Cr	PLACEBO	48±7	41±8	44±9	48±8
mmol/mmol	TYRAMINE	47±6	44±7	46±8	48±7
	PHENTOL	55±12	49±10	49±12	44±9
K/Cr	PLACEBO	11.6±1.8	12.7±1.6	13.5±1.8	13.4±1.8
mmol/mmol	TYRAMINE	13.5±1.9	14.9±1.8	14.9±2.1	13.8±2.0
	PHENTOL	11.9±1.6	12.7±1.3	12.2±1.2	11.7±1.4
U/Cr	PLACEBO	45±4	45±3	45±2	45±3
mmol/mmol	TYRAMINE	47±6	47±4	45±3	43±4
	PHENTOL	50±5	49±4	45±3	41±3
Mg/Cr	PLACEBO	0.93±0.15	0.81±0.10	0.85±0.08	0.85±0.08
mmol/mmol	TYRAMINE	1.0±0.21	0.91±0.13	0.89±0.10	0.87±0.10
	PHENTOL	1.05±0.21	0.93±0.11	0.89±0.12	0.81±0.09
Ph/Cr	PLACEBO	2.48±0.27	2.45±0.32	2.65±0.31	3.09±0.38
mmol/mmol	TYRAMINE	2.12±0.37	2.35±0.36	2.58±0.35	2.66±0.40
	PHENTOL	2.70±0.63	2.61±0.54	2.61±0.43	2.24±0.32
Cl/Cr	PLACEBO	50±10	38±7	43±8	45±5
mmol/mmol	TYRAMINE	51±8	45±7	48±8	47±7
	PHENTOL	59±12	50±8	46±11	40±8
Frus/Cr	PLACEBO	4.5±0.7	4.2±0.3	4.4±0.5	5.5±0.9
μmol/mmol	TYRAMINE	4.1±0.9	6.5±2.5*	6.0±1.9*	6.0±1.7
	PHENTOL	4.2±0.6	4.8±0.6	4.0±0.9	4.5±0.8
cGMP/Cr	PLACEBO	5±1	5±1	5±1	5±2
pmol/mmol	TYRAMINE	5±1	6±2	6±2	6±2
	PHENTOL	6±2	6±1	4±1	4±1

No significant differences were noted in serum sodium, potassium, urea,

creatinine, magnesium, phosphate or chloride concentrations with either intervention compared with placebo.

Table 8.4 Plasma electrolyte concentrations

Changes in plasma electrolyte concentrations during infusions of tyramine, phentolamine and placebo at one, two and three hours from the start of the study.

		1 HOUR	2 HOURS	3 HOURS
Na	PLACEBO	140±1	139±1	140±1
mmol	TYRAMINE	140±1	139±1	139±1
	PHENTOLAMINE	139±1	139±1	139±1
K	PLACEBO	3.9±0.2	3.8±0.2	3.9±0.2
mmol	TYRAMINE	3.7±0.2	3.6±0.2	3.5±0.2
	PHENTOLAMINE	3.5±0.1	3.5±0.2	3.4±0.1
Urea	PLACEBO	7.5±0.5	7.2±0.6	7.1±0.6
mmol	TYRAMINE	8.0±0.9	7.7±0.9	7.4±0.9
	PHENTOLAMINE	8.4±1.5	7.9±1.4	7.9±1.4
Creat	PLACEBO	92±4	90±4	89±4
mmol	TYRAMINE	96±6	93±6	93±5
	PHENTOLAMINE	91±8	88±7	86±7
Mg	PLACEBO	0.79±0.02	0.81±0.03	0.79±0.03
mmol	TYRAMINE	0.79±0.03	0.78±0.03	0.78±0.04
	PHENTOLAMINE	0.81±0.02	0.80±0.03	0.78±0.02
Phos	PLACEBO	0.91±0.1	0.94±0.09	0.97±0.07
mmol	TYRAMINE	0.86±0.07	0.90±0.06	0.96±0.05
	PHENTOLAMINE	0.84±0.07	0.89±0.05	0.90±0.05
Cl	PLACEBO	104±2	104±2	104±2
mmol	TYRAMINE	101±2	102±1	102±1
	PHENTOLAMINE	102±1	103±1	103±1

Plasma hormone concentrations: Table 8.5, Figure 8.1.

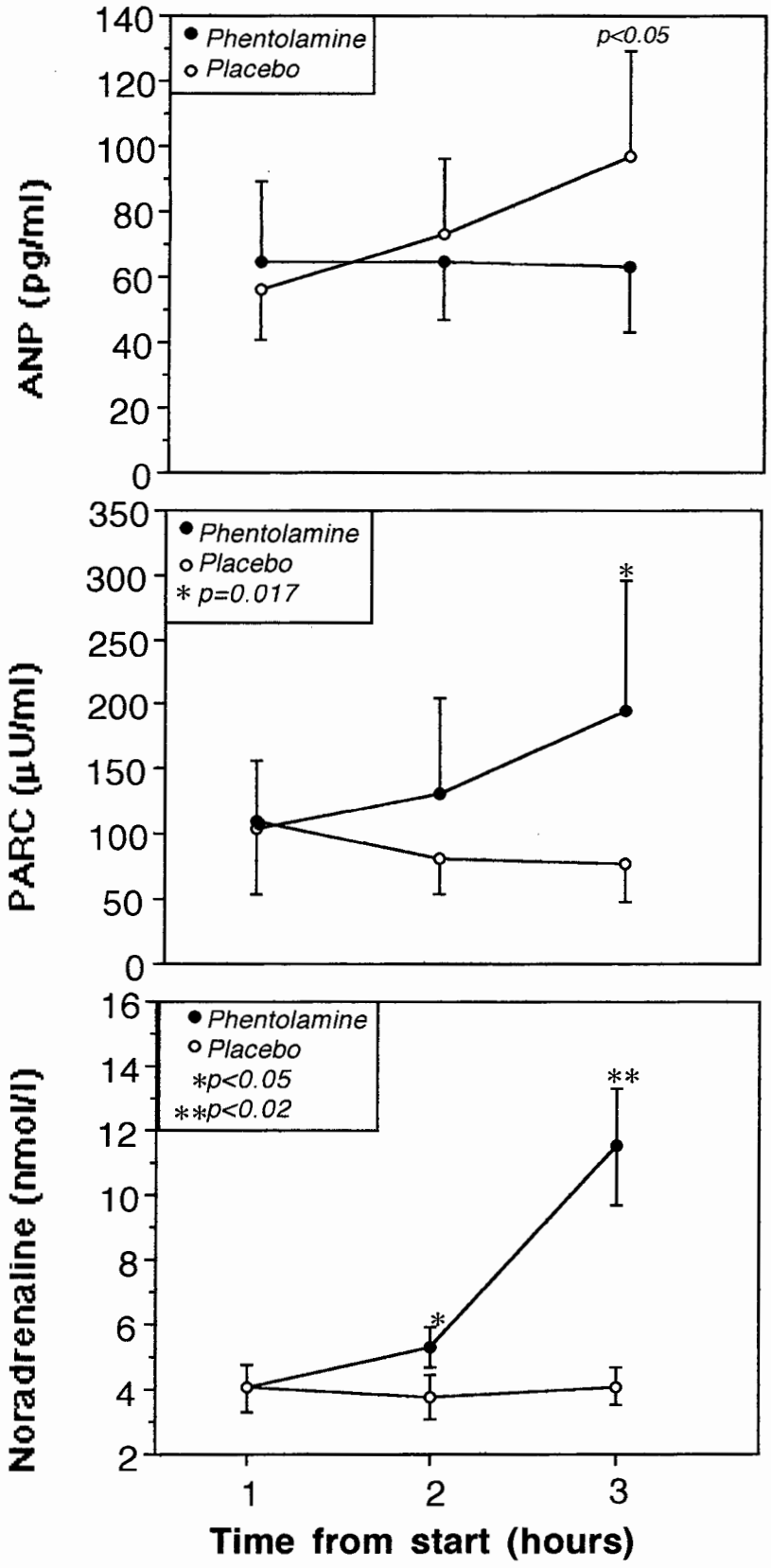
None of the plasma hormones measured were altered significantly by tyramine infusion but phentolamine infusion was associated with a rise in active renin concentration, a fall in ANP and a rise in noradrenaline and adrenaline concentrations.

Table 8.5 Plasma hormone concentrations

Changes in plasma concentrations of atrial natriuretic peptide (ANP), plasma active renin (PARC), noradrenaline (N ADREN) and adrenaline (ADREN) at one, two and three hours form the start of the study with tyramine or placebo. *p<0.05

		1 HOUR	2 HOURS	3 HOURS
ANP	PLACEBO	56±15	73±23	97±32
pg/ml	TYRAMINE	77±32	63±19	69±27
	PHENTOLAMINE	65±24	65±18	63±20*
PARC	PLACEBO	108±40	79±23	77±25
μU/ml	TYRAMINE	72±17	63±18	55±15
	PHENTOLAMINE	103±49	131±72	194±103*
N ADREN	PLACEBO	4.1±0.8	3.8±0.7	4.1±0.6
nmol/l	TYRAMINE	3.8±0.6	4.0±0.6	4.4±0.7
	PHENTOLAMINE	4.1±0.7	5.3±0.6*	11.5±1.8*
ADREN	PLACEBO	0.23±0.06	0.22±0.06	0.25±0.08
nmol/l	TYRAMINE	0.24±0.06	0.26±0.07	0.25±0.08
	PHENTOLAMINE	0.27±0.08	0.30±0.07	0.34±0.11

Figure 8.1 Changes in plasma concentrations of ANP, PARC and noradrenaline during infusion of phentolamine and placebo.



Haemodynamic variables

Blood pressure fell during high dose phentolamine infusion with effects persisting an hour afterwards (Figure 8.2). Mean arterial pressure rose during high dose tyramine infusion (Figure 8.3).

ERPF increased significantly during phentolamine infusion (218.9±101.3 to 272.7 ±131.2, and 222.9±80.6 to 272.0±122.1 for low and high dose respectively, p=.0357 for both doses). ERPF tended to fall during tyramine infusion although this did not reach statistical significance (table 8.6, figure 8.4).

Creatinine clearance did not change significantly with tyramine or phentolamine infusion (table 8.6).

Table 8.6 Renal haemodynamics

Changes in hourly glomerular filtration rate (GFR) and effective renal plasma flow (ERPF)during infusion of placebo, tyramine and phentolamine (PHENTOL). * p<0.05

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
GFR MLS/MIN	PLACEBO	87±13	67±9	71±8	63±8
	TYRAMINE	78±5	63±7	61±8	69±10
	PHENTOL	69±9	74±9	78±11	77±10
ERPF MLS/MIN	PLACEBO	227±33	219±36	223±29	228±30
	TYRAMINE	214±36	214±31	191±29	234±37
	PHENTOL	212±35	273±46*	272±43*	224±32

Figure 8.2 Changes in mean arterial pressure during infusion of placebo, phentolamine and tyramine

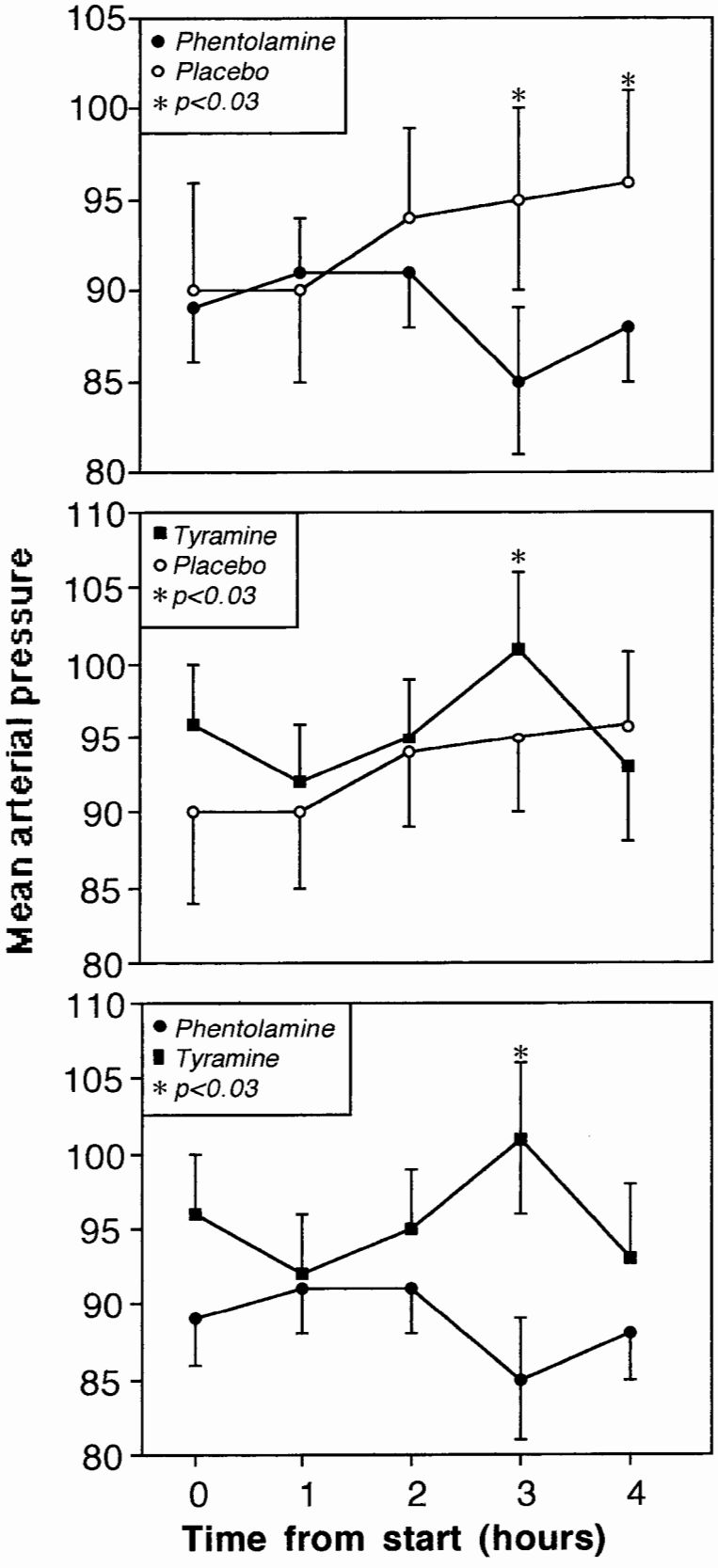


Figure 8.3 Changes in heart rate during placebo, phentolamine and tyramine infusions

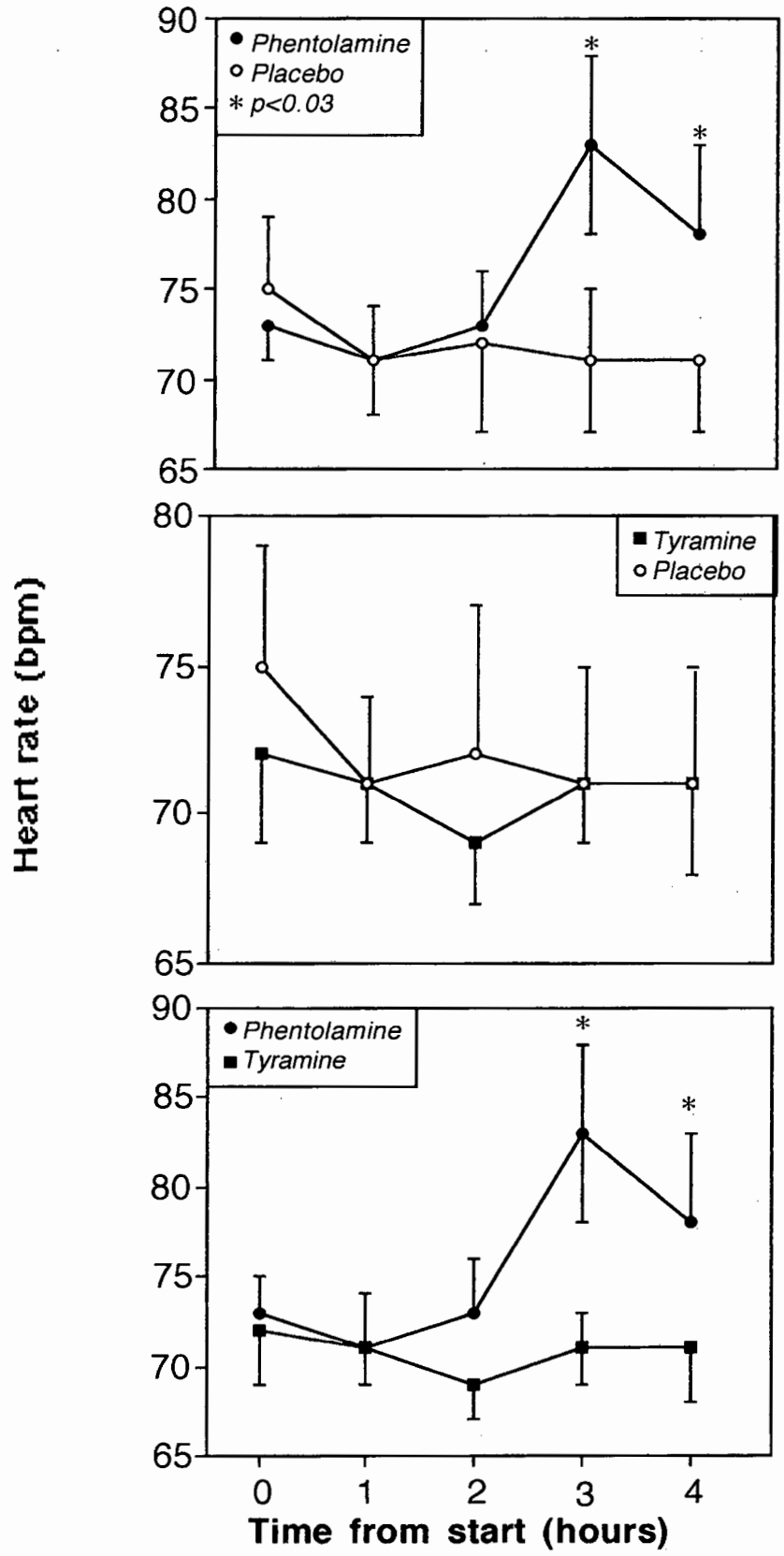
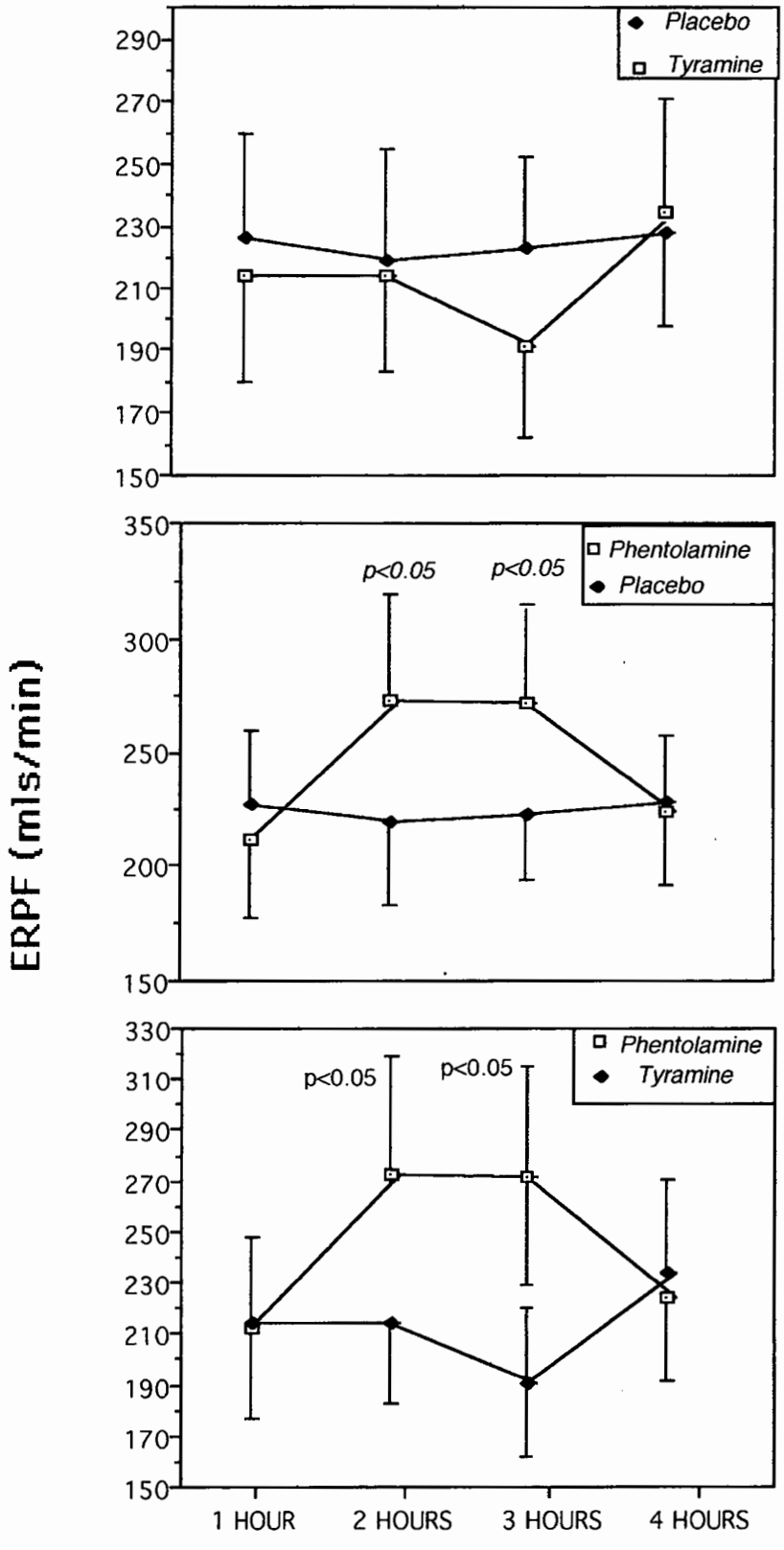


Figure 8.4 Changes in effective renal plasma flow during infusion of placebo, phentolamine and tyramine.



8.4 Discussion

Indirect sympathetic nervous system stimulation with tyramine did not alter diuresis or natriuresis in response to furosemide in this study. There was also no significant difference between the effect of stimulation with tyramine and α-adrenergic blockade with phentolamine on diuresis.

The interventions differed in effect as follows: phentolamine caused a marked and sustained fall in arterial pressure, while tyramine caused a modest increment in mean arterial pressure during high dose infusion. Tyramine had no effect on circulating renin activity, ANP or catecholamine concentrations while phentolamine caused significant suppression of ANP during high dose infusion with elevation of and renin and plasma catecholamine concentrations. Tyramine did not improve, or even tended to impair ERPF, while phentolamine augmented ERPF both at high and low dose. Neither intervention had any effect on renal cyclic GMP or furosemide excretion.

These results demonstrate that a fall in arterial pressure may override any diuretic or natriuretic forces resulting from an improvement in ERPF, yet an increase in arterial pressure without associated beneficial renal haemodynamic effects likewise fails to enhance diuresis or natriuresis. Phentolamine failed to enhance a furosemide induced diuresis or natriuresis despite improving renal plasma flow significantly but blood pressure was depressed during high dose infusion with compensatory tachycardia and a rise in catecholamines. Garg (1992) has suggested that enhancement of natriuresis and diuresis depends not only on total renal plasma flow, which is increased by a number of drugs which do not improve natriuresis or diuresis, but also on enhancement of renal papillary flow.

Although phentolamine inhibited the renal adrenergic sodium-retaining effects directly, the associated hypotension activated the renin-angiotensin system. The net effect on natriuresis was neutral.

Tyramine had modest effects on blood pressure and no effect on heart rate in the present study. Tyramine infusion titrated to achieve a rise in blood pressure of approximately 25mmHg in 9 patients with coronary disease failed to produce a significant fall in heart rate, in keeping with the present study (Forman, Robertson, Goldberg, et al 1984). Circulating noradrenaline levels did not change significantly during tyramine infusion, although there was an upward trend. This may have been due to receptor downregulation or inadequate dosing. Tyramine infusion at slightly higher dose than that used in the present study (15μg/kg/min) in 6 normal volunteers resulted in a rise in plasma noradrenaline concentration (0.8 to 1.4ng/ml) and a significant fall in plasma renin activity (Lang, Rahman, Balfour, et al 1993b). Lang, Rahman, Balfour et al also proposed that circulating noradrenaline (infused) and neuronally released noradrenaline (from tyramine administration) may have opposite effects on renal sodium handling in man.

The lack of suppression of plasma renin activity by tyramine infusion in the present study may reflect the smaller rise in arterial pressure which was achieved or blunting of baroreflex suppression of renin release. It has been shown that α-adrenoceptor-mediated renal vasoconstriction predominates over β-adrenoceptor-mediated vasodilatation (Drew, Whiting 1979). Renin release from juxta glomerular cells is controlled by β-adrenoceptors (Katz, Lindheimer 1977; Osborn, DiBona, Thames 1981). Selective α2-adrenergic agonists have been shown to cause diuresis in vivo in part due to inhibition

of vasopressin release, and via α2-adrenergic-mediated decreases in renin release (Keeton, Campbell 1980; Gellai 1990). Renal nerve stimulation results in augmentation of renin release (Osborn, Holdaas, Tharnes, et al 1983). The mechanism of tyramine-induced renin suppression is thought to be secondary to increased solute delivery to the macula densa as well as via the baroreceptor mechanism in response to increased blood pressure (Lang, Rahman, Balfour, et al 1993b). Ferrari, Ceconi, De-Guili, et al (1991) demonstrated that sympathectomised SHR and WKY rats had less pronounced pressor and tachycardic responses to tyramine than control rats. Patients with heart failure have decreased baroreceptor function (Eckberg, Drabinski, Braunwald 1971; Goldstein, Beiser, Stampfer, et al 1975; Manthey, Dietz, Opherk, et al 1992) and decreased receptor responsiveness to exogenous and endogenous catecholamines (Bristow, Ginsburg, Minobe, et al 1982; Bogaert, Fraeyman 1991). Exaggerated sympathetic activity may represent a pathogenetic factor contributing to baroreceptor reflex impairment in these patients. Manthey, Dietz, Opherk, et al (1992) provide evidence that arterial baroreceptor unloading with hydralazine may induce a dissociation between reflex responses of the sympathetic nervous system and vasopressin release in patients with severe heart failure. Reflex release of vasopressin in response to a significant fall in blood pressure was not impaired, yet sympathetic stimulation in response to the fall in arterial pressure was impaired. Vasopressin was not measured in the present study.

A study by Yusuf, Garg, Held, et al (1992) shows that tyramine blocks sodium channels by suppressing maximal sodium channel conductance in frog ventricular myocytes and it is open to speculation that similar effects in the kidney might act to impair natriuresis.

Limitations of the study

Adequate sympathetic stimulation may not have been achieved by tyramine infusion at the dose used as plasma catecholamine concentrations did not rise and the rise in blood pressure was relatively small. One patient with milder heart failure had a more marked hypertensive response to tyramine which was dose related. Thus it was suspected that patients with more severe failure exhibit a blunted hypertensive response to tyramine, as was the case with dopamine and dobutamine infusions. All the limitations relating to the study design documented in the previous chapters apply to this chapter as well but have not been repeated.

Conclusion

In patients with heart failure, neither sympathetic inhibition (phentolamine) nor indirect stimulation with tyramine had any clinically significant effect on the diuresis and natriuresis induced by furosemide.

Chapter 9 The effect of dopamine and dobutamine on a furosemide induced diuresis

9.1 Introduction

In animal models, increased renal sympathetic activity stimulates renin release, constricts the glomerular arterioles, alters the pattern of intrarenal blood flow and enhances proximal tubular sodium reabsorption. All these effects apparently act in concert to inhibit natriuresis (Zambraski, DiBona, Kaloyanides 1976; Bunag, Page, McCubbin 1967). Sympathetic denervation of the kidney (Gill 1979; Gottschalk 1979; Bello-Reuss 1980) or sympathetic inhibition with guanethidine and phenoxybenzamine reverses these changes in animal models (Zambraski,DiBona, Kaloyanides 1976; DiBona 1978). In contrast, inhibition of sympathetic nerve efferent activity with guanethidine results in sodium retention in patients with heart failure (Gill, Mason, Bartter 1965), as does α-adrenergic blockade (Riegger, Haeske, Kraus, et al 1987b). β-adrenergic blockade, although advocated by some for the management of heart failure, are known to exacerbate the condition in some patients (Ikram, Fitzpatrick 1981; Binkley, Lewe, Lima, et al 1986; Shanes 1987; Waagstein, Caidahl, Wallentin, et al 1989; Lang R 1990; Packer 1992). This may be attended by salt and water retention. Thus pharmacological interruption of sympathetic efferent pathways in human heart failure in contrast to animal models, generally results in sodium retention. This may be due to a reduction in blood pressure rather than effects of interfering with the local sympathetic nervous system, nonetheless it puts the importance of renal mechanisms into clinical perspective.

Dobutamine, an agent which stimulates β1, β2 and α1 adrenergic receptors

increases urine and sodium output in patients with heart failure (Leier, Webel, Bush 1977; Leier, Heban, Huss 1978; Applefeld, Newman, Sutton, et al 1987). No specific renal vasodilatation is believed to occur with dobutamine, suggesting that the diuresis and natriuresis is due to an increase in cardiac output, blood pressure and thus renal blood flow. Dopamine, the precursor of noradrenaline, activates α- and β-adrenergic receptors and binds to dopamine-1 and -2 receptors. The inotropic effects of dopamine in heart failure are mediated mainly by β1-adrenoceptor activation, probably indirectly through dopamine-induced release of endogenous noradrenaline (Brown, Lorenz, Erdmann 1985; Brodde 1991). The observed clinical effects of dopamine infusion in patients with heart failure depend on which receptor is activated predominantly. At very low doses (0.5-2.μg/kg/min) the dopamine receptors alone are activated, resulting in renal vasodilatation, decreased peripheral vascular resistance, a fall in blood pressure, increased renal blood flow and natriuresis. At doses from 2μg/kg/min some increases in myocardial contractility may be evident with improved left ventricular function. At larger doses (2-5μg/kg/min), cardiac β1-adrenoceptors are stimulated, with positive inotropic and chronotropic effects being manifested with preservation of decreased peripheral vascular resistance, however at doses of 10μg/kg/min, peripheral vasoconstriction and increased systemic vascular resistance results.

Dopamine and dobutamine are widely used in the management of decompensated, severe left ventricular failure. There are several anecdotal reports and uncontrolled studies in patients with heart failure showing beneficial diuresis and natriuresis during dopamine (Goldberg, McDonald, Zimmerman 1963; McDonald, Goldberg, McNay, et al 1964; Beregovich,

Bianchi, Rubler, et al 1974) and dobutamine infusion (Leier 1978, Applefeld 1987) although the precise mechanism for these renal effects is not known. Infusion of dobutamine caused greater increments in blood pressure and cardiac index (Leier, Heban, Huss 1978) than with similar doses of dopamine with an associated fall in peripheral vascular resistance. This suggests that the mechanism for natriuresis and diuresis with dobutamine is improved renal perfusion pressure. Whether there is an additional effect of specific dopamine receptor stimulation on a furosemide induced diuresis in patients with heart failure is not known. Dopamine receptor stimulation may specifically induce a natriuresis, especially when used in low doses. To differentiate this effect from that of increasing cardiac output alone, the renal effects of equivalent doses of dopamine and dobutamine were compared.

9.2 Patients and methods

8 patients with chronic stable heart failure requiring diuretics to control symptoms were studied on three occasions in single blind fashion as set out in Chapter 2. After the run-in period of one hour, dobutamine, dopamine or matching placebo infusions were commenced at 1μg/kg/min, for one hour. During the next (3rd) hour, the dose was increased incrementally to a maximum of 10μg/kg/min and maintained at this dose for the remainder of the hour for both agents. The infusions were discontinued for the final hour. Infusion rate was reduced if heart rate increased to over 110bpm, if frequent ventricular premature beats appeared or if symptoms such as chest pain or breathlessness occurred. Mean maximum infusion rate was 5μg/kg/min and 6μg/kg/min for dopamine and dobutamine respectively.

Hourly urine and blood samples were collected for electrolytes, urinary cGMP, urinary frusemide, urinary and plasma PAH and plasma ANP and

active renin. Heart rate and blood pressure were measured at 15 minute intervals.

Table 9.1 Patient characteristics

Individual patient characteristics: age, sex, New York Heart Association classification (NYHA), maintenance diuretic requirements in milligrammes per day (furosemide-F, metolazone-MET), type and dose of ACE-inhibitor in milligrammes per day (captopril-CAP, enalapril-ENAL, lisinopril-LISIN) and aetiology of heart failure (ischaemic heart disease-IHD, dilated cardiomyopathy-DCM).

PATIENT	AGE	SEX	NYHA	DIURETIC	ACE-I (mg)	AETIOLOGY
1	67	M	IIB	F 120	CAP 75	IHD
2	65	M	IIB	F 120	-	IHD
3	55	M	III	F 120	ENAL 20	IHD
4	62	M	III	F 40, MET 2.5	LISIN 10	IHD
5	63	M	III	F 120	ENAL 15	DCM
6	62	M	IIB	F 80	ENAL 10	IHD
7	77	M	III	F 40	CAP 37.5	IHD
8	54	M	IIB	F 40	ENAL 10	IHD

9.3 Results

Urine volume tended to rise with respect to placebo during high dose dopamine and dobutamine infusion, although this did not reach significance. There was a significant fall in urine volume during the hour after dopamine infusion. (Table 9.1).

Electrolyte excretion. There was no significant change in sodium or chloride excretion after dobutamine, although sodium excretion and chloride excretion increased after dopamine infusion compared to placebo at high dose ($p=0.036$ and $p=0.05$ respectively, figure 9.1 and 9.2). The difference in sodium and chloride excretion between dopamine and dobutamine was however not significant. Slight but statistically significant increases in potassium excretion were seen during high dose infusion of both dopamine

and dobutamine.

Furosemide excretion did not alter during dopamine or dobutamine infusion although fractional excretion of furosemide increased in the hour following high-dose dopamine infusion.

cGMP excretion was not altered by dobutamine infusion but both excretion and fractional excretion of cyclic GMP fell during high-dose dopamine infusion.

Table 9.2 Urine indices

Hourly changes in urine volume (millilitres) and excretion of sodium, potassium, urea, creatinine, chloride, magnesium, phosphate (millimols), furosemide (milligrammes per hour) and cyclic GMP (nanograms per minute). * denotes significance at $p<0.05$.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
URINE (MLS)	PLACEBO	228±29	231±26	232±30	251±26
	DOPAMINE	247±40	232±31	328±47	221±23*
	DOBUTAMINE	225±34	234±32	275±32	277±36
SODIUM mmol	PLACEBO	21±5	17±4	15±2	17±3
	DOPAMINE	24±4	21±3	31±6*	20±3
	DOBUTAMINE	21±4	22±4	20±4	22±4
POTASSIUM mmol	PLACEBO	6±0.7	6±0.7	5±0.9	6±0.8
	DOPAMINE	6±1	6±1	7±1*	6±1
	DOBUTAMINE	6±1.3	6±1.1	6±1.1*	6±1.3
UREA mmol	PLACEBO	21±9	17±2	15±2	16±2
	DOPAMINE	23±3	18±3	20±7*	15±3
	DOBUTAMINE	22±3	21±4	17±2	20±2
CREATININE mmol	PLACEBO	0.62±0.08	0.47±0.04	0.39±0.04	0.44±0.05
	DOPAMINE	0.63±0.07	0.46±0.05	0.51±0.04	0.39±0.07
	DOBUTAMINE	0.58±0.09	0.53±0.1	0.42±0.06	0.49±0.07
CHLORIDE mmol	PLACEBO	19±4	16±4	15±5	15±2
	DOPAMINE	24±5	21±3	29±5*	17±3
	DOBUTAMINE	20±4	21±4	18±4	20±3
MAGNESIUM mmol	PLACEBO	0.39±0.06	0.31±0.02	0.28±0.04	0.29±0.03
	DOPAMINE	0.41±0.07	0.28±0.06	0.34±0.05	0.20±0.04
	DOBUTAMINE	0.37±0.06	0.37±0.07	0.28±0.04	0.32±0.05
PHOSPHATE mmol	PLACEBO	1.3±0.2	1.0±0.07	1.0±0.09	1.1±0.07
	DOPAMINE	1.4±0.21	1.0±0.08	1.3±0.16	0.9±0.09
	DOBUTAMINE	1.3±0.09	1.2±0.09	0.9±0.05	1.0±0.08
FUROSEMIDE (mg/hr)	PLACEBO	8.3±1.3	9.0±1.4	7.8±1.2	7.7±1.1
	DOPAMINE	7.3±1.1	6.4±0.7	6.3±0.4	8.4±1.0*
	DOBUTAMINE	7.5±1.5	8.7±1.7	9.3±2.2	9.3±1.7
CGMP (ng/min)	PLACEBO	3.1±0.9	2.7±0.6	3.1±0.6	2.8±0.7
	DOPAMINE	3.8±0.7	2.8±0.7	2.9±0.7*	2.0±0.5
	DOBUTAMINE	3.3±0.5	2.9±0.6	3.2±0.8	3.0±0.9

Hourly excretion of sodium and chloride rose significantly after dopamine infusion compared to placebo ($p=0.036$ and $p<0.05$; figure 9.1 and 9.2).

Figure 9.1 Changes in hourly sodium excretion during infusion of placebo and dopamine

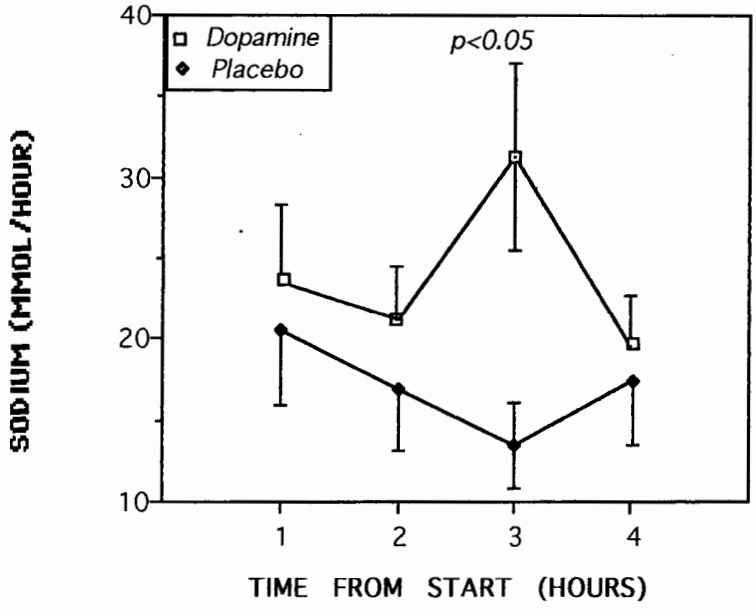
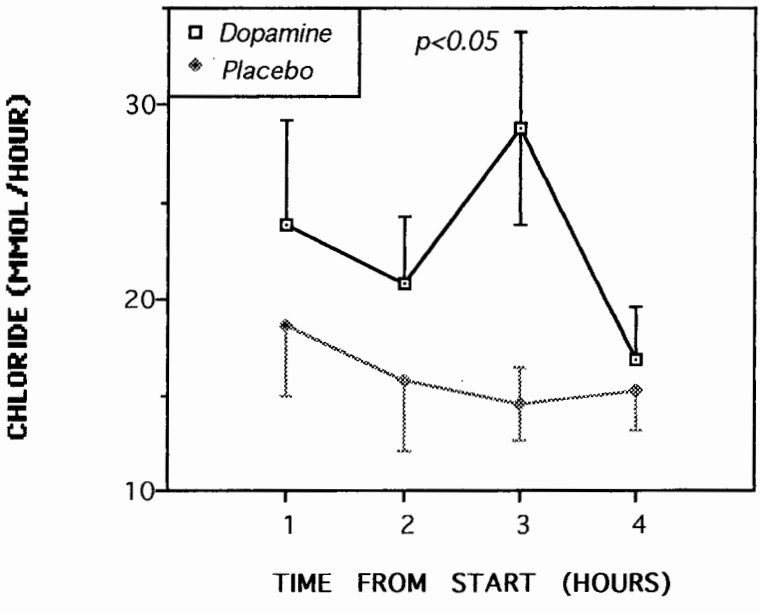


Figure 9.2 Changes in hourly chloride excretion during infusion of placebo and dopamine



Fractional excretion of sodium and chloride increased during high dose dopamine infusion compared with placebo while cGMP fell. The trend to increase with high dose dobutamine did not reach statistical significance. Fractional excretion of furosemide increased in the hour following high dose dopamine infusion compared with placebo.

Table 9.3 Fractional excretion of electrolytes, furosemide and cyclic GMP

Excretion of sodium (Na), potassium (K), urea, chloride (Cl), magnesium (Mg), phosphate (Phos), furosemide (FRUS) and cyclic GMP (cGMP)/creatinine (Cr). * denotes significance at $p<0.05$.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
Na/Cr	PLACEBO	34±7	37±8	37±8	42±6
mM/mM	DOPAMINE	37±5	48±7	58±7*	56±10
	DOBUTAMINE	39±8	46±7	49±7	48±8
K/Cr	PLACEBO	11±2	13±2	14±2	13±2
mM/mM	DOPAMINE	10±2	12±2	14±2	15±2
	DOBUTAMINE	11±2	13±2	13±2	13±2
U/Cr	PLACEBO	33±2	37±2	38±2	38±2
mM/mM	DOPAMINE	36±2	39±2	39±2	39±2
	DOBUTAMINE	39±2	41±2	42±2	41±2
Cl/Cr	PLACEBO	31±6	34±7	39±6	36±4
mM/mM	DOPAMINE	37±6	48±8	54±6*	48±8
	DOBUTAMINE	34±6	41±6	42±6	43±6
Mg/Cr	PLACEBO	0.64±0.08	0.68±0.05	0.72±0.06	0.67±0.05
mM/mM	DOPAMINE	0.65±0.07	0.63±0.11	0.66±0.09	0.60±0.10
	DOBUTAMINE	0.65±0.08	0.71±0.08	0.68±0.07	0.67±0.06
Phos/Cr	PLACEBO	2.06±0.17	2.24±0.19	2.67±0.28	2.70±0.33
mM/mM	DOPAMINE	2.29±0.21	2.34±0.21	2.56±0.21	2.82±0.56
	DOBUTAMINE	2.35±0.24	2.62±0.37	2.52±0.44	2.38±0.36
FRUS/Cr	PLACEBO	2.89±0.48	3.86±0.44	4.08±0.46	4.03±0.51
μg/mM	DOPAMINE	2.78±0.47	3.43±0.62	4.12±0.64	6.21±1.7*
	DOBUTAMINE	2.93±0.63	5.72±2.76	9.2±4.94	6.01±2.26
cGMP/Cr	PLACEBO	4.92±1.54	5.74±1.25	8.26±1.80	7.37±2.44
ng/mM	DOPAMINE	6.84±1.74	6.89±1.74	5.71±1.20*	8.38±3.73
	DOBUTAMINE	5.97±1.02	7.46±2.94	8.43±2.09	6.33±1.54

Plasma electrolytes: Plasma concentrations of sodium, potassium, urea, creatinine and magnesium did not differ significantly between interventions. Plasma chloride concentrations fell during high dose dopamine infusion with respect to placebo and plasma phosphate concentrations were significantly lower during low and high dose dobutamine infusion and during high dose dopamine infusion.

Table 9.4 Plasma electrolyte concentrations

Changes in plasma sodium (Na), potassium (K), urea, creatinine (Creat), chloride (Cl), magnesium (Mg) and phosphate (Phos) at one, two and three hours from the start of each study. * indicates significance at $p<0.05$.

		1 HOUR	2 HOURS	3 HOURS
Na	PLACEBO	138±1	137±1	137±1
mmol/l	DOPAMINE	138±1	138±1	137±1
	DOBUTAMINE	137±1	137±1	137±1
K	PLACEBO	4.0±0.2	3.9±0.1	3.8±0.1
mmol/l	DOPAMINE	4.0±0.1	3.9±0.2	3.7±0.1
	DOBUTAMINE	3.9±0.2	3.8±0.1	3.6±0.1
Urea	PLACEBO	8.0±0.7	7.9±0.7	7.7±0.7
mmol/l	DOPAMINE	8.0±0.6	7.9±0.6	7.8±0.6
	DOBUTAMINE	8.1±0.7	8.0±0.7	7.8±0.7
Creat	PLACEBO	114±8	113±8	110±9
μmol/l	DOPAMINE	110±10	108±10	111±9
	DOBUTAMINE	112±10	110±10	109±9
Cl	PLACEBO	99±1	101±1	101±1
mmol/l	DOPAMINE	100±1	100±1	98±1*
	DOBUTAMINE	100±2	101±1	101±1
Mg	PLACEBO	0.79±0.03	0.80±0.03	0.79±0.03
mmol/l	DOPAMINE	0.78±0.04	0.76±0.04	0.76±0.04
	DOBUTAMINE	0.79±0.03	0.79±0.03	0.78±0.03
Phos	PLACEBO	1.02±0.08	1.03±0.07	1.07±0.06
mmol/l	DOPAMINE	0.96±0.04	0.97±0.04	0.97±0.04
	DOBUTAMINE	0.92±0.05	0.90±0.05	0.87±0.04

Plasma hormone concentrations: There was no change in ANP or active renin concentrations during high or low dose dopamine infusion but there was a significant rise in plasma renin after high dose dobutamine infusion (figure 9.3, table 9.5).

Table 9.5 Plasma hormone concentrations

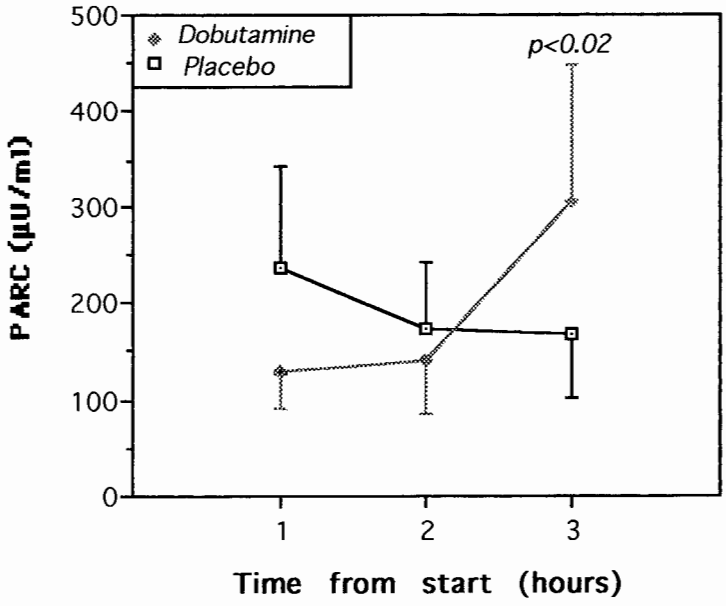
Plasma concentrations of active renin (PARC) measured in micro-units per millilitre and atrial natriuretic peptide (ANP) measured in picograms per millilitre at one, two and three hours from the start of the studies. * denotes significance at $p<0.05$.

		1 HOUR	2 HOURS	3 HOURS
ANP	PLACEBO	96±43	103±47	91±30
pg/ml	DOPAMINE	104±45	92±34	70±19
	DOBUTAMINE	109±46	92±38	77±34
PARC	PLACEBO	235±108	172±70	167±66
μU/ml	DOPAMINE	181±81	146±61	133±49
	DOBUTAMINE	128±38	140±54	306±142*

Adverse responses

Dopamine infusion was poorly tolerated at doses above 4μg/kg/min with only one patient able to remain on the maximum infusion rate of 10μg/kg/min for the entire third hour. Two patients developed angina and five developed significant ventricular arrhythmia. Dobutamine was also poorly tolerated at doses greater than 6μg/kg/min with similar adverse effects including paradoxical hypotension in one patient and profound bradycardia in two.

Figure 9.3 Changes in plasma renin during infusion of placebo and dobutamine



Haemodynamics:

Creatinine clearance did not change significantly after dopamine or dobutamine (table 9.7).

Effective renal plasma flow increased during low and high dose dopamine infusion while the differences with dobutamine were only significant during high dose infusion (figure 9.4).

Heart rate did not change significantly during dopamine infusion but increased significantly during high dose dobutamine infusion (figure 9.5).

Systolic blood pressure rose significantly during high dose dobutamine infusion (table 9.6).

Table 9.6 Haemodynamic variables

Hourly changes in creatinine clearance (CR. CLEAR), ERPF, systolic, diastolic and mean blood pressure (BP) and heart rate. * denotes significance at $p<0.05$.

		0-1 HRS	1-2 HRS	2-3 HRS	3-4 HRS
CR.CLEAR (MLS/MIN)	PLACEBO	98±17	75±8	61±8	69±12
	DOPAMINE	99±16	71±9	78±7	62±15
	DOBUTAMINE	91±15	85±18	67±11	80±14
ERPF (MLS/MIN)	PLACEBO	174±14	171±11	192±14	207±16
	DOPAMINE	179±13	220±17*	297±27*	209±29
	DOBUTAMINE	171±14	189±18	288±31*	208±20
SYSTOLIC BP (mmHg)	PLACEBO	115±7	118±6	113±5	115±7
	DOPAMINE	115±6	112±5	119±6	113±6
	DOBUTAMINE	116±5	119±5	123±6*	118±6
DIASTOLIC BP (mmHg)	PLACEBO	74±3	77±3	76±3	74±2
	DOPAMINE	73±3	73±3	73±2	73±3
	DOBUTAMINE	77±3	77±3	75±4	77±4
MEAN BP (mmHg)	PLACEBO	88±4	91±4	89±3	89±4
	DOPAMINE	88±4	86±4	89±3	87±4
	DOBUTAMINE	92±4	93±3	92±4	92±4
HEART RATE (bpm)	PLACEBO	75±3	73±2	73±2	70±2
	DOPAMINE	74±3	72±2	77±3	76±3
	DOBUTAMINE	77±3	79±3	90±5*	78±3

Figure 9.4 Changes in ERPF during placebo, dopamine and dobutamine infusion

Dopamine increased ERPF at low and high dose while the differences were only significant during high dose dobutamine infusion

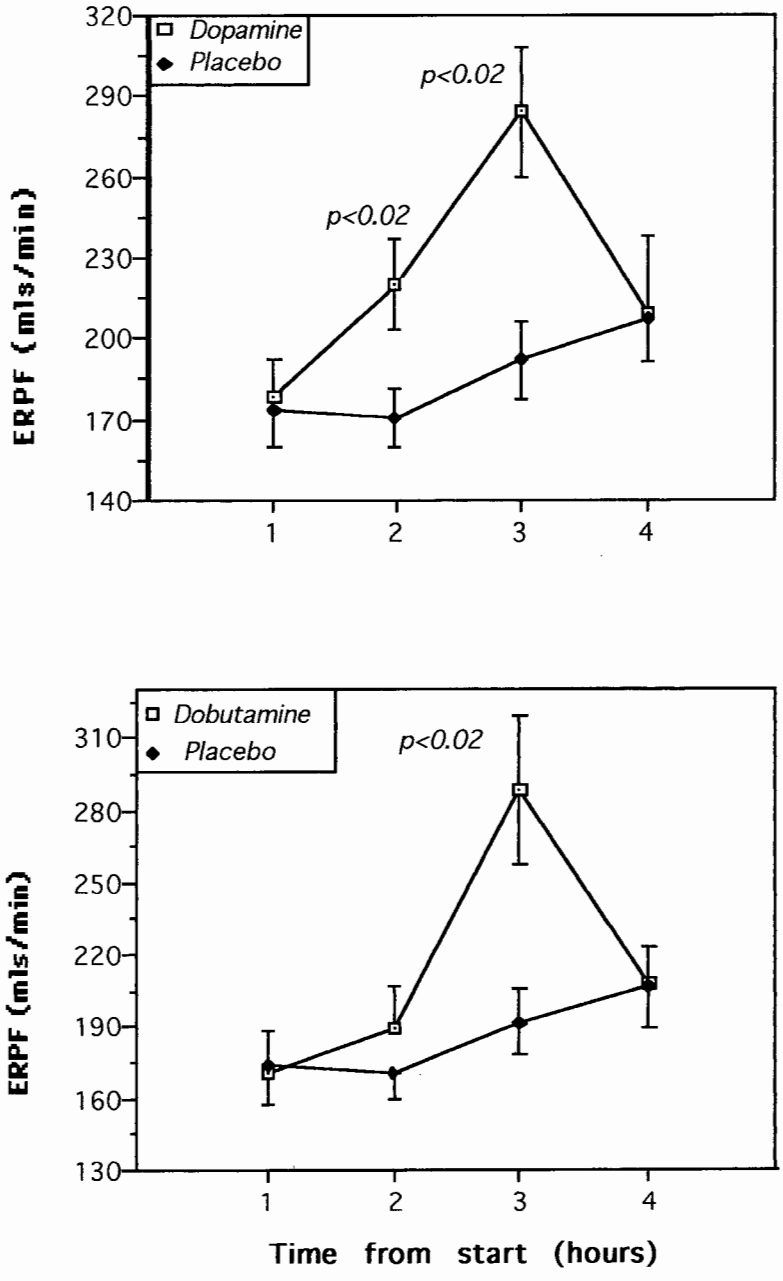


Figure 9.5 Changes in hourly heart rate during placebo and dobutamine infusion

High dose dobutamine infusion resulted in a significant increase in heart rate

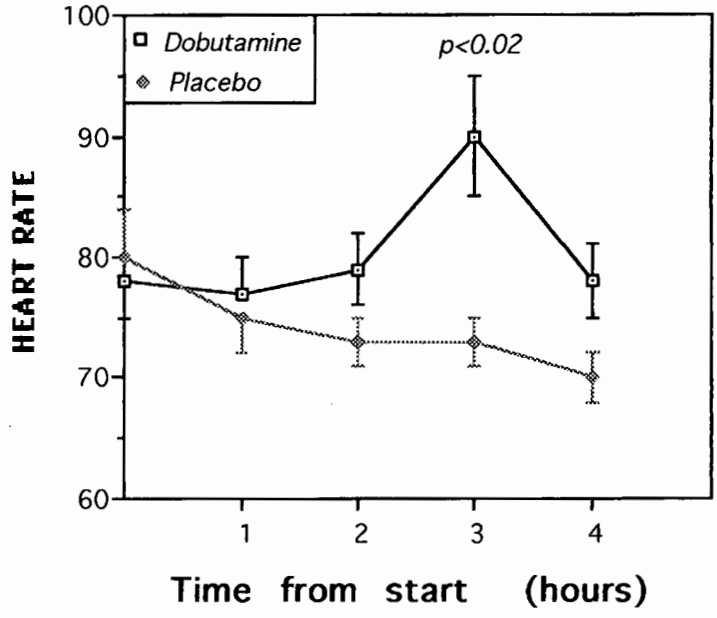
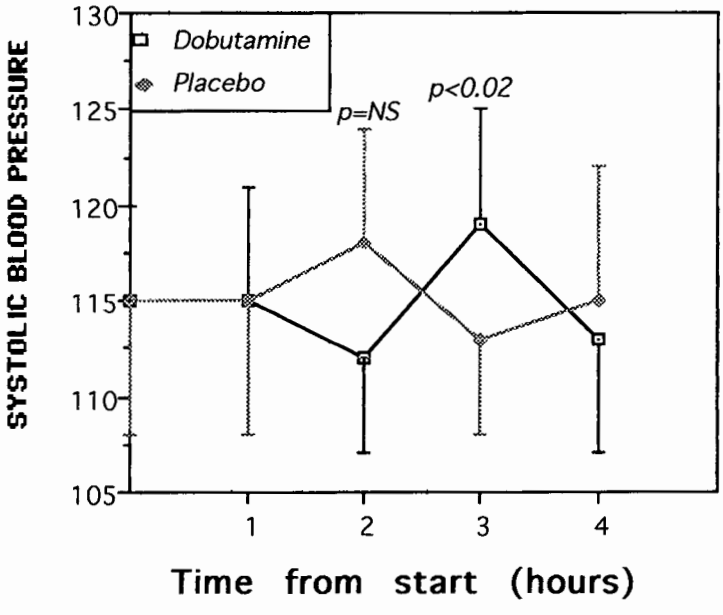


Figure 9.6 Changes in hourly systolic blood pressure during placebo and dobutamine infusion

High dose dobutamine infusion lead to a significant increase in systolic blood pressure



9.4 Discussion

Dobutamine is used extensively for inotropic support in patients with decompensated heart failure with or without acute myocardial ischaemia and intermittent dobutamine infusions have been used on an outpatient basis for control of symptoms in patients with severe heart failure (Applefeld, Newman, Sutton, et al 1987). While side effects such as tachycardia, ventricular extrasystoles and angina have been reported with its use, dobutamine is usually well tolerated at doses of 10-20μg/kg/min. The frequency of adverse effects experienced in the present study at relatively low infusion rates was thus unexpected.

Patients with and without ischaemic heart disease have been shown to develop idiosyncratic hypotension with or without bradycardia during dobutamine infusion (Marcovitz, Bach, Mathias, et al 1993). The exact mechanism is debated. Peripheral vasodilatation, a fall in pulmonary capillary wedge pressure and ventricular mechanoreceptor stimulation leading to reflex bradycardia and vasodilatation are possible mechanisms. Heart failure patients may be particularly sensitive to these effects due to the abnormal baroreceptor sensitivity which is found in chronic heart failure (Eckberg, Drabinsky, Braunwald 1971; Mancia 1990).

A high proportion of the study patients developed tachyarrhythmias during relatively low dose dobutamine infusion. These results suggest that an arrhythmic mechanism may have played a part in the increased incidence of sudden death noted in the treatment arm of the study of chronic dobutamine therapy by Dies (1988).

Leier, Heban, Huss, et al (1978) reported similar undesirable effects with

infusions of dopamine as low as 4μg/kg/min in their patients with severe non-ischaemic congestive failure but their patients were able to tolerate dobutamine infused at 8μg/kg/min for 24 hours. Beregovich, Bianchi, Rubler, et al (1974) described an increase in urine flow and renal sodium excretion in patients with heart failure but only at dopamine doses of greater than 5μg/kg/min, concentrations which from the present study are not safely achieved or are poorly tolerated.

Dopamine augmented the furosemide induced diuresis in the final hour of the present study compared with placebo, however dobutamine did not. Dobutamine had no significant effect on natriuresis compared with placebo but dopamine caused a 30% increase in sodium and chloride excretion during high dose infusion. This contrasted with the 4.8 fold increase in sodium excretion after dopamine infusion described by Rosenblum, Tai and Lawson (1972) in patients not receiving concomitant diuretics and the 2.8 fold increase in urine flow and 4.6 fold increase in urinary sodium excretion with dopamine infusion relative to dobutamine infusion in a study of patients after open-heart surgery (Hilberman, Maseda, Spencer, et al 1984). A more recent study of low-dose dopamine infusion in elderly patients with congestive heart failure showed no diuretic or natriuretic effect (Robinson, Gariballa, Fancourt, et al 1994) and Graves, Cioffi, Vaughan, et al (1993) found inconsistent natriuretic effects in hyperdynamic thermally injured patients.

There are several mechanisms by which dopamine could cause a diuresis and natriuresis:

a) Stimulation of renal dopamine receptors. Selective renal vasodilatation by dopamine was suggested by the increase in ERPF

during low dose dopamine but not dobutamine infusion. However renal vascular resistance and filtration fractions were no different from those seen with dobutamine. Hilberman, Maseda, Spencer, et al (1980a) suggested that the most likely mechanism for the diuresis and natriuresis produced by dopamine was a direct action on renal tubular solute transport, although they did not rule out an effect on intrarenal redistribution of blood flow. The data from the present study do not support this mechanism for enhanced natriuresis as it was only seen during infusion of higher doses and the effects were no different from those observed during dobutamine infusion.

b) Improved renal perfusion pressure. ERPF increased with low dose and high dose dopamine infusion, supporting an effect of dopamine-1 receptor stimulation in the kidney at both doses, but natriuresis was enhanced only at high dose infusion, thus other factors such as arterial pressure appear to have a permissive role. ERPF also increased during high dose dobutamine infusion.

c) Increased tubular delivery of furosemide. Although no change in urinary furosemide excretion was apparent, fractional excretion of furosemide rose in the final hour after dopamine infusion. This was a period when diuresis fell, making increased tubular delivery of furosemide an unlikely mechanism. There may also have been an alteration in intrarenal haemodynamics or a direct effect on the renal tubule.

d) A fall in filtration fraction. Ramdohr, Schüren, Biamino, et al (1973) showed that dopamine infusions result in a fall in filtration fraction and

a rise in ERPF which is more marked in patients with heart failure than in normal subjects. Filtration fraction was not calculated in the present study as creatinine clearance was used to estimate GFR rather than a more accurate method such as inulin clearance.

e) Improvement in GFR. Creatinine clearance did not change significantly although there was a trend during high dose dopamine infusion. Beregovich, Bianchi, Rubler, et al (1974) noted a dose-related increase in creatinine clearance, urine flow and sodium excretion with dopamine administration in patients with congestive cardiac failure. However McDonald, Craig and Watson (1989) demonstrated a four-fold increase in sodium excretion during dopamine infusion in patients with congestive heart failure without evidence of significant increases in glomerular filtration rate or ERPF. Thus changes in GFR were probably not responsible for the observed natriuresis with dopamine infusion although the mechanism may have played a part in other patient groups.

f) Neuroendocrine modulation. A fall in plasma renin activity has been demonstrated in patients with heart failure during low dose dopamine infusion, although the opposite is usually seen in normal subjects. Concentrations fell during both placebo and dopamine infusions in the present study,without any statistical difference between the two. Plasma renin was elevated during high dose dobutamine infusion however these opposite effects had little impact on diuresis or natriuresis, hence this is unlikely to be an important mechanism. Aldosterone secretion is inhibited by dopamine (McKenna 1979) and the oral dopamine analogue, ibopamine,

reduced aldosterone levels in an uncontrolled study of heart failure patients (Nakano, Morimoto, Kakuta, et al 1986). Concentrations were not measured in the present study and some patients were taking ACE-inhibitors which could have influenced results, although these were stopped 18 hours before each study. In patients with congestive heart failure, ibopamine lowers noradrenaline levels (Nakano, Morimoto, Kakuta, et al 1986; Van Veldhuisen, Girbes, Crijns, et al 1989) but catecholamines were not measured in the present study. ANP concentrations were unaffected by either agent. Urinary cyclic GMP actually fell during dopamine-enhanced natriuresis. ANP concentrations may fall during dopamine infusion in congestive heart failure (Fontana 1991) but this probably depends on whether significant changes in right atrial pressure occur during infusion.

g) Pressure natriuresis. Blood pressure increased only with dobutamine infusion in the present study and changes were modest, probably because the mean maximum dose of dopamine tolerated for the third hour was only 5μg/kg/min, which has little effect on peripheral vascular resistance or blood pressure. While maintaining a minimum arterial pressure clearly has a vital permissive role the diuretic and natriuretic actions of other agents, raising pressure alone does not appear to have a natriuretic action in patients with heart failure.

h) Decreased proximal tubular reabsorption of sodium leads to increased distal (and thus the macula densa) delivery of sodium and thus natriuresis, which is probably the mechanism for the diuresis caused by saline infusion (Martinez-Maldonado, Tsaparas, Eknoyan,

et al 1972) as well as that induced by prostaglandin infusion (Vander 1968; Daugherty, Belleau, Martino, et al 1968; Martinez-Maldonado, Tsaparas, Eknoyan, et al 1972). It is possible that this was the mechanism for the observed natriuresis in the present study.

Limitations of the study

(The limitations applying to all the studies have not been repeated). In Chapter 5, dobutamine infusion potentiated diuresis and natriuresis compared to sodium nitroprusside. In the present chapter, changes in diuresis and natriuresis with dobutamine were not significant but were directionally similar. The reason for the discrepancy between this study and the previous one is not clear, but probably relates to the small sample size and the modest changes in urine volume and natriuresis effected by the infusions. The role of chronic dopamine or dobutamine infusion was not addressed but recent clinical trials of inotropic agents in heart failure patients have demonstrated increased mortality in the treatment arm (Dies 1988; The Xamoterol in Severe Heart Failure Study Group 1990; Uretsky, Jessup, Konstam, et al 1990; Packer, Carver, Rodeheffer, et al 1991). The small sample size did not allow any assessment of the effect of other maintenance therapy such as digoxin or calcium antagonists although cardioactive drugs were omitted on study days to reduce possible errors.

Conclusion

Dopamine infusion at doses of 4-5μg/kg/min has a modest additive natriuretic and diuretic effect when administered with furosemide. The trend with dobutamine infusion failed to reach significance compared with placebo although differences between dobutamine and dopamine were not significant (with the exception of ERPF during low dose infusion). Significant

natriuretic actions similar to those seen with dopamine were recorded with dobutamine infusion in Chapter 5. This raises the question of whether any true difference exists between the effects of the two agents on diuresis and natriuresis or whether differences were due to the small sample size. The mechanism for the natriuresis probably relates to the beneficial adrenergic systemic and renal haemodynamic effects of dopamine and dobutamine increasing solute delivery to Henle's Loop where furosemide can act.

Chapter 10 Final discussion

Diuretic agents remain one of the mainstays of therapy for symptoms of heart failure and no-one would contest that they are lifesaving in acute heart failure. There is little direct evidence to support a beneficial effect of diuretics on mortality in patients with chronic heart failure, probably because it would be unethical to perform such a trial (Taylor 1993). The neurohormonal activation induced by diuretic administration may be harmful in the long-term (Gavras, Kremer, Brown, et al 1975; Packer 1992).

Blunting of the renal effects of furosemide is an important and costly clinical problem in heart failure. Treatment with combinations of loop diuretics and thiazide diuretics may restore diuresis but at the expense of further activation of the renin-angiotensin system and development of hyponatraemia. The diuretic sparing effects of infusion of dopamine and dobutamine are largely anecdotal and these agents are not practical for routine outpatient treatment (Unverferth, Blanford, Kates, et al 1980; Unverferth, Magorien, Altschuld, et al 1983; Robinson, Gariballa, Fancourt, et al 1994). A better understanding of the renal mechanisms in heart failure is of academic importance, while restoration of diuretic efficacy without further activation of the renin-angiotensin system remains a clinical challenge.

At the time of starting the project there was a lack of studies in this area. For this reason, a large number of small studies were performed so that future investigators would have some insights from which to work. The mechanisms of diuretic resistance were addressed, correlating changes in a furosemide induced diuresis and natriuresis with changes in urinary furosemide excretion, cyclic GMP excretion, neuroendocrine activation,

changes in arterial pressure and changes in renal haemodynamics. The decision to perform several studies with small numbers of patients rather than fewer studies with larger numbers was based on a need to explore potential therapeutic stategies without devoting the entire thesis to one or two negative studies, a situation which could easily have arisen had only the phentolamine and ANP studies been performed. The conflicting reports from animal, normal volunteer and heart failure studies in the literature show that it is impossible to predict which agents would be beneficial.

The studies presented do not have great statistical power, but the trends indicated in the digoxin and captopril studies can be confirmed in larger studies. Both diuresis and natriuresis were measured. In health, free water clearance occurs when plasma osmolality is low, and sodium excretion in excess of water excretion in the setting of high plasma osmolality and normal plasma volume. In heart failure, plasma volume is expanded and although there is total body sodium excess, plasma sodium is normal or reduced. Administration of furosemide prevents reabsorption of sodium, which enhances water clearance. Because of the technical difficulty in obtaining precise hourly urine volumes without indwelling urinary catheters, changes in urine volume were somewhat imprecise, while changes in sodium could be corrected for urine volume by expressing them as a ratio of creatinine excretion and as such were a more sensitive measure of changes in salt and water excretion. It is likely that diuresis and natriuresis occurred simultaneously.

Several of the agents investigated, drugs used in the treatment of acute decompensated heart failure, had little or no effect on the diuresis and natriuresis induced by furosemide in well controlled patients. One of the

reasons may be precisely because of the patient group chosen: stable patients with compensated heart failure. The omission of patients' morning diuretic dose on study days allowed a small positive fluid balance to develop during the course of the day. It should thus have been possible to increase a very modest diuresis with low-dose intravenous furosemide by the addition of other pharmacological agents. Although these negative results may be a reflection of the small sample size and lack of statistical power to detect changes, they do challenge the assumption of the benefit of such agents in chronic stable heart failure.

Captopril, digoxin and dobutamine appeared to improve natriuresis but not diuresis. These results were not unexpected, as all these agents have been used to treat heart failure, and have been shown to be natriuretic in some clinical settings. However no unifying hypothesis could be invoked for the mechanism. If the captopril study was excluded, the pressure hypothesis could be invoked, as in all the other studies a fall in blood pressure was associated with failure of the agent to enhance diuresis and natriuresis.

ACE inhibition with captopril potentiated natriuresis despite causing a fall in GFR and arterial pressure; this finding was in disagreement with the results of other investigators who maintain that a fall in blood pressure and GFR negates the enhancement of natriuresis by captopril (Motwani, Fenwick, Morton, et al 1992b). Although not measured in this study, increased concentrations of vasodilator prostaglandins induced by chronic therapy with captopril could have protected renal function despite the fall in perfusion pressure and GFR. Bradykinin is inactivated by two kininases, one being identical to angiotensin converting enzyme, thus it accumulates during ACE inhibitor treatment. Bradykinin has vasodilator properties in part due to

an increased conversion of arachidonic acid to prostaglandins. Prostaglandins have an important role in renal autoregulation and may be important modulators of the renal response to furosemide during chronic ACE inhibition. While it would have been interesting to study the effect of supression of prostaglandin synthesis by a non streroidal anti-inflammatory agent such as indomethacin, such a study was deemed unethical. Renal vasodilator prostaglandins may alter intrarenal haemodynamics and prevent the fall in blood pressure and glomerular filtration rate from causing a fall in renal plasma flow and diversion of blood to the medullary nephrons. Non steroidal anti-inflammatory drugs abolish the diuresis and natriuresis caused by captopril administration, at least in normal volunteers, although studies investigating the effects of chronic use are not available.

ACE inhibition may also influence vascular remodelling and alter baroreceptor function, maintaining renal perfusion pressure. Reduction in renal angiotensin II concentrations may thus cause enhanced sodium excretion because the kidney is in some way protected from the fall in blood pressure and glomerular filtration rate by chronic but not acute ACE inhibitor treatment

Potentiation of the natriuretic effect of furosemide by digoxin has not, to our knowledge been reported before, although several authors have reported natriuretic effects of cardiac glycosides on their own (Hyman, Jaques, Hyman 1956; Gavey, Parkinson 1939; Hull, Mackintosh 1977; Lloyd, Sandberg, Edwards 1992). The most probable cause for this was inhibition of the renal tubular Na^+-K^+-ATPase and improvement in renal perfusion. Although it was not possible to document any significant change in cardiac output or arterial pressure after intravenous digoxin, ERPF increased.

Although plasma renin concentrations did not change after digoxin infusion in the present study, digoxin does inhibit renin release from the kidney (Montanaro, Antonello, Baggio, et al 1980; Covit, Schaer, Sealey, et al 1983). It is possible that chronic administration of digoxin may confer additional renal benefits as a result of neuroendocrine modulation, particulary of the renin-angiotensin system and baroreceptor function.

The role of pressure-natriuresis in heart failure was assessed using infusions of dopamine, tyramine and dobutamine to elevate systemic pressure. Tyramine raised systolic blood pressure but had no significant effect on natriuresis and dobutamine raised blood pressure marginally, but improved natriuresis no more than dopamine, which failed to augment blood pressure. The failure of tyramine to enhance natriuresis may have been because renal sympathetic stimulation caused enhanced sodium retention and masked the effects of increased renal perfusion pressure (Lang, Rahman, Balfour, et al 1993a; Lang, Rahman, Balfour, et al 1993b). These results do not exclude a beneficial diuretic effect of raising blood pressure in heart failure.

In experimental models of heart failure, renal sympathectomy restores diuresis but in the setting of this study, the systemic effects of phentolamine caused a fall in perfusion pressure, stimulated the RAAS, diminished creatinine clearance and reduced solute delivery to the Loop of Henle in addition to blocking the renal α-adrenergic outflow. These sodium retaining effects outweighed any natriuretic effects.

In the present study, dopamine and dobutamine had little impact on a furosemide induced diuresis in stable compensated heart failure. In

addition, they were poorly tolerated at doses usually safely prescribed for decompensated heart failure. Ventricular bigeminy was the limiting factor in several patients with infusions of both agents, while sudden hypotension occurred in two patients during dobutamine infusion. Dopamine infusion caused chest pain when the dose was increased above 5μg/kg/min in 3 patients who had underlying ischaemic heart disease. Although these studies did not address the usefulness of dopamine infusions in acute heart failure, a recent study in the elderly did not show consistent benefit (Robinson, Gariballa, Fancourt, et al 1994). The high incidence of arrhythmias seen in our patients may also provide a mechanism for the adverse effect these agents have been shown to have on survival in patients with heart failure when administered on a chronic basis. The natriuretic properties of dobutamine differed little from those of dopamine in the present study and were seen at doses of 5-10μg/kg/minute but not at concentrations likely to stimulate the dopamine receptors specifically. These findings were more consistent with the concept of pressure natriuresis, raised ERPF and possible additional dopamine effect.

Afterload reducing agents such as sodium nitroprusside and phentolamine which improve cardiac output in situations of acute pulmonary congestion and cardiac decompensation cause significant hypotension when infused into stable, well diuresed patients, without any detectable diuretic-sparing effect despite an improvement in renal plasma flow. This once again highlights the importance of renal perfusion pressure in modulating diuresis and natriuresis.

The general lack of change in renal furosemide excretion in our studies suggests that increased delivery of furosemide to the loop of Henle is not the

mechanism for the improved natriuresis observed with digoxin, dopamine, dobutamine and captopril administration. We were not able to support Nomura's findings of a relationship between urinary furosemide levels and sodium excretion after different treatment modalities in our patient population.

Renal cyclic GMP excretion did not change significantly during enhanced natriuresis with digoxin and captopril and was thus probably not involved in the enhanced natriuretic response. Conversely, the pronounced rise in cyclic GMP excretion seen during ANP infusion was not accompanied by enhanced diuresis or natriuresis. The relatively low dose of ANP infused did not augment natriuresis or diuresis with furosemide. Although it is possible that larger doses of ANP might have augmented natriuresis, just as the blunted effects in heart failure can be partially overcome, it is more likely that hypotension would again have offset any natriuretic effects. The rise in concentrations of plasma active renin measured during high dose ANP infusion may be explained by the fall in blood pressure as the effect of ANP otherwise is to leave plasma renin activity unaffected or to suppress it.

Attempts to modify the diuretic response to furosemide in patients with heart failure by suppression of antinatriuretic systems tend to be confounded by activation of additional sodium conserving neuroendocrine systems, thus isolation and inhibition of a single system does not reverse the inappropriate sodium and water retention found in heart failure. Natriuresis was enhanced by agents with diverse effects on neuroendocrine activation but preservation of adequate renal perfusion appeared to be essential for the response.

Strategies for the future

The role of arginine vasopressin in diuretic resistance was not investigated. Elevated plasma concentrations of arginine vasopressin are associated with vasoconstriction and antidiuresis in heart failure but as yet, no suitable receptor antagonist is available for clinical studies (Laszlo, Laszlo, De Wied 1991). Such antagonists may have an adjunctive role in the treatment of heart failure in the future.

Neutral endopeptidase (NEP) is a protease which inactivates a number of peptides including ANP. Inhibitors of NEP cause concentrations of ANP to rise and the diuretic and natriuretic response is greater in relation to the plasma concentration of ANP than is found with infusion of ANP in patients with heart failure. The combination of an ACE inhibitor and a neutral endopeptidase inhibitor may be beneficial. This could result in supression of the renin-angiotensin system, the sympathetic nervous system and argenine vasopressin as well as protection of GFR and ERPF and appropriate natriuretic and diuretic properties. Whether these actions will all be preserved when the agents are combined remains to be seen, but a recent study in a rat model of heart failure proved disappointing (Helin 1993).

The assumption is made that the statistically significant and insignificant results were genuine. This may not have been the case as the sample size was small and the power to detect differences may not have been adequate in all studies for all measurements. However the mean differences were generally small where no significant changes were found which suggests that the lack of differences were genuine.

The patients studied had adequately treated, compensated, stable heart failure, unlike patients being admitted for relief of acute left ventricular failure. The effect of a fall in blood pressure and thus renal perfusion pressure offset any gains from neuroendocrine manipulation. There is no evidence that salt restriction is any less harmful than increasing the diuretic dosage in stable, compensated patients.

Table 10.1 Haemodynamic effects with all interventions

The table shows increases (>>), decreases (<<) or lack of change (NC) of blood pressure (BP), heart rate (HR), effective renal plasma flow (ERPF), glomerular filtration rate (GFR) and urinary sodium excretion (UNa) for each intervention.

INTERVENTION	BP	HR	ERPF	GFR	UNa
SALT RESTRICTION.	≪	NC	≪	NC	≪
CAPTOPRIL	≪	NC	≫	≪	≫
DOBUTAMINE	≫	≫	≫	NC	≫
SNP	≪	NC	≫	NC	NC
DIGOXIN	NC	≪	≫	NC	≫
ANP	≪	NC	≫	NC	NC
TYRAMINE	≫	NC	NC	NC	NC
PHENTOLAMINE	≪	≫	≫	NC	NC
DOPAMINE	NC	NC	≫	NC	≫

Table 10.2 Endocrine effects with all interventions

The table shows increases (>>), decreases (<<) or lack of change (NC) of plasma concentrations of atrial natriuretic peptide (ANP), plasma active renin (plasma renin), noradrenaline (NADREN), of urinary cyclic GMP (cGMP) and of urinary sodium excretion (UNa) during the interventions.

INTERVENTION	ANP	renin	NADREN	cGMP	UNa
SALT RESTRICTION	≪	≫	≫	-	≪
CAPTOPRIL	-	≫	-	NC	≫
DOBUTAMINE	NC	≫	-	NC	≫
SNP	≪	NC	≫	NC	NC
DIGOXIN	NC	NC	NC	NC	≫
ANP	≫	≫	NC	≫	NC
TYRAMINE	NC	≪	NC	NC	NC
PHENTOLAMINE	≫	≫	≫	NC	NC
DOPAMINE	NC	NC	-	NC	≫

Abbreviations used in the text

ACE	Angiotensin converting enzyme
ADREN	Adrenaline
A-I	Angiotensin-I
A-II	Angiotensin II
ANP	Atrial natriuretic peptide
AVP	Arginine vasopressin
BP	Blood pressure
cGMP	Guanosine 3',5'-cyclic monophosphate
Cl	Chloride
Cr, Creat	Creatinine
ERPF	Effective renal plasma flow
GFR	Glomerular filtration rate
HR	Heart rate
K	Potassium
Mg	Magnesium
Na	Sodium
NADREN	Noradrenaline
Na-K-ATPase	Sodium, potassium adenosine triphosphatase
PARC	Plasma active renin concentration
Phos	Phosphate
RAAS	Renin-angiotensin-aldosterone system
SNP	Sodium nitroprusside
TBI	Thoracic bioimpedance
U	Urea

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