

ELECTROPHYSIOLOGICAL MECHANISM OF VENTRICULAR
AUTOMATICITY. A MODEL FOR VENTRICULAR ARRHYTHMIAS

A Thesis submitted

by

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ABSTRACT

Motivation

Ventricular arrhythmias are difficult to study in man. The current experimental models are arrhythmias induced by electrical stimulation, coronary artery ligation or by subsequent reperfusion. An electrophysiological model will be useful for exploring the cellular mechanism of arrhythmias and for studying the mechanism of action of new anti-arrhythmic drugs. This project seeks to establish automaticity as a model for studying ventricular arrhythmias.

Objectives

1. To review the literature on the mechanism of ventricular arrhythmias.
2. To explore ventricular automaticity induced by "reperfusion" after O_2 and substrate deprivation.
3. To explore beta-adrenoceptor mediated ventricular automaticity.
4. To evaluate possible new anti-arrhythmic drugs, carminomycin and ketanserin.

Findings

ELECTROPHYSIOLOGICAL MECHANISM OF VENTRICULAR ARRHYTHMIAS. Presently three concepts are proposed to explain the trigger for ventricular arrhythmias: (1) abnormal pacemakers evoked by oscillatory after-potentials; (2) the injury current which is believed to be induced by the electrical polarity across the border zone between infarcted and normal myocardium; and (3) re-entry of a delayed impulse to a region suffering a reduced refractory period. None of these mechanisms have been directly related to ventricular arrhythmias. Nevertheless

it is possible that one or all three mechanisms might play a role.

"REPERFUSION"-INDUCED AUTOMATICITY. The effects of simulated "ischemia" (hypoxia, O_2 tension <35 mmHg and glucose-free medium) and simulated "reperfusion" (return to normal medium) were investigated in the guinea-pig papillary muscle. "Ischemia" depolarized the resting membrane potential, reduced action potential amplitude and duration and contractile force and increased resting tension. "Reperfusion" reversed the effects of "ischemia" on the action potential and muscle tension, but induced oscillating afterpotentials which preceded the onset of and appeared to evoke full non-driven depolarizations (extrasystoles) which could develop into self-sustained rhythm (automaticity). The automaticity occurred in all of 13 experiments within 10-20 sec of "reperfusion" after 40 min of hypoxic glucose-free medium. Reducing the duration of "ischemia" from 40 to 30 or 25 min, decreased the incidence of automaticity (100 to 60 or 33%) but adding isoprenaline ($1 \mu\text{M}$) or increasing the stimulation rate (3 versus 0.5 Hz) during the 25 min of "ischemia" induced "reperfusion" automaticity in all the experiments. With only hypoxia or only glucose-free medium (40 min), there was no "reperfusion" automaticity. Adding (in the "ischemic" period) a high concentration of lidocaine (0.25 mM) completely, and verapamil ($5 \mu\text{M}$) partially prevented the "reperfusion" rhythm abnormalities, whereas propranolol ($0.1 \mu\text{M}$) had no effect. My findings suggest that "reperfusion" automaticity might be mediated by calcium-dependent afterpotentials.

I propose that ventricular automaticity occurring after temporary withdrawal of glucose and O_2 might be a model for reperfusion ventricular arrhythmias.

ISOPRENALINE-INDUCED AUTOMATICITY. Isoprenaline-induced oscillations of membrane potential and resting tension in the guinea-pig papillary muscle are described. The afterpotentials were elicited by increasing external stimulation from the basic rate of 0.5 Hz. When the afterpotentials reached a threshold potential of about -75 mV they evoked non-driven extrasystoles which could develop into automaticity. Aftercontractions corresponding to the afterpotentials, and automaticity were reflected in the contractile force. The incidence of automaticity was 10, 40 or 63% when the isoprenaline concentration was 0.1, 1.0 or 10 mM respectively. When the medium potassium was reduced (5.9 versus 2.7 or 1.2 mM), the automaticity induced by isoprenaline (1 μ M) was increased (40 versus 47 or 85%). In the presence of ouabain (0.7 μ M), the automaticity induced by isoprenaline also increased (40 versus 86%). Verapamil (1 μ M) or lidocaine (100 μ M) completely prevented the isoprenaline-ouabain-induced automaticity, whereas the reduction by lidocaine 10 μ M was not significant. It is concluded that membrane oscillations evoked by an elevated cytosolic calcium might play a role in the automaticity induced by beta-adrenoceptor stimulation in ventricular myocardium.

CARMINOMYCIN. In the papillary muscle, carminomycin (10 μ M) reduced the incidence of isoprenaline-ouabain-induced automaticity (40 versus 86%).

Carminomycin was further investigated for acute cardiotoxicity in the isolated working rat heart. Carminomycin (17.5 μ M) after 55 min of addition, reduced cardiac output by 56%, left ventricular power production by 64% and efficiency of work by 61%. O_2 consumption, heart rate and coronary flow were not affected. Fall in tissue ATP and phosphocreatine was 38% and 10% respectively.

Coenzyme Q₁₀, a mitochondrial extract, restored the high energy compounds but could not prevent mechanical failure. From the preceding data, it was inferred that since afterload and preload were constant in this model, heart failure occurred because the inotropic state was depressed. Procedures that increased cytosolic calcium, relieved heart failure: viz. pre-treatment with digoxin (62.4 µg), isoprenaline (1 µM) and increased perfusate Ca²⁺ (5 mM versus 2.5 mM), all prevented carminomycin-induced fall in cardiac output (41 ± 1, 47 ± 5 and 52 ± 1 respectively versus 26 ± 2 ml/min).

Conclusion: A lowered cytosolic calcium and decreased energy stores might cause the contractile failure. The cytosolic calcium and high energy phosphate compounds were lowered by separate mechanisms.

KETANSERIN (10 µM) reduced the incidence of isoprenaline-ouabain-induced automaticity (29 versus 86%). However, ketanserin (10 µM) after 40 min reduced cardiac output (by 16%) and heart rate (by 39%) in isolated working hearts. The ketanserin-induced fall in cardiac output was not associated with alteration in myocardial O₂ consumption, high energy compound stores, cyclic AMP or left ventricular power production. Heart failure was most probably due to the reduction in heart rate.

CONCLUSIONS

1. "Reperfusion" or isoprenaline may induce ventricular automaticity.
2. Automaticity may arise from membrane afterpotentials.
3. Elevated internal calcium may provoke afterpotentials.
4. "Reperfusion" and isoprenaline induced automaticity could be a model to explore the mechanism of arrhythmias and new anti-arrhythmic drugs.

5. Carminomycin and ketanserin might have anti-arrhythmic potential but in a dose range that produces acute cardiotoxicity. The drugs may nevertheless present a tool to explore the cellular mechanism of automaticity.

INTRODUCTION

Ideally ventricular arrhythmias should be investigated in humans. But safety, cost and ethical considerations have restrained the use of invasive studies in clinical research. Consequently, for over a century, cardiac arrhythmias have been explored in animal experiments. Attention has been focussed mainly on ventricular fibrillation because it is fatal. Premature extrasystoles and ventricular tachycardia might also be important because they are believed to evoke ventricular fibrillation. However, this view has not been supported by direct evidence. As for mechanism, abnormal pacemaker foci and re-entrant excitation have been proposed but neither mechanism has been causally related to ventricular arrhythmias.

Mechanism of Ventricular Arrhythmias: Re-entry versus Focal Automaticity

At about the middle of the nineteenth century, it was thought that a "metabolic poison" was responsible for fibrillation. In 1884, Kronecker and Schemy observed fibrillation when the ventricular septum was pierced at the junction of the upper- and middle-thirds. They concluded fibrillation had occurred because they had destroyed the "coordination center" of the ventricle. This theory was laid to rest by McWilliam in 1887. He said that permanent damage to a "coordination center" was unlikely because in several species recovery from fibrillation was possible. The idea of a "coordination center" became more unlikely when it was demonstrated that the amputated lower two-thirds of the ventricular septum was made to fibrillate by electrical stimulation. McWilliam attributed fibrillation to altered contractile properties of muscle fibres. His explanation was

that when the myocardium became "excited", fibrillation was initiated by a rapid succession of incoordinated peristaltic contractions that travelled along complex inter-communicating bundles to return from several directions to fibres that it had already passed. In 1894, Porter postulated that conduction blocks caused the impulse to take a devious path. This idea reinforced McWilliam's conception and laid the basis for the circus theory which holds that fibrillation is sustained by an impulse that travels in a circuitous path and returns to re-excite its site of origin to repeat the circus.

In 1896, Engelmann proposed the alternate hypothesis that each heart muscle fibre was an independent focus of impulse formation during fibrillation. This view was upheld for over a decade by many prominent workers including Lewis who in 1910 emphasized the importance of single or multiple ectopic impulses in the origin of fibrillation.

Mines and Garrey (1913 to 1914) re-introduced the circus hypothesis. Mines observed in auricular electrograms that electrically induced ectopic impulses had shorter cycles and, therefore, shorter refractory periods. Also, these impulses conducted more slowly than the sinus excitation. He proposed that such an ectopic impulse could, because of slow conduction, return to the site of origin which is ready to restart the cycle because of the shortened refractory period. In 1914, Garrey independently came to the same conclusions using different techniques. He demonstrated that bits of tissue shaved off a fibrillating heart ceased to fibrillate. The pieces could be made to contract rhythmically by an external electrical stimulus. He also found that fibrillation was more likely to be sustained in larger hearts. From these findings he inferred that a mass of myocardium was required

to establish the circus movement necessary to sustain fibrillation. Thereupon he examined the behaviour of contraction in excised rings of auricular and ventricular myocardium. Heart strips are capable of conducting in both directions with equal facility. But Garrey showed circular conduction in one direction. He, therefore, assumed a uni-directional conduction block. Thus Mines and Garrey re-established the circus theory. Garrey, in 1924, gave us the following very full description of ventricular fibrillation:

"an incoordinate, disorderly and extremely bizarre contractile process in which normal systole and diastole no longer occur, the impression being given that individual fibres or groups of fibres are contracting independently (hence the name fibrillation). While certain regions of the fibrillating tissue are at rest, other adjacent areas or areas widely separated from each other may show synchronous contraction. The surface of the fibrillating chamber shows areas of fine twitchings, of flickering and tremulous movements, combined with coarser undulating waves of muscular contraction which progress slowly through the muscle mass, moving now in one direction, now in another, being continually blocked in their progress by interference with other waves".

In 1928, Schmitt and Erlanger presented more direct evidence for decremental conduction and re-entry. They passed strips of ventricle through 4 chambers (1, 2, 3, 4) separated by rubber curtains that fitted snugly around the muscle. Contractions were monitored in each chamber. On stimulation at one end, contraction would be conducted in one direction and in the opposite direction when stimulated at the other end. By placing a high potassium solution in chambers 1 and 2, they were able to slow conduction more in one direction than in the other. They were also able to show that slow conduction travelling in the direction

of chamber 4 could return to elicit a response in chamber 2 and thereafter in chamber 1. Schmitt and Erlanger believed that this experiment demonstrated the phenomenon of re-entry. Their explanation was based on the arrangement of muscle bundles. They postulated that when a sinus impulse is blocked in a depressed muscle fibre, it might be conducted slowly in the adjacent fibre to return along the fibre suffering the forward block. Arriving back at the myocardium which by that time has recovered from the sinus excitation, the reflected impulse can excite the myocardium before the next sinus wave. In this way, a repetitive re-entrant circus could be established. These workers also conceived that slowed conduction in Purkinje fibres could also evoke re-entry circuits. An impulse travelling along Purkinje fibre might reach the same ventricular fibre by two branches. If one branch suffers unidirectional conduction block, the impulse will reach the ventricular fibre by the second branch. Excitation from the ventricular fibre might travel up the first branch to the second branch and thereby establish a repetitive re-entry circuit.

In the period 1930 to 1943, Wiggers, Harris and Moe, after extensive experimentation, proposed that whereas ventricular fibrillation was sustained by re-entrant circuits, it was initiated by single or multiple extrasystoles. By simultaneous cinematographic and electrogram techniques, they studied the evolution of ventricular fibrillation which they induced electrically or by coronary artery ligation (Wiggers, 1930; Moe et al, 1941; Harris and Rojas, 1943). Their main observation was that fibrillation introduced by electrical shock or coronary artery ligation was always "introduced by an accelerating series of ventricular ectopic beats". The ectopic beats in the coronary artery ligation model originated in the border zone. Recently, it has also been shown that

ventricular fibrillation occurring soon after coronary occlusion is associated with premature extrasystoles (Janse et al, 1980; Janse and Capelle, 1982). These extrasystoles originate from normal myocardium on the non-ischemic side near the border zone.

A similar prelude to ventricular fibrillation has also been found in humans. Holter monitoring has revealed that increasing salvos of ventricular extrasystoles and ventricular tachycardia almost always precede ventricular fibrillation (Panidis and Morganroth, 1983; Pratt et al, 1983).

Current Theories of Abnormal Impulse Formation

It is now appropriate to consider how premature extrasystoles are evoked and how single or multiple extrasystoles might cause re-entry. Premature extrasystoles might arise by three possible mechanisms. They might be triggered by an injury current, by re-entry of a slowly conducted impulse or by oscillating membrane potentials.

INJURY CURRENT. This mechanism might operate during regional ischaemia and was first proposed by Harris in 1950. He reasoned that the difference in potential that exists between the ischemic zone and non-ischemic zone might generate a current which he called the "injury current". He suggested that the injury current would evoke the ectopic impulses in the border zone. The disparity in potential across the border zone is reflected in epicardial electrograms. ST-segment elevation is recorded over a region of evolving infarction whereas ST-segment depression occurs over the non-ischemic zone (Brofman, 1956). Intracellular microelectrode recordings demonstrate that the current flow will change direction during the cardiac cycle. In the ischemic region there is marked reduction of amplitude and duration of the action potential and maximum resting potential is depolarized (Kardesch et al,

1958; Samson and Scher, 1960). Therefore, in systole, the cells in the ischemic region are more negative than cells in the non-ischemic region. In diastole the reverse occurs. Due to the depolarized resting potential, the cells in the ischemic region are more positive than the non-ischemic cells. The earlier repolarization of the ischemic cells further increases the polarity between the ischemic and non-ischemic cells. By direct-current magnetocardiogram techniques it has been shown that the S-T shift during coronary artery occlusion is due to an injury current (Cohen and Kaufman, 1975). Other workers have demonstrated that the injury current might induce automaticity in the papillary muscle (Katzung et al, 1975). Recently premature beats arising from the normal side of the border zone have been thought to be triggered by injury currents (Janse and Capelle, 1982). However, there is no evidence that the injury currents directly induce automaticity during myocardial infarction.

RE-ENTRY. Another possibility is that extrasystoles might be triggered by an impulse reaching repolarized cells at the border zone after being conducted slowly through the infarct. Conduction in the ischemic region may be delayed by as much as 200 msec (Durrer et al, 1971; Boineau and Cox, 1973). The possibility that such a delayed impulse might evoke extrasystoles at the border zone is strengthened by the observation of persistent electrogram activity, over the infarct, between the sinus beat and the extrasystole (Boineau and Cox, 1973; Waldo and Kaiser, 1973). However, a direct connection between a delayed impulse and a premature extrasystole has not been demonstrated. Instead extrasystoles arising from the non-ischemic side of the border are separated from the impulse in the ischemic region by an inexcitable zone (Janse and Capelle, 1982). It is, therefore, argued

that it is unlikely that extrasystoles are induced by a delayed impulse.

OSCILLATORY MEMBRANE POTENTIALS. Oscillating afterpotentials were first associated with arrhythmias in 1943 by Harris and Rojas. The oscillations appeared on epicardial electrograms, the leads of which were placed at the sites where premature beats originated. The premature beats were induced by electrical shock or coronary occlusion. However, it was not clear if the oscillations gave rise to the ectopic impulses. In the same year, similar oscillatory afterpotentials were recorded by external electrodes in ventricular strips (Bozler, 1943). These afterpotentials which are induced by high calcium solutions and enhanced by adrenaline, trigger single or bursts of impulses. After the introduction of intracellular microelectrode techniques, membrane oscillatory afterpotentials have been recorded under a variety of conditions. Membrane oscillations are described at depolarized potentials or at normal resting potentials. At the less negative potential (-60 to -40 mV) membrane oscillations in Purkinje fibres are described as being produced by the interplay of the slow inward current and the delayed rectifier (Hauswirth et al, 1969). In ventricular muscle similar membrane oscillations are evoked by passing depolarizing DC currents (Katzung and Morgenstern, 1977). At normal resting potentials and following driven action potentials, membrane oscillations are induced in Purkinje fibres by cardiotonic steroids (Davis, 1973; Ferrier et al, 1973), pacing in the presence of norepinephrine (Vassalle and Carpentier, 1972), or external potassium depletion (Eisner and Lederer, 1979a); in mitral valve and coronary sinus by catecholamines (Wit and Cranefield, 1976 to 1977); in ventricular muscle by digoxin (Adamantidis et al, 1983) or by potassium free, high calcium medium (Hiraoka et al, 1981); and in isolated single ventricular cells by injection of intracellular calcium (Matsuda et al, 1982).

How Might Premature Extrasystoles Cause Ventricular Fibrillation?

When a premature extrasystole interrupts a normally propagated sinus excitation, it might set the stage for re-entrant circuits. It is believed that multiple re-entrant circuits sustain ventricular fibrillation. The properties of premature extrasystoles that induce re-entry are as follows: First, by action potential recordings it has been shown that the phase of rapid depolarization (the fast sodium channel activity) is reduced (Noble, 1979). Slowing of the rapid depolarization phase of the action potential results in reduced conduction velocity (Weidmann, 1951). Secondly, since the premature impulse is conducted in the wake of the propagated sinus excitation, its own wave of excitation will in different directions meet cells that are at various stages of repolarization. This dispersion of refractoriness is demonstrated by the unequal recovery of excitability in the cells surrounding an ectopic focus (Han and Moe, 1964).

In addition, inexcitability in some groups of cells might cause conduction block. Thirdly, the shortened action potential duration of a premature impulse will result in a shortened refractory period (Noble, 1979). It is conceivable that the slowed conduction, conduction block and dispersion may deflect the premature impulse to travel a devious and circuitous path to return to the site of origin where excitability is prematurely restored due to the shortened refractory period (Han, 1969). Multiple re-entrant circuits are thought to sustain ventricular fibrillation (Surawicz et al, 1967).

Other Factors That Might Contribute to Arrhythmogenesis

Acidosis, free fatty acids, lactate, lysophosphatides, fatty acyl

carnitine, cyclic 3'5' monophosphate and reduction of cellular high energy phosphate compounds have all been incriminated in the mechanism of ventricular arrhythmias (Corday et al, 1977; Corr and Sobel, 1979; Hjalmarson, 1980; Opie et al, 1979; Russell, 1982). Although in regional ischemia the biochemical changes correspond to profound alterations of electrogram and intracellular microelectrode recordings, the changes could not be related to arrhythmias (Moréna et al, 1980).

It has also been proposed that contraction abnormalities might initiate ventricular arrhythmias. Ventricular arrhythmias associated with abnormalities of ventricular contraction or direct mechanical stimuli have been extensively reviewed (Goldberg, 1977) but the cellular mechanism is not known.

Possible Role of Excessive Beta-Adrenoceptor Activity

Considerable evidence has accumulated to link excessive beta-adrenoceptor activity with ventricular fibrillation. In humans, attention has been focussed on arrhythmias during anxiety (Goble, 1965; Horan and Venables, 1962; Järvinen, 1955). Serum catecholamine levels are elevated when ventricular arrhythmias occur during physical and emotional stress (Taggart et al, 1972 to 1973). In post mortem studies of hearts from victims of instantaneous coronary death (without evidence of acute myocardial infarction), a focal coagulative myocytolysis thought to be induced by excessive catecholamine stimulation was observed (Baroldi, 1975). More direct evidence was obtained when ventricular fibrillation was induced by catecholamines in children having the Adams-Stokes syndrome (Coumel et al, 1978). Reduction in the risk of sudden death by giving beta-adrenoceptor blocking drugs to patients

surviving acute myocardial infarction (Multi-Centre International Study, 1975; Wilhelmsson et al, 1974) also suggest a role for catecholamines.

In animal experiments, the evidence for a beta-adrenoceptor mechanism is as follows: (1) In conscious dogs, psychological stress produces ventricular fibrillation (Corbalan et al, 1974). Stress during coronary occlusion induced spontaneous arrhythmias in the same dogs. The fall in ventricular fibrillation threshold due to stress was prevented by beta-adrenoceptor blocking drugs (Matta et al, 1976). (2) In open chest dogs, cardiac nerve stimulation causes abnormal pacemaker activity (Armour et al, 1972). (3) During coronary artery ligation, sympathetic nerve stimulation is associated with ventricular arrhythmias (Gillis, 1971) and fall in ventricular fibrillation threshold (Kliks et al, 1975). Sympathetic ablation reduces coronary occlusion-induced fall in ventricular fibrillation threshold (Kliks et al, 1975), and spontaneous arrhythmias (Harris et al, 1951). (4) Catecholamine infusion in dogs reduces the ventricular fibrillation threshold (Hoffman et al, 1955). (5) Ventricular arrhythmias induced with acute coronary artery occlusion are associated with increased release of catecholamines (Ceremużynski et al, 1968). (6) Catecholamine-induced arrhythmias are blocked by beta-adrenoceptor blocking drugs (Somani and Lum, 1965).

Approaches Adopted in the Present Studies

In spite of extensive investigation, it is clear that injury currents, re-entry or oscillatory afterpotentials have not been directly shown to be causally related to ventricular arrhythmias. Nevertheless, these concepts deserve careful evaluation. Moreover, if these phenomena are easily established, they may be of value for the preliminary in vitro testing and definition of the mechanism of new anti-arrhythmic drugs. This approach is particularly valuable since presently no ideal anti-

arrhythmic drug exists. Although in some studies anti-arrhythmic drugs reduce the mortality after myocardial ischemia (Lie et al, 1974; Multi-Centre International Study, 1975; Wilhelmsson, 1974), other reports question the value of anti-arrhythmic therapy in patients (Adgey and Webb, 1979; Clausen et al, 1966; Dhurandhar et al, 1971; Pantridge and Geddes, 1974). In general, currently available anti-arrhythmic drugs have a doubtful protective effect and have side-effects.

The automaticity induced by oscillatory afterpotentials might be a useful model of ventricular arrhythmias. But, as already described, afterpotentials are only produced by exceptional conditions in ventricular muscle. In the present studies, for the first time, are described automaticity induced by return to normal medium ("reperfusion") after hypoxic glucose-free superfusion and automaticity induced by isoprenaline in the isolated guinea-pig ventricle. Hypoxia and glucose deprivation were the minimum components of regional ischemia required to induce the "reperfusion" automaticity which might correspond to the ventricular arrhythmias evoked by release after regional ischemia. Reperfusion ventricular fibrillation has been used as a model because it is a highly reproducible phenomenon.

Also described are indirect procedures used to relate internal calcium elevation to the automaticity. Isoprenaline was used to provoke automaticity to strengthen the argument for the role of calcium.

Finally, two new drugs, carminomycin and ketanserin, were explored for possible anti-arrhythmic potency and for adverse cardiac effects.

CARMINOMYCIN has recently been added to the anthracycline group of drugs used to treat cancer. A preliminary study revealed that carminomycin prevented ventricular arrhythmias induced by coronary occlusion or by subsequent reperfusion in isolated Langendorff hearts. The effects of carminomycin on ventricular automaticity and myocardial function were studied.

KETANSERIN is a serotonin antagonist used to treat hypertension. Ketanserin also prevented ischemic and reperfusion arrhythmias in the Langendorff model. The effects of ketanserin were studied on ventricular automaticity and myocardial function.

EXPERIMENTAL MODEL AND METHODS

The mechanism of serious ventricular arrhythmias should ideally be resolved in humans. But the unexpected onset and rapid progress of ventricular fibrillation to death, preclude the fulfilment of the rigorous demands of scientific discipline. Cost, ethical and safety considerations place a further restraint on the use of invasive techniques in man. As an alternative, animal models have been extensively used to study ventricular arrhythmias. In vitro preparations may clarify pathophysiological mechanisms as long as rational objectives are defined. Experimental models may also be of value for the preliminary assessment of the efficacy and toxicity of new drugs.

When drawing conclusions about sudden coronary death from animal experiments, one should be mindful that ventricular fibrillation occurs in hearts that might have focal areas of necrosis from the long standing coronary atherosclerosis. There is no corresponding animal model that is suitable. Nevertheless, when extreme caution is applied in extrapolating from the tissue bath to the clinical situation and careful consideration is given to the goals set for a particular model, useful advances in the clarification of a cellular mechanism may be made by animal experimentation.

In the present studies, further clarity on the electrophysiological mechanism of ventricular arrhythmias was sought in the superfused guinea pig papillary muscle by micro-electrode techniques. The papillary muscle has been used as a model for cellular electrophysiology for over three decades. The rhythm disturbances that were induced in this preparation were also used to explore the anti-arrhythmic potential of carminomycin and ketanserin. Undesirable cardiac effects were looked for in the isolated perfused working rat heart. The working heart was used to

correlate mechanical and biochemical function (Neely et al, 1967).

Papillary Muscle Model

Guinea-pigs (200 to 450 g; 5 to 8 weeks old), fed ad lib were killed by cervical dislocation. The hearts were excised rapidly and arrested in ice-cold Krebs-Henseleit buffer. Small right ventricular papillary muscles were dissected in oxygenated ice-cold Krebs solution and mounted horizontally in a 3 ml organ bath. The basal end of the muscle was pulled firmly against a platinum stimulating electrode by a silk thread which was affixed to an immovable mount. A second stimulating electrode was adjusted alongside the muscle. Both stimulating electrodes were isolated, except for the parts exposed to the muscle. A silk thread tied to the tendinous end attached the muscle to a force displacement transducer (Grass-FTO3G). Once the muscles were mounted, resting tension was adjusted to the peak of their length-tension curve by a micrometer screw attached to the transducer. Resting and developed tension were continuously recorded on a Grass ink pen recorder. A stimulator and a stimulus isolation unit (Grass-588 and SIU5) were used to drive the muscle at a basic rate of 30/min, the stimulus voltage being 10% above threshold.

Glass micro-electrodes were pulled and filled with 2.7 mM KCl solution using conventional techniques. Impalements were made (in the superficial cell layer) with floating micro-electrodes, having DC tip resistance between 10 and 30 m Ω , suspended by flexible Ag.AgCl wire. The tissue bath was grounded with another Ag.AgCl wire. The action potential signal was amplified by a modified Frederick Haer band pass amplifier and was displayed on a storage oscilloscope (Tektronix 5103). Polaroid photographs were taken of individual action potentials. In addition, the maximum diastolic potential and action potential amplitude plateau duration (at 0 mV) and duration (at 90% repolarization) were

estimated by a microprocessor system based on the Motorola 6800 family. The analogue action and diastolic potential was converted to a digital format and analysed by software and resident firmware. Data was displayed on a video display unit and at a touch of a key, the parameters of ten action potentials obtained over a period of thirty seconds would be printed.

The perfusate used was a Krebs-Henseleit (Krebs and Henseleit, 1932) bicarbonate buffer with 10 mM glucose as external substrate, and with an ionic composition of NaCl 118.5 mM; KCl 4.7 mM; CaCl₂ 2.5 mM; KH₂PO₄ 1.19 mM; MgSO₄ 1.19 mM; and NaHCO₃ 25 mM. Flow rate in the bath was 20 ± 1 ml/min. Glucose-free medium was obtained by substituting equimolar D-mannitol to prevent osmolality changes. The temperature in the bath ranged between 34.8 and 35.2°C. When the medium was bubbled with 95% O₂ and 5% CO₂ the bath O₂ tension was over 420 mmHg and pH ranged between 7.33 and 7.47. Hypoxia (O₂ under 35 mmHg) was obtained by equilibrating the medium with a gas mixture of 95% N₂ and 5% CO₂ without a change in pH.

Critique of the Papillary Muscle Model

The papillary muscle may not be a reliable model because of the possible effects of a hypoxic core (Paradise et al, 1981). We, therefore, took the following precautions: (1) small muscles (of less than 1.2 mm² cross-sectional area) were selected; (2) the basic stimulation rate was 0.5 Hz; (3) bath temperature was 35°C and in some experiments reduced to 25°C to further ensure adequate oxygenation. At the latter temperature, rhythm disturbances occurred suggesting that it was unlikely that hypoxic core played a role. In spite of these precautions a quantitative evaluation of the resting and developed tension was not attempted and instead the muscle tension results are presented

quantitatively. The microelectrode recordings were made from superficial cells which were adequately oxygenated. Therefore, reliable conclusions could be made from the membrane resting and action potential data.

It is possible that Purkinje fibres were present in the papillary muscles. Histological examinations were not done to resolve this point. However, the cellular recordings were definitely made from ventricular cells as determined by the action potential configuration. The features that indicated that ventricular fibres were impaled were the shorter action potential duration and the more positive level of the plateau than would be expected for a Purkinje fibre. Also, diastolic depolarizations (phase 4) that are characteristic of a Purkinje fibre were not present.

Conclusions from the papillary muscle can only be made for endocardial ventricular cells and not for epicardial or medial myocardium. Heart cells from different sites may not have the same response to artificial medium since the endothelial cells also derive oxygen and energy substrates from cavity blood.

The advantages of the superfused papillary muscle are:

(1) the effects of anaesthetics, and changes in blood pressure or heart rate are excluded; (2) autonomic and hormonal homeostatic reflexes are not present; (3) a simple trace of resting and contractile force could be related to micro-electrode recordings; (4) energy production could be reduced simply by omitting oxygen and glucose from the superfusate; and (5) the effects of external ion concentrations could easily be varied in the medium.

Isolated working heart

Hearts (excised from male Long-Evans rats, weight 300 ± 50 g) were rapidly arrested in Krebs-Henseleit buffer at 4°C . The hearts were pre-perfused via the aorta by the Langendorff method at a hydrostatic

pressure of 65 cmH₂O for 15 minutes and thereafter perfused by the left atrial method (Neely et al, 1967) for a further 75 minutes at a filling pressure of 10 cmH₂O, working against a hydrostatic pressure of 100 cm. 100 ml of perfusing medium was recycled. The perfusate was a Krebs-Henseleit bicarbonate buffer equilibrated with 95% O₂ 5% CO₂. 5.0 mM CaCl₂ solution was achieved by doubling the CaCl₂ added to the solution.

Aortic pressure was measured by a Statham P23DB pressure transducer and monitored on a Device M2 direct writer, Welwyn Garden City, Hertfordshire, UK. Power production (Kannengiesser et al, 1979) was measured as the sum of pressure and kinetic power by the formulae:

$$\text{Pressure power} = \dot{W}_p = 0.002222 \times P_s \times \text{CO}$$

$$\text{Kinetic power} = \dot{W}_k = \frac{1}{432 \times 10^7} \times \frac{d (\text{CO})^3}{A^2} \times \left(\frac{T}{T_e} \right)^2$$

where \dot{W}_p = pressure power; P_s = peak systolic pressure; CO = cardiac output; \dot{W}_k = kinetic power; d = density of perfusate; A = internal cross-sectional area of aortic cannula; T = cycle time and T_e = ejection time. Units are MW (mJ/s) for kinetic power, mmHg for pressure power, ml/min for cardiac output, g/cm³ for density and cm² for area. The perfusate density was taken as 1 g/cm³.

The efficiency of mechanical work was the total power production (pressure plus kinetic) divided by the oxygen uptake.

Required concentrations of carminomycin (10 μM and 17.5 μM) were achieved in the perfusate by addition of a solution to the atrial perfusion at 20 minutes of working heart. Solutions were prepared from the mannitol-stabilized powder of carminomycin HCl. In one series, rats were pre-treated with vehicle-free coenzyme Q₁₀ (10 μg/kg) via the tail vein at 2 hours and again at half-an-hour before sacrifice, in

two equally divided doses. In another series rats were pre-treated with digoxin (62.4 μg) via the tail vein 1 hour before sacrifice (Burns and Dow, 1980), when the mean serum concentration was measured to be 20 ± 2.8 nmol/l. Isoprenaline (1 μM) was achieved in the perfusate by addition 10 minutes before carminomycin was added.

Required concentrations of ketanserin (0.1 to 10 μM) were obtained by adding a solution of ketanserin present as a tatarate (Janssen Pharmaceutica).

At the end of the experiments, the hearts were freeze-clamped by aluminium tongs (Wollenberger et al, 1960) cooled to the temperature of liquid nitrogen, and analysed for ATP and phosphocreatine (Opie et al, 1971). cAMP was assayed by the method of Tovey et al (1974). Results were expressed in terms of the initial wet weight.

Statistical Analysis

Results were expressed as means \pm SEM. Statistical analysis was done by the paired t test, one way analysis of variance, two way analysis of variance or Chi-squared test (with Fisher's modification when necessary). $P < 0.05$ was considered significant.

REPERFUSION-INDUCED AUTOMATICITY

REPERFUSION-INDUCED AUTOMATICITY

Abnormal automaticity rather than re-entrant excitation is thought to underlie ventricular fibrillation that occurs on release of coronary artery ligation (Corr and Witkowski, 1983; Penkoske et al, 1978). Abnormal impulse formation arising out of oscillating potentials were reported as early as 1943 by Bozler. Intracellular microelectrode recordings revealed that in cardiac tissue a variety of conditions could induce oscillating afterpotentials (for review see Cranefield, 1977), which in turn may give rise to automaticity (Cranefield and Aronson, 1974).

More clarity about the role of automaticity or microcircuits is not possible in a model of regional ischemia because of the limited resolution of standard electrogram techniques. The present study describes a new model of "reperfusion arrhythmias". Contractile force and action potentials were recorded during 25 to 40 min of hypoxic glucose-free superfusion (simulated "ischemia") and the return to normal medium (simulated "reperfusion") in a guinea-pig papillary muscle. This model may be useful for studying the mechanism of reperfusion arrhythmias for the following reasons. First, a simple contractile trace may be correlated with intracellular electrophysiological recordings; secondly, metabolites were constantly washed away (toxic effects from their accumulation could not have occurred, as in regional ischemia); thirdly, the independent role of substrates could be determined by omitting or adding them to the medium singly or in combination. It is proposed that the production of afterpotential mediated abnormal pacemaker activity during "reperfusion" can be a model of reperfusion ventricular arrhythmogenesis.

Protocol

Hypoxic glucose-free medium (simulated "ischemia") was begun after a stabilization/healing over period (de Mello, 1972) of at least

30 min. Thereafter "reperfusion" (return to normal medium) was started at 25, 30 or 40 min. Effects determined were of hypoxia alone; glucose-free medium alone; low external calcium (0.25 mM); increased drive rate (180 versus 30/min); isoprenaline (Winthrop Laboratories); verapamil (Holpro Pharmaceuticals); lidocaine (Astra Pharmaceuticals); and propranolol (ICI). All interventions were made only in the "ischemic" period. In the low calcium group, calcium was also reduced in the "reperfusion period". Microelectrode recordings were started in the stabilization period but electrodes frequently dislodged at 30-40 min of "ischemia", possibly due to the onset of contracture as reflected by a marked rise in resting tension. However, in some experiments, trans-membrane potential recordings were continued into the "reperfusion period". Observations were made for 15 min of "reperfusion".

RESULTS

Figure 1 shows the changes in contractile force and membrane and action potential during 40 min of hypoxic glucose-free superfusion and subsequent "reperfusion". Similar results were obtained in at least 13 experiments.

The Hypoxic Glucose-Free Period

In the typical trace presented (Figure 1) resting tension increased gradually until 15 min of hypoxic glucose-free medium and thereafter increased sharply until it reached a plateau at 25 min. The rate and extent of increase in resting tension varied in each experiment. The contractile force declined almost completely in 10 min of hypoxic glucose-free superfusion. Of interest is the recovery of contractile force between 18 and 30 min in all experiments. The action potential duration began shortening after 5 min of the "ischemic" medium and continued linearly until a marked maximum shortening was

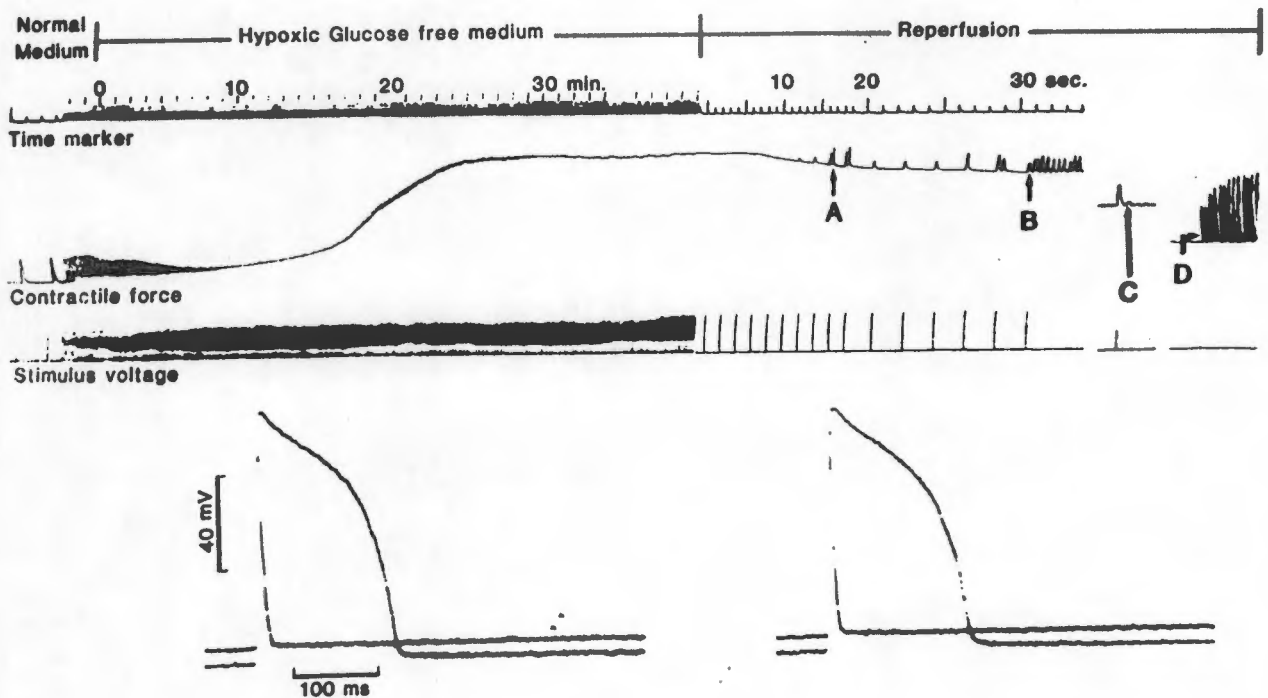


Figure 1

Upper panel. Pen recordings of the developed and resting tension of guinea-pig papillary muscle. The trace shows typical features that were observed in 13 experiments during hypoxic glucose-free superfusion followed by "reperfusion" (see text). When the extrasystolic contractions (A) triggered new beats, the stimulator was switched off and automaticity (B) was sustained. The aftercontraction (C) and spontaneous automaticity (D) shown are from different preparations. Note that the spontaneous automaticity was not triggered by the external stimulator.

Lower panel. On the left, an action potential during normal medium is superimposed on action potential altered by "ischemia" (40 min). On the right, an action potential showing recovery after "reperfusion" is superimposed on the action potential changed by "ischemia".

reached at about 20 to 30 min. The shift of the maximum diastolic potential to a more negative level occurred at about 15 min and generally reached a maximum of 5 to 10 mV during the rest of the "ischemic" period. The overshoot potential and maximum dV/dt (not shown) were also observed to decrease.

Effects of "Reperfusion"

The membrane and action potential changes induced by "ischemia" reversed within 2 min of "reperfusion" (Figure 1). The action potential duration, overshoot, the maximum dV/dt increased and the maximum diastolic depolarization became more negative. Full recovery usually occurred within 15 min.

Action potentials that were not caused by the external stimulus (extrasystoles) appeared within 30 sec of "reperfusion". The extrasystoles shown in Figure 2a appeared to arise from the peak of oscillating afterpotentials, which had amplitudes of about 5 mV. The potential (threshold potential) at which the extrasystoles took off from the afterpotential was about -75 mV. In Figure 2b the afterpotentials are shown on an expanded time scale. The interval between full repolarization of the preceding action potential and the first of the damped oscillation of the membrane potential was about 200 msec. The interval between full repolarization of the stimulated action potential and the take off of the extrasystole was also about 200 msec (Figure 2a) suggesting that the extrasystoles originated from the afterpotential. Moreover, the afterpotentials appeared earlier than extrasystoles, which were presumably triggered when the afterpotentials enlarged to threshold. The contractile force recovered immediately after "reperfusion" and within 1 to 2 min was greater than even prior to change to "ischemic" medium (not shown). Aftercontractions and extrasystolic contractions occurred in association with the afterpotentials and extrasystolic action

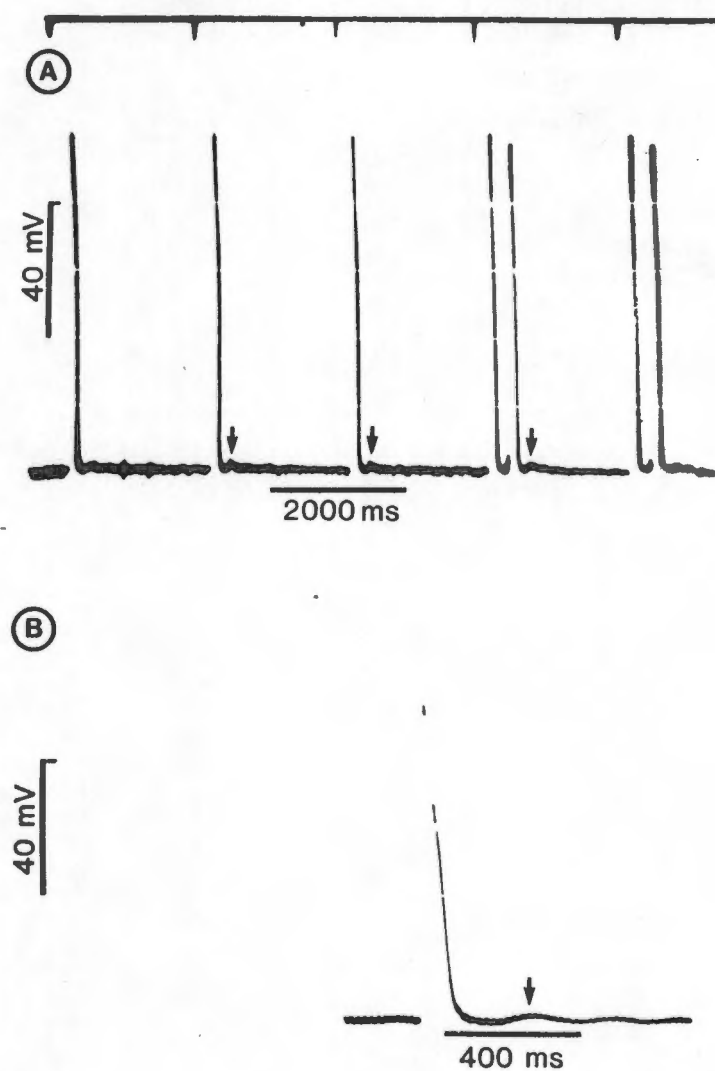


Figure 2

Oscilloscope recordings at the onset of "reperfusion" showing (A) oscillatory afterpotentials which preceded and appeared to evoke full extrasystolic depolarization. Note the afterdepolarization after the evoked depolarization; and (B) an afterpotential on an expanded time scale.

potentials. Self-sustained activity (automaticity) was recorded mainly on the contractile trace because of the difficulty of continuing micro-electrode recordings during "reperfusion". The automaticity could be demonstrated by turning off the external stimulus when extrasystoles appeared (Figure 1). In some experiments after the quiescent period following an automatic burst, automaticity would be renewed spontaneously (i.e. without an external stimulus). This untriggered automaticity could also be demonstrated if the preparation was not externally stimulated from the onset of "reperfusion" (Figure 1).

The incidence of the "reperfusion" abnormalities depended on the duration of the "ischemic" period. In Figure 3, extrasystoles occurred less often when the "ischemic" period was 25 min (42%) and 30 min (80%) than when it was 40 min (100%). Similarly, automaticity occurred more often when the "ischemic" period was increased from 25 to 30 to 40 min (33% to 60% to 100%).

To determine if the deleterious effects during "ischemia" that induced "reperfusion" dysrhythmia were due to the hypoxia or the absence of glucose from the medium, experiments were done with either intervention alone. Only hypoxia or only glucose-free medium produced less decline in contractile force and tended to shift the resting tension curve to the right (not shown). In Figure 4, hypoxia alone or glucose-free medium alone did not induce "reperfusion" extrasystoles or automaticity.

Effect of Interventions

"Reperfusion" after 25 min of hypoxic glucose-free medium only induced automaticity and extrasystolic contractions in 33% and 42% of the experiments respectively. In Figure 5, adding isoprenaline (1 μ M) or increasing stimulation rate (3 Hz) during the 25 min "ischemic" period induced "reperfusion" abnormalities in all experiments.

At a basic stimulation rate of 0.5 Hz and without intervention, the

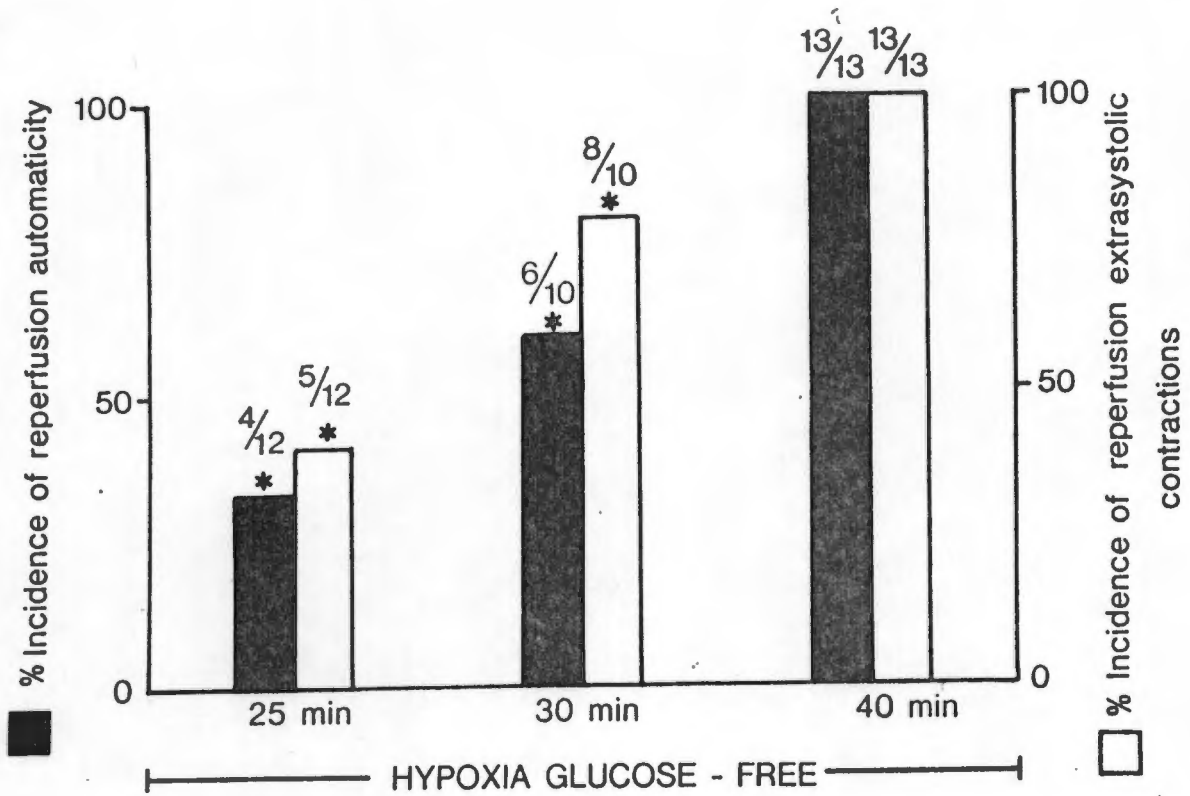


Figure 3

"Reperfusion-induced automaticity and extrasystolic contractions after 25 to 40 min of "ischemia". Significance (*P < 0.001) by overall comparison (gradient response) suggested that the incidence of "reperfusion" abnormalities depended on the duration of "ischemia". (Chi-squared test).

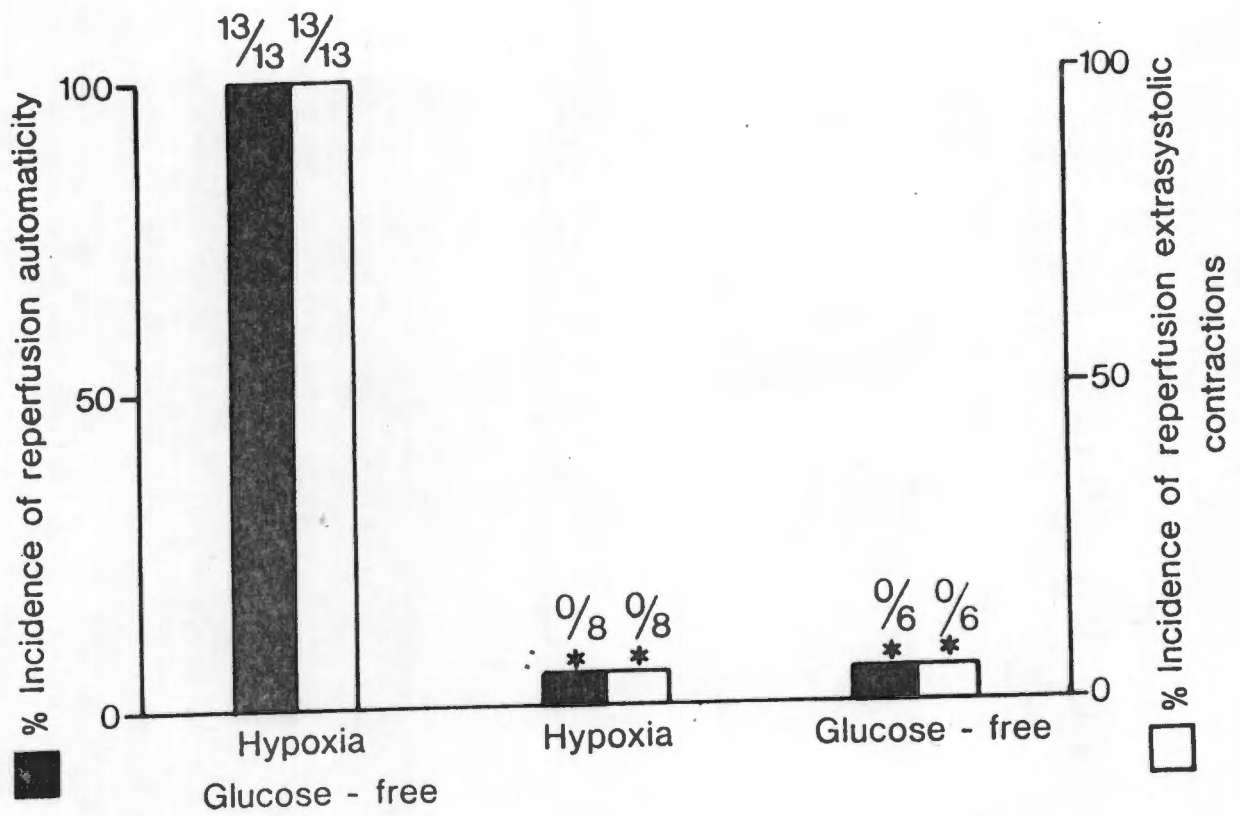


Figure 4

"Reperfusion" effects after 40 min of hypoxic medium containing glucose or glucose-free medium saturated with O_2 . * $P < 0.01$ for individual comparison with hypoxic glucose-free group. (Chi-squared test).

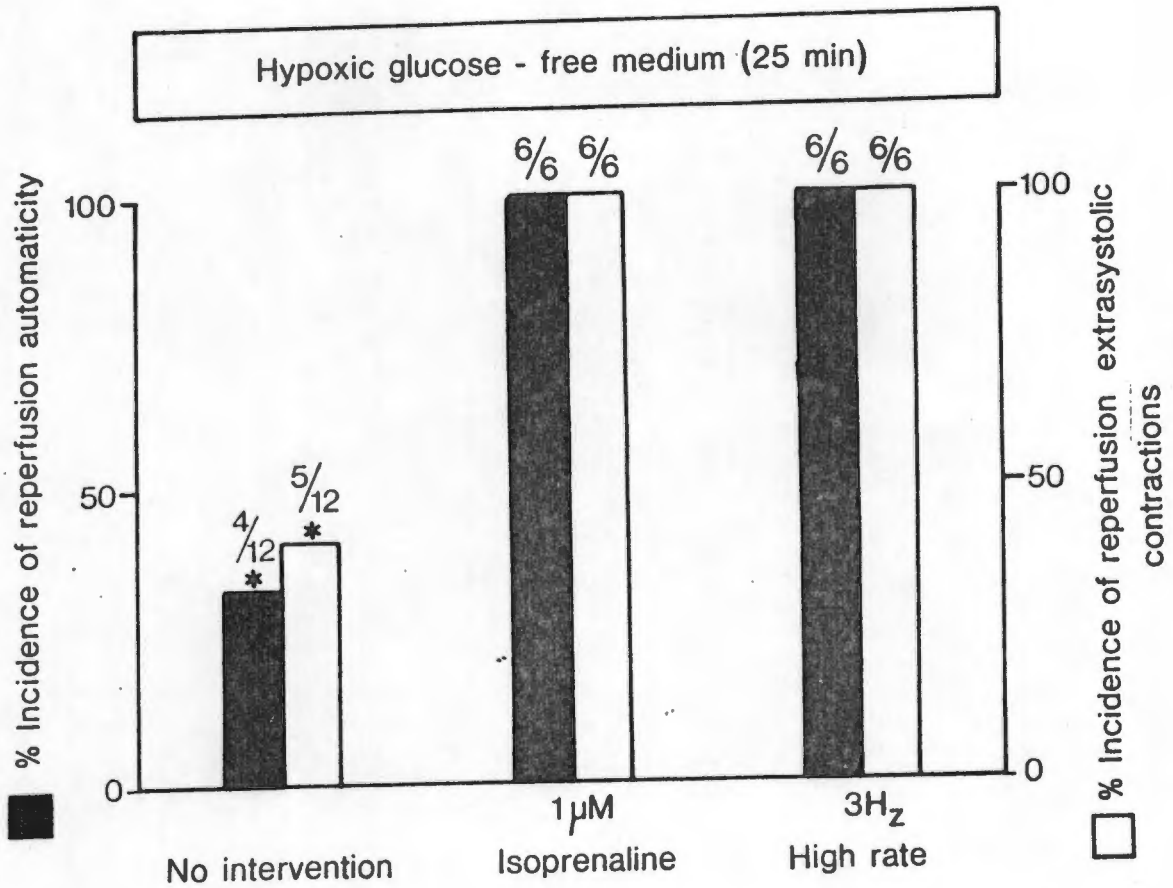


Figure 5

Effect of "reperfusion" after adding isoprenaline (1 μM) or increasing stimulation rate (3 vs 0.5 Hz) during hypoxic glucose-free medium for 25 min. *P<0.01 for individual comparison with the no intervention group. (Chi-squared test).

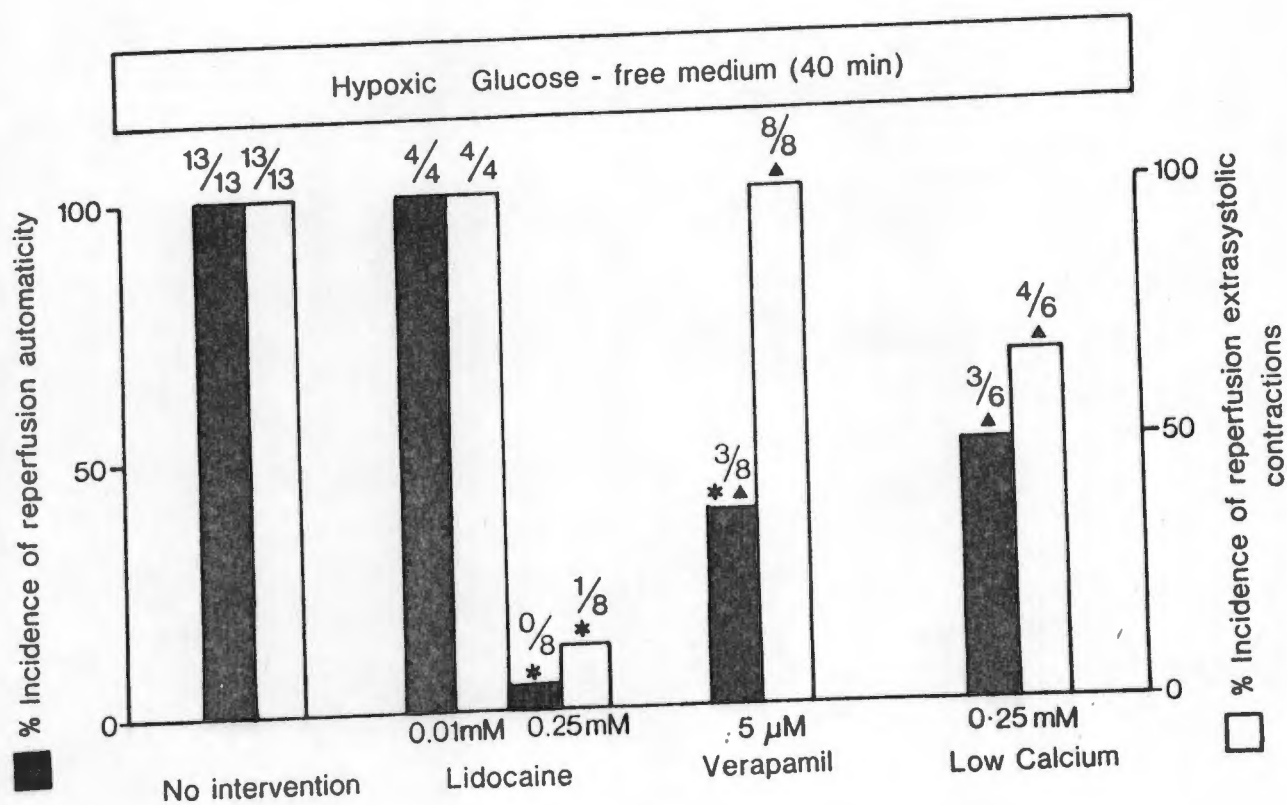


Figure 6

Effect of lidocaine, verapamil or low external calcium (0.25 mM vs 2.5 mM) on incidence of automaticity and extrasystolic contractions induced by "reperfusion" after 40 min of ischemia. * $P < 0.01$ for individual comparison with the no intervention group. ▲ $P < 0.01$ for individual comparison with lidocaine (0.25 mM). (Chi-squared test).

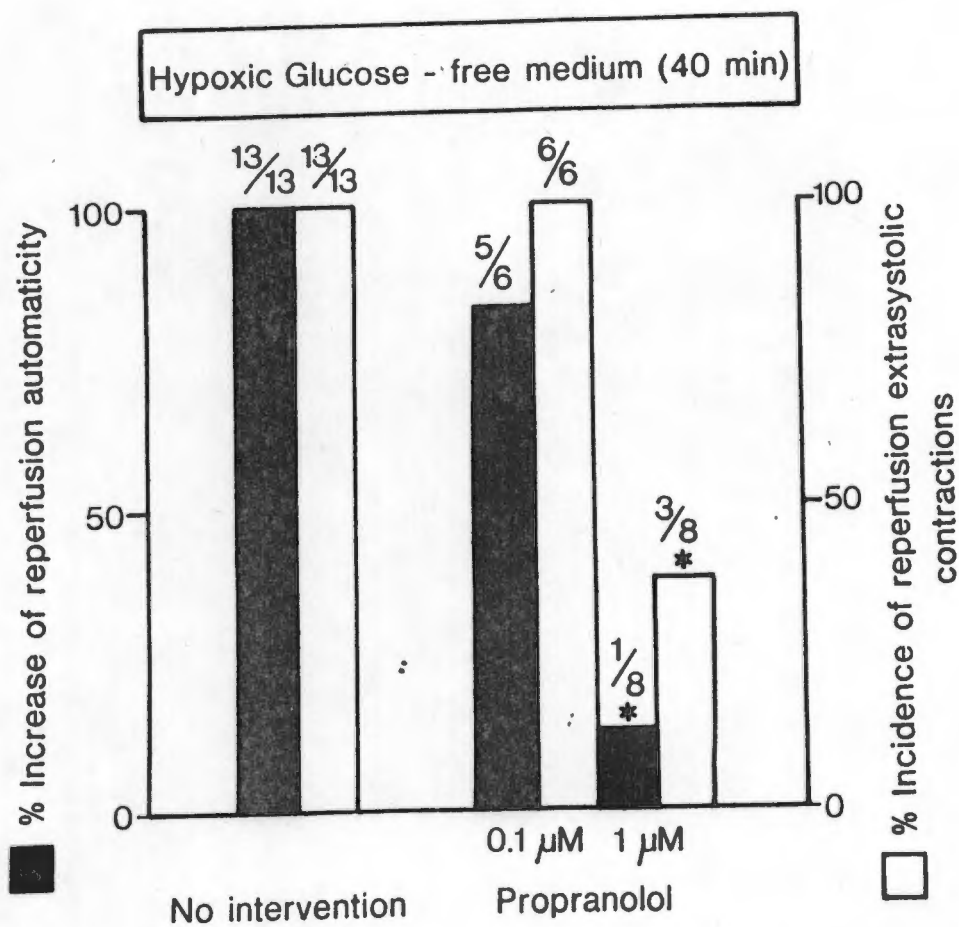


Figure 7

Effect of propranolol (0.1 to 1 μ M) on incidence of "reperfusion" abnormalities after 40 min of "ischemia". *P<0.01 for individual comparison with the no intervention group.

hypoxic substrate-free medium for 40 min induced "reperfusion" automaticity and extrasystoles in 100% of the preparations. Adding lidocaine (0.01 mM) during the 40 min ischemic period did not block the "reperfusion" abnormalities (Figure 6). Lidocaine (0.25 mM) reduced the incidence of extrasystoles by 88% and automaticity by 100%, whereas verapamil (5 μ M) reduced automaticity by 62% but did not prevent extrasystoles. When the external calcium was low during the hypoxic substrate-free period and during "reperfusion" (0.25 mM versus 2.5 mM), extrasystoles and automaticity was blocked in 50% of the preparation (Figure 6). When the external calcium was normal during reperfusion (2.5 mM), automaticity and extrasystoles occurred in all of 3 experiments. Propranolol (1 μ M) reduced the incidence of extrasystoles and automaticity by 63% and 88% respectively, whereas propranolol (0.1 μ M) had no effect (Figure 7).

The high stimulation (3 Hz) and isoprenaline (1 μ M) tended to shift the resting tension curve to the left, whereas lidocaine (0.25 mM) and propranolol (1 μ M) tended to shift it to the right. With the verapamil (5 μ M) and the low calcium (0.25 mM) - containing superfusate, the resting tension curves appeared to be similar to those of the "ischemic" preparations.

DISCUSSION

In the present study on guinea-pig papillary muscle, extrasystoles and automaticity occurred within seconds of return to normal after glucose-free hypoxic medium. The extrasystoles appeared to arise from oscillatory afterpotentials.

Oscillatory Afterpotentials and "Reperfusion"-Induced Arrhythmias

Membrane oscillation might be induced at two levels of resting

membrane potential (less or more negative than -70 mV). At the less negative potential, in Purkinje fibres oscillations are induced by an interplay of the slow inward current and the delayed rectifier (Hauswirth et al, 1969). In ventricular muscle similar membrane oscillations were evoked by passing depolarizing DC currents (Katzung and Morgenstern, 1977). At the more negative resting potentials, oscillatory afterpotentials, following normal action potentials, are induced in Purkinje fibres by cardiotonic steroids (Davis, 1973; Ferrier et al, 1973), pacing (Vassalle and Carpentier, 1972) or potassium depletion (Eisner and Lederer, 1979a); in motral valve and coronary sinus by catecholamines (Wit and Cranefield, 1976-77); in ventricular muscle by digoxin (Adamantidis et al, 1983) or potassium-free, high calcium medium (Hiraoka et al, 1981); and in isolated single ventricular cells by injection of intracellular calcium (Matsuda et al, 1982). We describe reperfusion-induced membrane oscillations at the more negative resting potential in ventricular muscle. The membrane oscillations in our preparation that gave rise to extrasystoles and automaticity might be comparable to the afterpotentials previously noted to evoke automaticity (Cranefield and Aronson, 1974).

Oscillating afterpotentials correspond to a transient inward current in voltage clamp studies (Karagueuzian and Katzung, 1982). Power spectrum analysis of the transient inward current suggests a role for the oscillating release of calcium from the internal stores in Purkinje fibres (Kass and Tsien, 1982) and in isolated single ventricular cells (Matsuda et al, 1982). The internal calcium oscillation might activate a non-specific inward current passing channel, thereby causing the oscillating afterpotentials (Colquhoun et al, 1981). An alternate possibility is that the fluctuation of internal calcium might modulate

the $\text{Na}^+/\text{Ca}^{2+}$ exchange (Karagueuzian and Katzung, 1982) which might be electrogenic (Mullins, 1979).

The present study does not exclude that microcircuits (re-entry) caused the automaticity. Re-entry could explain the extrasystoles which did not appear to be preceded or caused by delayed afterdepolarizations. However, this possibility is unlikely because, first, oscillatory afterdepolarizations preceded and appeared to evoke extrasystolic depolarizations in most instances. Secondly, the spontaneous (unstimulated) onset of "reperfusion"-automaticity, after a quiescent period, suggests that the automaticity originated in a single pacemaker. Thirdly, afterpotentials could occur in isolated single cells (Matsuda et al, 1982). Fourthly, automaticity was blocked by lidocaine (0.25 mM), a concentration that blocks the transient inward current thought to underlie aftercontractions induced by potassium-free solution (Eisner et al, 1983).

Cytosolic Calcium Oscillations in the Reperfusion Period

How does "ischemia" and reperfusion induce cytosolic calcium oscillations? It was previously suggested that reperfusion arrhythmias are related to the preceding ischemic metabolic damage (Balke et al, 1981; Carbonin et al, 1981) of which contracture could be used as an index (Lowe et al, 1979). Our observations support this idea: first, the incidence of reperfusion automaticity decreased when the "ischemic" period was shortened; secondly, an increase in the metabolic demand by increasing the stimulation rate or isoprenaline enhanced the incidence of reperfusion automaticity and contracture development; and thirdly, when energy metabolism may still occur during the only-hypoxic or only-glucose-free medium, reperfusion automaticity did not occur and contracture tended to be less. High energy compound depletion has been

associated with ischemic contracture (Lowe et al, 1979; Allison and Holsinger, 1983). In our study loss of high energy compounds was reflected in a decline of contractile force and an increase in resting tension (Jennings et al, 1960) and was found to occur at about 10 min of "ischemia". The shortening of action potential duration during this time might also reflect high energy compound depletion. During ischemia and hypoxia shortening of the action potential duration is thought to be caused mainly by an increase in the background potassium current (Vleugels et al, 1980) although a decrease in the slow inward current may contribute (McDonald and MacLeod, 1972). Recently, patch clamp studies revealed sarcolemmal potassium channels which opened when ATP fell below 1 mM and with an increased opening probability with a further decrease in ATP (Noma, 1983). In our preparation the decrease in action potential duration after the first 5 min of "ischemia" might have indicated a fall in ATP below 1 mM with a probable further subsequent decrease. Cellular energy failure during "ischemia" is thought to elevate intracellular calcium (Nayler et al, 1978), possibly by inhibiting the active calcium pump (Tada et al, 1978) of the sarcoplasmic reticulum or the sarcolemmal Na-K pump. Higher cytosolic sodium resulting from Na-K pump blockade indirectly elevates intracellular calcium by stimulating the $\text{Na}^+/\text{Ca}^{2+}$ exchange (Reuter, 1974; Langer, 1982). The rise in intracellular calcium might partially explain the increase in resting tension during "ischemia" in the present study. In a recent study using aequorin (Allen and Orchard, 1984), the finding that resting calcium does not rise during the first 20 min of hypoxia does not contradict this hypothesis, because the present observations were made for 40 min. Moreover, the recovery of contractile force at 25 min in our preparation probably reflects the

the rise in intracellular calcium.

In addition to the calcium accumulation during ischemia, reperfusion in itself induces a considerable influx of calcium (Shen et al, 1982). The high cytosolic calcium might be driven into the depleted sarcoplasmic reticulum by the ATP regenerated at the onset of reperfusion (Allison and Holsinger, 1983; Danforth et al, 1960), thereby evoking the exaggerated cytosolic calcium oscillations responsible for the oscillatory afterdepolarizations and sustained automaticity. Recovery of ATP in our study might be reflected in the recovery of action potential duration and contractile force.

Explanations of the Effects of Interventions

We found that the calcium reduction only in the "ischemic" period was ineffective whereas low calcium during both the "ischemic" and "reperfusion" blocked "reperfusion" abnormalities. High dose lidocaine might have prevented "reperfusion" automaticity by the following possible mechanisms: (1) because local anaesthetics might reduce internal calcium by blocking calcium release from the sarcoplasmic reticulum (Caputo et al, 1979; Hunter et al, 1982); (2) by blocking the transient inward current (Eisner et al, 1983), possibly by reducing the internal sodium activity (Deitmer and Ellis, 1980); or (3) by indirectly blocking the sarcolemmal slow calcium channels (Josephson and Sperelakis, 1976; Thorens, 1971). Similarly the high dose of propranolol (1 μ M) might have acted as a local anaesthetic since membrane stabilizing properties have been attributed to high dose of this drug (Iansith et al, 1983). The lack of effect of lower doses of propranolol suggests that a beta-adrenoceptor mechanism did not play a role in the genesis of "reperfusion" automaticity. The inability of verapamil or a tenfold reduction in external calcium to completely block "reperfusion"-

induced abnormalities (compared to high dose lidocaine) suggests that calcium from sources other than the slow inward channel (eg. $\text{Na}^+/\text{Ca}^{2+}$ exchange and/or internal stores) might have contributed to the genesis of "reperfusion" automaticity.

A Model for Reperfusion?

Reperfusion is considered to be the re-supply of O_2 , substrates and electrolytes and the washing away of accumulated metabolites after transient ischemia. The present experiments were similar to ischemia only in the O_2 and glucose deprivation. In a superfused preparation, it is not possible to determine the effect of washing away metabolites (lactate, H^+ , K^+), that accumulates during ischemia. Nevertheless, automaticity was observed always after 40 min of glucose-free hypoxic superfusion.

The coupling interval between afterpotentials or extrasystoles and the previous driven impulse in the papillary muscle preparation was about 200 msec. In all of 10 Langendorff guinea-pig hearts, premature ventricular extrasystoles and ventricular tachycardia (followed by fibrillation) appeared within seconds after glucose-free hypoxic perfusion (unpublished). The coupling interval between the extrasystoles and the previous propagated beat was also about 200 msec. The similar time of onset and coupling interval in the papillary muscle and the isolated heart suggests that the same phenomenon was observed in the two models.

In the present study, automaticity occurred within seconds of reperfusion after glucose-free hypoxic medium for 25 to 40 min. Ventricular fibrillation induced by reperfusion after regional (Penkoske et al, 1978; Murdock et al, 1980) or global (Carbonin et al, 1981) ischemia also appears within seconds and occurs maximally when the period of

ischemia is between 20 and 30 min. The similarity in the time of onset of dysrhythmias and in the ischemic duration between the present study and the latter studies suggests that automaticity might play a role in ventricular fibrillation induced by reperfusion.

ISOPRENALINE-INDUCED AUTOMATICITY

ISOPRENALINE-INDUCED AUTOMATICITY

Considerable evidence links ventricular arrhythmias to the autonomic nervous system. Excessive beta-adrenoceptor stimulation is an established cause of ventricular fibrillation in animal experiments and has been suggested to play a role in sudden death (Lown et al, 1977). Catecholamines induce severe ventricular tachyarrhythmias in children with Adams-Stokes syndrome (Coumel et al, 1978). Local lesions, remarkably resembling the lesions induced experimentally by catecholamines, are observed in the hearts of victims of instantaneous death (Baroldi, 1975). In dogs, sympathetic nerve stimulation causes focal ventricular activity (Armour et al, 1972).

It has already been suggested that the ventricular automaticity induced by reperfusion might be mediated by elevation of internal calcium. It was logical to consider that beta-adrenoceptor stimulation, which increases the probability of open calcium channels during excitation (Reuter, 1982), might induce ventricular automaticity. For this part of the study, the papillary muscles were superfused with medium containing both oxygen and glucose throughout. The effect of isoprenaline and other procedures that might modulate internal calcium (low K^+ , ouabain, high stimulation rate, verapamil and lidocaine) were explored. It was concluded that isoprenaline-induced automaticity arose out of afterpotentials which might have been evoked by cytosolic calcium elevation.

Protocol

Isoprenaline was added after a half-hour stabilization period and its effects were observed for 15 min. In the ouabain and low potassium experiments, either intervention was begun after the stabilization period and isoprenaline was added 15 min thereafter. In another two series,

verapamil or lidocaine were added simultaneously with ouabain and continued after the addition of isoprenaline.

To heighten the possibility of eliciting afterpotentials, the stimulus rate was increased from the basic rate of 0.5 Hz to 2 Hz over 2 min. The procedure was performed at the end of the stabilization period and in the first 2 min of the isoprenaline period. For the rest of the isoprenaline period, observations for afterpotentials and automaticity were made at a stimulation rate of 2 Hz. In the ouabain and low potassium series, the procedure of increasing the rate was also done immediately before isoprenaline was added to the ouabain or low potassium solution.

RESULTS

Effects of Isoprenaline

In Figure 8 are demonstrated the contractile and membrane oscillations that were produced by isoprenaline (1 μ M). Afterpotentials were accompanied by aftercontractions. Non-driven impulses, accompanied by contractions (extrasystoles) arise from the crest of afterpotentials (marked by arrow). The extrasystole was followed by the normal driven impulse. A non-driven self-sustained rhythm (automaticity) was demonstrated (at point C) by switching off of the existing stimulation when extrasystoles began to appear. The onset of the extrasystoles and automaticity was always preceded by the appearance of afterpotentials.

In Figure 9 an isoprenaline-induced afterpotential occurring at a high level of membrane potential is shown to induce automaticity. At a stimulation rate of 0.5 Hz the amplitude of the afterpotential was 10 mV and the coupling interval (the time between full repolarization of the preceding driven action potential and the crest of the afterpotential) was about 200 msec. As the stimulation rate was gradually increased

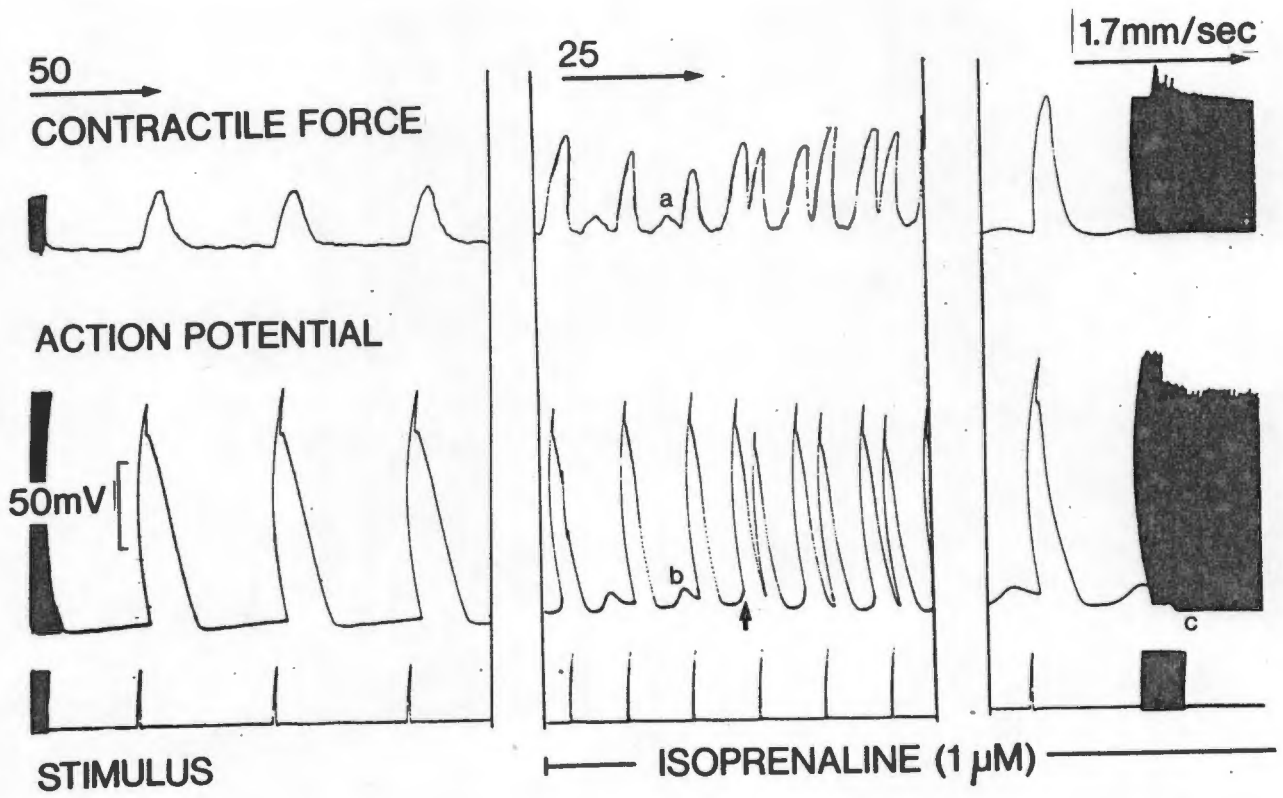


Figure 8

Isoprenaline-induced oscillations of membrane potential and resting tension. Parts of an action potential and contractile trace were selected to demonstrate an aftercontraction (a), afterpotential (b) and the onset of automaticity (c). Arrow indicates a non-driven depolarization arising from the crest of the afterpotential.

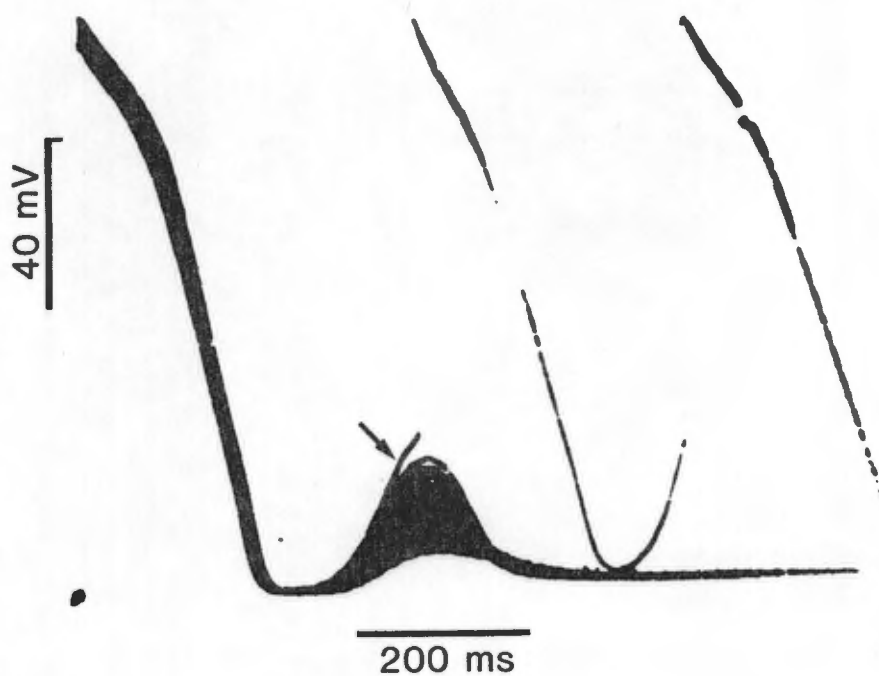


Figure 9

Effect of increasing external drive rate on an isoprenaline-induced afterpotential. Shown is the gradual increase in the amplitude of the afterpotential as the drive rate was increased from 0.5 Hz to 1.3 Hz over 3 min. Arrow indicates the take off of a non-driven depolarization. Medium K^+ was 1.2 mM.

from the basic 0.5 Hz over 3 min to 1.3 Hz, the amplitude of the afterpotential increased from 10 to 32 mV and the coupling interval shortened to about 160 msec. At the rate of 1.3 Hz, the afterpotential gave rise (arrow) to a non-driven full depolarization which repolarized completely and induced a second non-driven impulse. This second depolarization, like the afterpotential and unlike the driven action potentials, had a gradual onset, reaching 32 mV in about 80 msec after its take off from the level of maximum diastolic potential. In this preparation automaticity continued after the second non-driven impulse. Medium K^+ in this experiment was 1.2 mM. The amplitude of afterpotentials induced by isoprenaline in normal medium K^+ (5.9 mM) was in the region of 5 mV.

The incidence of afterpotentials, extrasystoles and automaticity depended on the dose of isoprenaline (Figure 10). Afterpotentials occurred in 33 or 75% of preparation when the isoprenaline used was 0.1 μ M or 1 μ M respectively. The incidence of extrasystoles and automaticity was 10, 47, and 40 or 63% when the added isoprenaline was 0.1, 1.0 or 10 μ M respectively. At the highest dose of isoprenaline used (10 μ M), excessive contractile force dislodged microelectrodes and afterpotentials were not observed. Isoprenaline 0.01 μ M did not induce membrane oscillation.

The response of the action potential and contractile force to isoprenaline (0.01 to 1 μ M) are presented in Table I. Shown are changes that occurred 2 min (the time when the changes appeared to peak) after isoprenaline was added. Isoprenaline (0.01 μ M) increased contractile force ($159 \pm 14\%$) but there was no significant change in the action potential. Isoprenaline in a dose of 0.10 μ M increased contractile force ($405 \pm 74\%$), action potential amplitude (109 ± 1.8 vs 114 ± 1.5 mV)

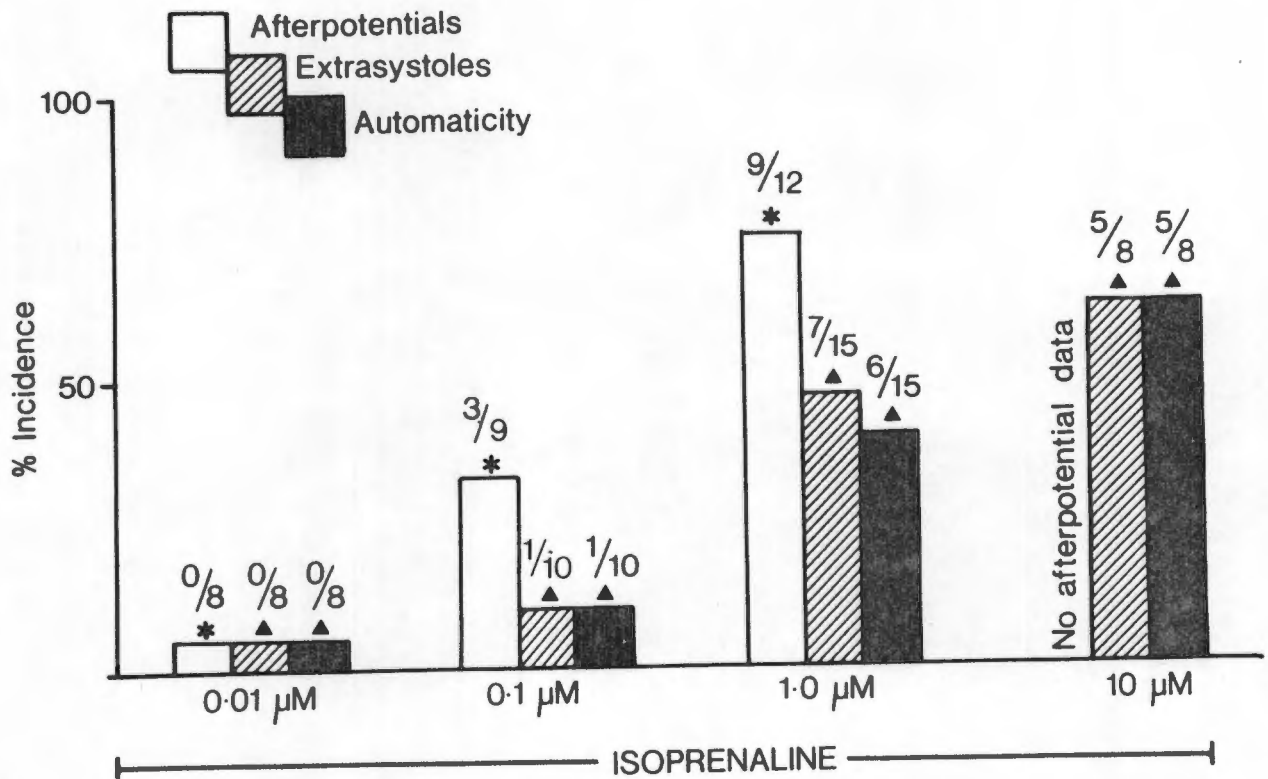


Figure 10

Incidence of afterpotentials, extrasystoles and automaticity in response to the dose of isoprenaline. * $P < 0.01$ for overall comparison.

▲ $P < 0.05$ for overall comparison. (Chi-squared test).

Table I

	Resting Potential (mV)	Action potential		Plateau Duration (ms)(O _m V)	Contractile Force (% of control)
		Amplitude (mV)	Duration (ms) (90% RP)		
<u>Isoprenaline</u> (N= 8-12)					
Control	83 ± 0.5	109 ± 1.2	145 ± 7.2	76.5 ± 6.5	
10 ⁻⁸ M	84 ± 0.4	110 ± 1.1	140 ± 8.2	87.7 ± 2.44	159 ± 14
Control	84 ± 0.6	109 ± 1.87	142 ± 5.2	70.9 ± 6.97	
		P < 0.05		P < 0.01	
10 ⁻⁷ M	84 ± 0.6	114 ± 1.5	138 ± 6.4	82.9 ± 4.3	405 ± 74
Control	84 ± 0.9	109 ± 1.8	141 ± 5.5	69.9 ± 5.97	
		P < 0.05	P < 0.01	P < 0.05	
10 ⁻⁶ M	83 ± 1.9	115 ± 2.4	115 ± 4.2	82.6 ± 4.5	672 ± 185

P values <0.05 were regarded as significant - paired t test.

and plateau duration at 0 mV (70.9 ± 6.9 vs 82.9 ± 4.3 ms). Isoprenaline $1 \mu\text{M}$ increased contractile force ($672 \pm 185\%$), action potential amplitude (109 ± 1.8 vs 115 ± 2.4) and plateau duration (69.9 ± 5.9 vs 82.6 ± 4.5 mV) and reduced action potential duration (141 ± 5.5 vs 115 ± 4.2 ms). Isoprenaline had no effect on the resting potential which ranged between -82 and -85 mV in all experiments.

Effect of Intervention

Since isoprenaline even in the highest dose did not induce automaticity in all experiments, we used procedures that might in addition elevate internal calcium indirectly. Papillary muscles were treated with medium containing low potassium (1.2 or 2.7 vs 5.9 mM) or ouabain (0.7 mM) which are known to inactivate the membrane Na-K pump. It is thought that the consequent increase in internal sodium might increase the internal calcium via the $\text{Na}^+/\text{Ca}^{2+}$ exchange. The increase in contractile force that occurred (not shown) after the low potassium or ouabain reflected the increase in internal calcium. When the external stimulation rate was increased during low potassium or ouabain treatment, no oscillations of membrane potential or resting tension occurred. The incidence of isoprenaline-induced extrasystoles and automaticity depended on the concentration of external potassium (Figure 11). Adding isoprenaline ($1 \mu\text{M}$) 15 min after the medium potassium was reduced from 5.9 to 2.7 to 1.2 increased the incidence of automaticity (40 to 57 to 85%). Similarly, the incidence of extrasystoles also increased (47 to 57 to 92%). The addition of isoprenaline in the presence of ouabain, increased the incidence of both automaticity and extrasystoles (40 and 47 vs 86%). The effects of verapamil and lidocaine on the abnormalities induced by ouabain and isoprenaline are shown in Figure 12. Verapamil ($1 \mu\text{M}$) blocked

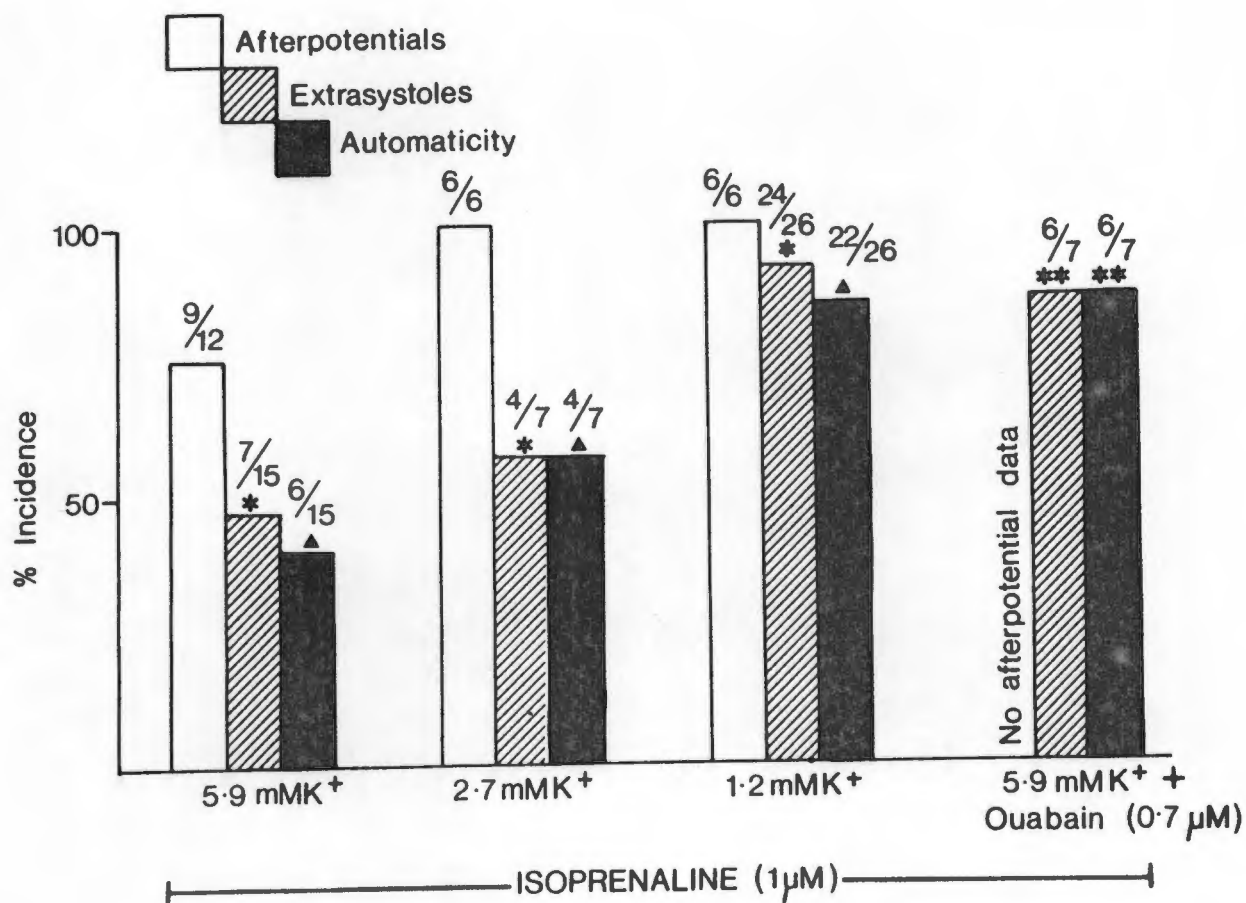


Figure 11

Effect of reducing external potassium and adding ouabain on the incidence of afterpotentials, extrasystoles and automaticity induced by isoprenaline (1 μM). *P<0.01 for overall comparisons of the different external potassium groups. ▲ P<0.05 for overall comparison. **P<0.05 vs the group with 5.9 mM K⁺ and without ouabain. (Chi-squared test).

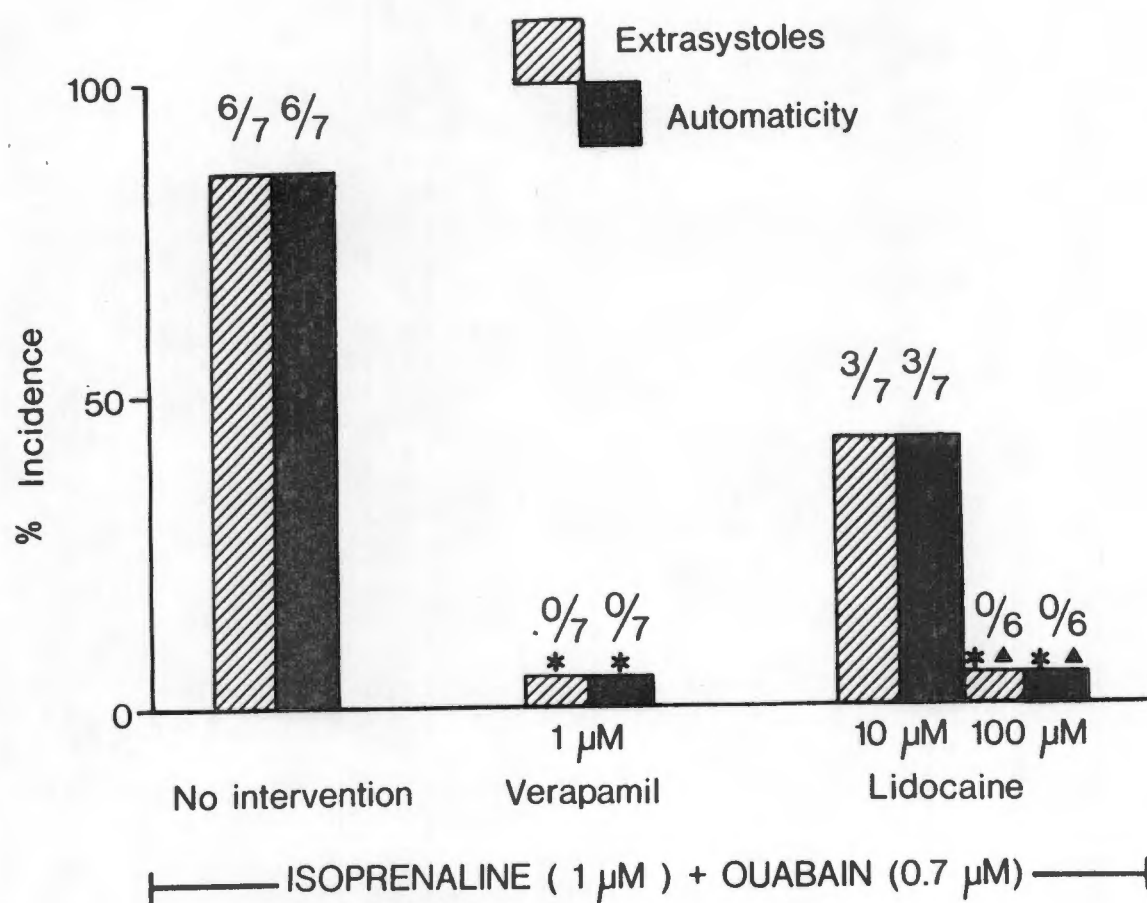


Figure 12

Effect of verapamil and lidocaine on the isoprenaline-ouabain-induced extrasystoles and automaticity. * $P < 0.01$ vs the non-intervention group.

▲ versus lidocaine (10 μM). (Chi-squared test).

automaticity and extrasystoles completely. Lidocaine (10 μM) reduced extrasystoles and automaticity (43 vs 86%) but this effect was not significant whereas the higher dose of lidocaine (100 μM) completely blocked extrasystoles and automaticity (0 vs 86%).

DISCUSSION

In 1943, using extracellular techniques, Bozler recorded oscillating afterpotentials from ventricular strips. The afterpotentials which were initiated by high calcium solution and enlarged by adrenaline gave rise to single or bursts of fresh impulses. These observations raised the possibility that abnormal ventricular pacemakers might be evoked by afterpotentials. With the advent of intracellular micro-electrode techniques for the direct study of transmembrane potentials, membrane oscillations were described at depolarized potentials or at normal resting potentials. At the less negative potential (-60 to -40 mV), membrane oscillations in Purkinje fibres is described as being produced by the interplay of the slow inward current and the delayed rectifier (Hauswirth et al, 1969). In ventricular muscle similar membrane oscillations are evoked by passing depolarizing DC currents (Katzung and Morgenstern, 1977). At normal resting potentials and following driven action potentials, membrane oscillations are induced in Purkinje fibres by cardiotonic steroids (Davis, 1973; Ferrier et al, 1973), pacing in the presence of norepinephrine (Vassalle and Carpentier, 1972), or external potassium depletion (Eisner and Lederer, 1979a); in mitral valve and coronary sinus by catecholamines (Wit and Cranefield, 1976 to 1977); in ventricular muscle by digoxin (Adamantidis et al, 1983) or by potassium free, high calcium medium (Hiraoka et al, 1981); and in isolated single ventricular cells by injection of intracellular calcium (Matsuda et al, 1982). In the present study, isoprenaline-induced

afterpotentials are described in ventricular muscle at normal resting membrane potentials. The afterpotentials, when they enlarged to a critical threshold depolarization, initiated a non-driven impulse which became self-sustained. This sequence was similar to the previous observation that delayed afterdepolarizations gave rise to automaticity in Purkinje fibres (Cranefield and Aronson, 1974). It must be pointed out that injury currents or re-entry microcircuits might also have induced automaticity in our preparation. Such a mechanism might arise out of the electrical inhomogeneity caused by a hypoxic core, developing when the contractions induced by isoprenaline were excessive. But, re-entry as a mechanism was unlikely in our study because the non-driven impulse was clearly demonstrated to arise from the crest of an afterdepolarization. Also the demonstration of afterpotentials in single isolated cell preparations (Matsuda et al, 1982) argues strongly against the possibility that afterpotentials represent electronic current changes and that automaticity was induced by an hypoxic core.

In voltage clamp studies, a transient inward current is shown to accompany afterpotentials (Karagueuzian and Katzung, 1983). In Purkinje fibres power spectrum analysis of the transient inward current has attributed it to cytosolic calcium oscillations caused by phasic release of calcium from internal stores (Kass and Tsien, 1982). These findings are confirmed by spectrum analysis in single isolated ventricular cells (Matsuda et al, 1982). The internal calcium oscillations might induce the transient inward current by activating non-specific inward current passing channels (Colquhoun et al, 1981) thereby causing oscillatory afterpotentials. An alternate possibility is that the fluctuation of internal calcium might modulate the $\text{Na}^+/\text{Ca}^{2+}$ exchange (Karagueuzian and Katzung, 1982) which might be electrogenic (Mullins, 1979).

In the present study the beta-adrenoreceptor agonist, isoprenaline, increased internal calcium by acting on the sarcolemmal calcium channels. In patch clamp studies, it has been shown that isoprenaline might increase the influx of calcium by increasing the probability of the calcium channels to remain open upon depolarization (Reuter et al, 1982). In the present study, isoprenaline-induced increase in internal calcium is suggested by the increase in contractile force, the prolongation of the action potential plateau duration at a voltage at which the slow calcium channels are activated (Beeler and Reuter, 1970a) and possibly by the shortening of the action potential duration at 90% duration. Catecholamine-induced elevation in intracellular cyclic 3'5' adenosine monophosphate increases the slow outward plateau current, i_x (Tsien et al, 1972). It is possible that the cAMP effects on i_x are mediated by the increase in internal calcium.

Although internal calcium might be elevated by increase in the slow inward current, several features in the present study suggest that calcium from sources other than the slow inward current might also contribute to the genesis of isoprenaline-induced afterpotentials and automaticity. First, isoprenaline in as high concentrations as 10 μ M could only induce automaticity in 60% of experiments. Secondly, by increasing the stimulation rate the afterpotential was enlarged until a full depolarization was triggered. It has been shown that the increase in calcium influx that accompanies the increase in contractile force induced by increasing the drive rate (Winegrad and Shanes, 1962) is not via the slow inward current (Beeler and Reuter, 1970b). In the latter study it was observed that when quiescent myocardium was stimulated repetitively, the contractile force increased until steady-state was reached in 6 to 8 beats, whereas the slow inward current was

maximal in the first beat, suggesting that the calcium was accumulating and being released from internal stores. Thirdly, isoprenaline in the presence of ouabain or low external potassium produced larger after-potentials and enhanced the incidence of automaticity. Isoprenaline, in the presence of cardiac glycosides, produces membrane oscillation in Purkinje fibres (Tse and Han, 1974). Ouabain or low external potassium, because they increase internal sodium activity (Ellis, 1977), might thereby indirectly elevate internal calcium by enhancing the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchange (Mullins, 1979). Ouabain or low potassium solution have been previously shown to induce membrane oscillations, because they might increase the internal sodium by inhibiting the membrane Na-K pump (Eisner and Lederer, 1979b). Fourthly, high dose lidocaine completely prevented automaticity. High dose local anaesthetic agents, in addition to indirectly reducing cytosolic calcium by reducing the internal sodium activity (Deitmer and Ellis, 1980) may directly block calcium release from the sarcoplasmic reticulum (Caputo et al, 1979; Hunter et al, 1982).

It was concluded that the isoprenaline-induced ventricular membrane oscillations were: (1) prevented by procedures that might reduce cytosolic calcium (verapamil and high dose lidocaine); and (2) enhanced by procedures that might increase cytosolic calcium (low K^+ , ouabain and increased stimulation rate).

EVALUATION OF CARMINOMYCIN AND KETANSERIN

EVALUATION OF CARMINOMYCIN AND KETANSERIN

Carminomycin

Carminomycin has recently been added to the anthracycline group of drugs. Anthracyclines are presently used to treat cancer.

Anti-Arrhythmic Potency of Anthracyclines

The following evidence identifies anthracyclines (in concentrations over 5 μM) as possible new anti-arrhythmic agents: (1) Carminomycin (5 μM) reduced the duration of ventricular tachycardia and the incidence of ventricular fibrillation during coronary artery ligation and during subsequent reperfusion in isolated Langendorff perfused rat hearts (unpublished); (2) Carminomycin (10 μM) prevented automaticity induced by ouabain-isoprenaline, in guinea-pig papillary muscle (Table 2); and (3) Adriamycin (another anthracycline) in a concentration of 50 μM prevented aftercontractions and afterpotentials in Purkinje fibres and ventricular muscle (Binah et al, 1983). The anti-arrhythmic effect of anthracyclines might be related to their internal calcium lowering effect. Anthracyclines (5 to 50 μM) antagonize the calcium-dependent slow response action potentials (Azuma et al, 1981) and block the membrane $\text{Na}^+/\text{Ca}^{2+}$ exchange (Caroni et al, 1981).

Carminomycin Cardiotoxicity

Carminomycin was explored for undesirable cardiac effects in isolated working hearts by the following procedures: (1) the role of coronary flow was studied by relating coronary flow rates to myocardial mechanical performance; (2) the possible role of myocardial energy metabolism was evaluated by measuring the tissue content of high energy phosphates and of cyclic 3'5' adenosine monophosphate

Table 2Isoprenaline-Ouabain-Induced Automaticity

No intervention	86%
Carminomycin (10 μ M)	40%
Ketanserin (10 μ M)	29%

(cyclic AMP); (3) the possible toxic effect of anthracycline on mitochondria was indirectly studied by observing the effects of coenzyme Q₁₀ (Co Q₁₀), an agent thought to stabilize the mitochondrial membrane (Lenaz et al, 1982); and (4) the possible role of calcium ions was studied by altering the perfusate calcium concentration or by administration of isoprenaline or digoxin. Carminomycin was added after 20 min of working heart and observations were made for a further 55 min.

RESULTS

Control Working Heart

In control hearts cardiac output and coronary flow rates were stable during the 75 minute perfusion period that hearts were made to work. (Figure 13). At 60 minutes left ventricular power production and efficiency were the same as 15 minute values (Figure 14). Heart rate was 244 ± 5 at 5 minutes but stabilized at between 220 and 230 per minute after 15 minutes. Left ventricular stroke volume was 0.24 ± 0.01 ml at 5 minutes and stabilized at 0.26 ± 0.01 ml after 15 minutes. Peak aortic systolic pressure was not changed for the duration that hearts worked. Oxygen consumption was 166 ± 4 , 164 ± 6 and 153 ± 4 U/g/min at 15, 30 and 60 minutes. Phospho-creatine, ATP and cyclic AMP values were 5.1 ± 0.8 μ mol/g, 4.7 ± 0.2 μ mol/g and 0.40 ± 0.02 nmol/g respectively (Table 3) at the end of the perfusion period (75 minutes).

Effect of Carminomycin

Carminomycin which was added 20 minutes after the onset of heart work, produced mechanical heart failure which was concentration related (Fig.13). Fifty-five minutes after carminomycin (17.5 μ M) was added, cardiac output was reduced by 56%. Left ventricular stroke volume was reduced by 56% and peak aortic systolic pressure

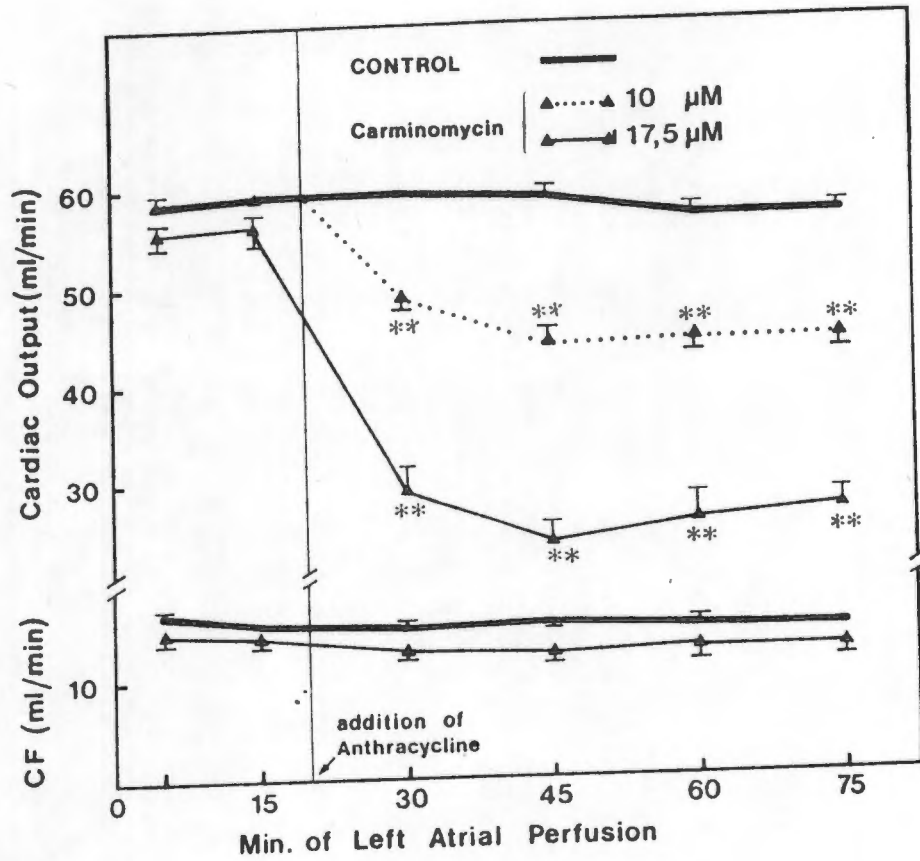


Figure 13

Effect of carminomycin on cardiac output and coronary flow (CF) rates. Results are means \pm SEM (\downarrow) $n = 14-16$. ** $P < 0.01$ vs control. Statistical analysis was by two-way analysis of variance.

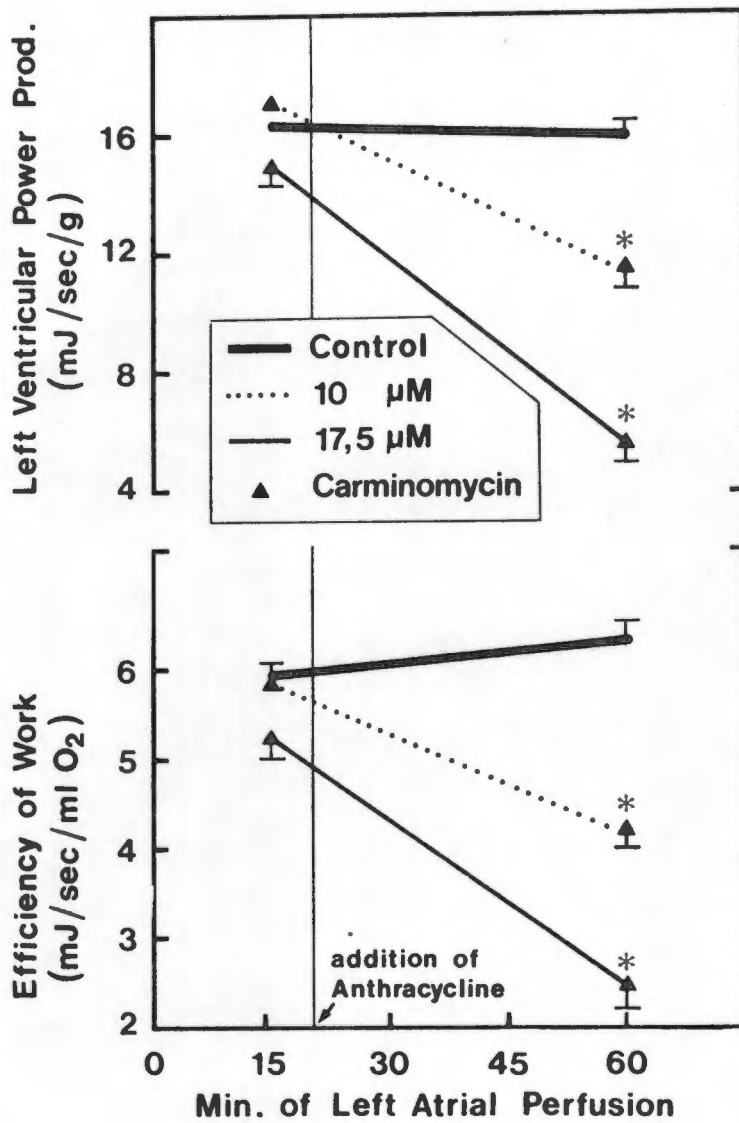


Figure 14

Effect of anthracycline on left ventricular power production (prod) (upper panel) and efficiency of heart work (lower panel). n = 14.

*P < 0.01 vs control. (Two-way analysis of variance).

Table 3 Effect of anthracyclines (55 min) and added Q_{10} on myocardial energy metabolism and mechanical function

	ATP ($\mu\text{mol/g wet wt}$)	Phospho- creatine ($\mu\text{mol/g wet wt}$)	Cyclic AMP (nmol/g wet wt)	Peak systolic pressure (% of predrug value)	Stroke volume (ml)	Heart rate (beats/min)	Cardiac output (ml/min)
Control (n=8-16)	4.7 \pm 0.2 ^a	5.1 \pm 0.8	0.40 \pm 0.04	101 \pm 1	0.26 \pm 0.01	223 \pm 5	57.1 \pm 0.8
Carminomycin (n=8-16)	3.3 \pm 0.2 ^b	3.7 \pm 0.2 ^c	0.36 \pm 0.02	97 \pm 2	0.20 \pm 0.01 ^b	219 \pm 3	44.3 \pm 1.6 ^b
10 μM	2.9 \pm 0.2 ^b	4.6 \pm 0.4	0.36 \pm 0.02	86 \pm 2 ^b	0.14 \pm 0.01 ^b	196 \pm 11	26.7 \pm 2.0 ^b
17.5 μM							
Coenzyme Q_{10} + carminomycin (n=7)	4.2 \pm 0.1 ^d	4.6 \pm 0.2	-	84 \pm 2 ^b	0.12 \pm 0.01 ^b	214 \pm 12	24.7 \pm 2.3 ^b
17.5 μM							

a = Mean \pm SE

b = $P < 0.001$ versus control. Statistical analyses by one-way analysis of variance

c = $P < 0.05$ versus control

d = $P < 0.001$ versus carminomycin 17.5 μM

- = absence of data

was reduced by 16% (Table 3). Heart rate did not fall so that the decreased cardiac output was caused by a fall in the stroke volume. Coronary flow rates were not reduced. Carminomycin reduced power and efficiency by 64% and 61% respectively (Figure 14). However, the oxygen consumption was not altered by carminomycin (Table 4). Fifty-five minutes after added carminomycin, the fall in ATP was 38% and the fall in phosphocreatine was 10% (not significant).

Effect of Interventions

The following interventions were tested: a higher perfusate calcium ion concentration (5 mM versus 2.5 mM), addition of isoprenaline to the perfusate and pre-treatment of rats with either digoxin or coenzyme Q₁₀. The aim was to prevent the fall in cardiac output caused by carminomycin (17.5 μM decreased cardiac output to 44% of the control value at 55 minutes). Figure 15 shows that the cardiac output was restored towards normal by a high calcium, isoprenaline, and digoxin pre-treatment, but not by pre-treatment with CoQ₁₀. Of the successful interventions, calcium was the most effective with a cardiac output of 91% of control value, whereas digoxin pre-treatment gave 74% of the control. The high calcium treatment also partially prevented the fall in left ventricular power production and efficiency of work caused by carminomycin (Figure 16).

DISCUSSION

An early hypothesis for the acute myocardial contractile failure caused by anthracyclines was that of coronary vasoconstriction. Mhatre et al proposed that an anthracycline metabolite, which required blood for its production, was responsible for contractile failure (Mhatre et al, 1971). Yet when their isolated hearts were perfused with a blood-free solution, the coronary perfusion pressure did not change. In

Table 4 Oxygen consumption related to cardiac output 40 min after anthracycline was added

	O ₂ consumption (μ l/g/min)	Cardiac output (ml/min)
Control ^a	153 \pm 4 ^b	57.5 \pm 1.0
Daunomycin (10 μ M)	168 \pm 3	36.2 \pm 1.8 ^c
Carminomycin (17.5 μ M)	147 \pm 8	25.9 \pm 2.1 ^c

^a n = 13 to 16

^b = Mean \pm SE

^c = P<0.001 versus control, statistical analysis by one-way analysis of variance

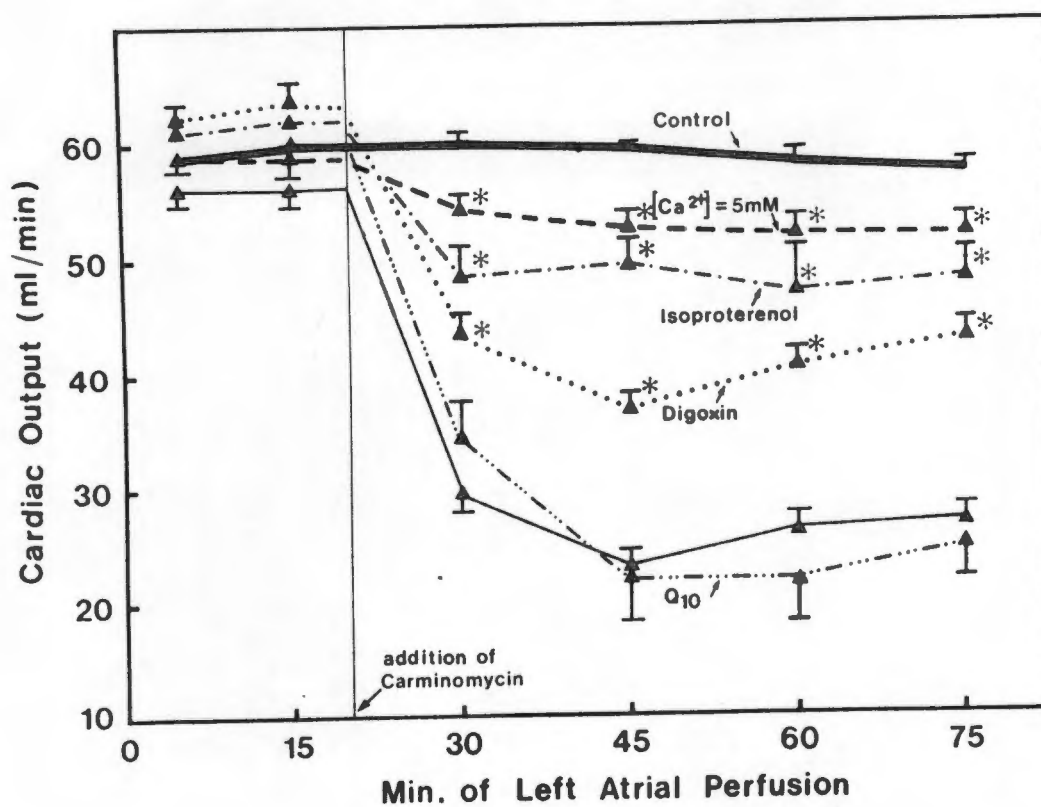


Figure 15

Protection by higher perfusate Ca^{2+} (5 mM vs 2.5 mM; $n = 8$), isoprenaline ($n=5$) and digoxin ($n=6$) against the fall of cardiac output produced by carminomycin (c) ($n=16$). Calcium concentration other than as indicated was 2.5 mM. $n=14$ in control. $n=7$ in CoQ_{10} series. $*P < 0.01$ vs carminomycin 17.5 μM alone. (Two-way analysis of variance).

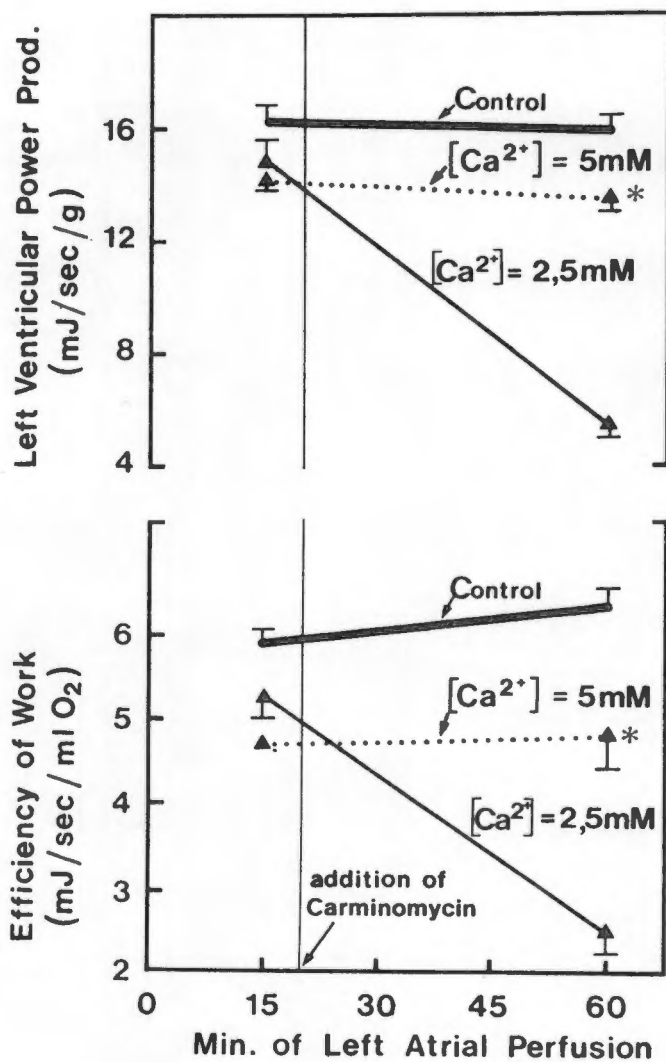


Figure 16

Effect of 5 mM perfusate Ca^{2+} ($n=8$) on fall of left ventricular power production (prod; upper panel) and efficiency of heart work (lower panel) produced by carminomycin $17.5 \mu\text{M}$. ($n=14$). Control ($n=14$) perfusate Ca^{2+} was 2.5 mM. * $P < 0.01$ vs carminomycin $17.5 \mu\text{M}$ alone. (Two-way analysis of variance).

the present study, in hearts perfused by an artificial medium, there was contractile failure even though the coronary flow did not fall (Figure 13). Since the preload (left atrial pressure) and afterload (height of perfusion column) were unchanged, and heart rate did not fall (Table 3) the acute heart failure could be ascribed to a reduced inotropic state rather than to any coronary vasoconstriction.

A disturbance of cellular energy stores could play a role as suggested by recent studies with nuclear magnetic resonance in isolated rabbit hearts (Jackson et al, 1983). Anthracyclines might impair the production of high energy components by acting on the mitochondria (Revis and Marusic, 1979). In the present hearts the contractile failure produced by treatment with anthracyclines was associated with a fall in adenosine triphosphate (ATP). But it is unlikely that a fall in ATP was the only factor causing the contractile failure since coenzyme Q₁₀ restored the high energy phosphate compounds towards normal but did not improve mechanical function (Table 3).

Anthracyclines may depress the inotropic state by modulating the ambient cytosolic calcium concentration. This hypothesis was supported by the present finding that procedures that increased cytosolic calcium relieved heart failure, viz. higher perfusate calcium, isoprenaline or digoxin. A higher perfusate calcium could increase the inward flux of calcium and thereby elevate the cytosolic calcium ion concentration. Isoproterenol might do this by enhancing calcium entry by the slow inward current (Sperelakis and Schneider, 1976) whereas digoxin might achieve the same effect by inhibiting the sarcolemmal Na⁺/K⁺-ATPase (Bachur et al, 1975; Boxtel et al, 1978; Glynn, 1964). The negative inotropic effect of acute anthracycline administration has also been reported in in vitro preparations studying contractile force (Azuma et al, 1981; Boxtel et al, 1978; Kobayashi et al, 1972; Mhatre et al, 1971.

The mechanism could be that anthracyclines either sequester calcium at intracellular sites (Revis and Marusic, 1979) or interfere with the transmembrane flux of calcium ions (Caroni et al, 1981). It is unlikely that anthracyclines altered calcium flux by modulating cyclic AMP because the myocardial levels of the latter were unchanged.

Conclusion: In the present model, anthracyclines lowered cytosolic calcium ion concentration and impaired the production of high energy phosphates by separate mechanisms. It must be emphasized that conclusions, based on the acute effects of anthracyclines on an in vitro heart preparation may not necessarily be relevant to the situation in patients receiving anthracyclines.

Ketanserin

Serotonin mediates peripheral arteriolar constriction. The serotonin antagonist, ketanserin, is used to treat hypertension and heart failure as an afterload reducing agent. Serotonin receptors have recently been described to occur in the myocardium (Kaumann, 1983).

Anti-Arrhythmic Potency of Ketanserin

The following evidence suggests an anti-arrhythmic role for ketanserin: (1) Ketanserin (5 μM) protected against ventricular tachycardia and fibrillation induced by coronary occlusion and by subsequent release in Langendorff perfused rat hearts (to be published); (2) Ketanserin (10 μM) reduced the incidence of automaticity induced by ouabain and isoprenaline in guinea-pig papillary muscle (Table 2); (3) Serotonin (10 μM) induces aftercontractions in kitten papillary muscle (Kaumann, 1983); and (4) Serotonin infusion in dogs with three day old infarcts, provokes ventricular arrhythmias (Ross and Wilkerson, 1979).

It is unlikely that the anti-arrhythmic action of ketanserin is

mediated by the S_2 receptors. The anti-arrhythmic concentration of ketanserin (5 to 10 μM) is several-fold higher than that required to cause S_2 serotonergic receptor antagonism (1 to 10 nM) (Leysen et al, 1981). It is probable that the anti-arrhythmic effect of ketanserin (5 to 10 μM) might be due to its alpha-1 adrenoceptor antagonism. Ketanserin binds to alpha-1 adrenoceptor (van Neuten et al, 1980; van Neuten et al, 1981), the ED_{50} for alpha-1 receptor affinity in coronary artery being 0.7 μM . Moreover, the alpha-1 receptors mediate elevation of internal calcium (Sharma et al, 1983).

Cardiac Effects of Ketanserin

The effects of ketanserin (0.1, 1 and 10 μM) on mechanical function and metabolic profile were studied in isolated rat hearts. Ketanserin was added at 20 min of working heart and observations were made for a further 40 min.

RESULTS

In control hearts aortic flow, coronary flow and peak aortic pressure were stable for the 60 min that hearts were made to work (Figure 17). Heart rate fell by 5% at 5 min but stabilized thereafter. At the end of 60 min left ventricular work was $17.4 \pm 0.6 \mu\text{l/g/min}$, ATP was $2.49 \pm 0.1 \mu\text{M/g}$, phosphocreatine was $3.11 \pm 0.16 \mu\text{M/g}$ and cyclic AMP was $0.40 \pm 0.02 \text{ nm/g}$.

Ketanserin (0.1 μM) had no effect and 1 μM reduced only heart rate at 5 min (248 ± 7 vs 290 ± 6 beats/min). Ketanserin (10 μM) reduced heart rate even at 5 min (214 ± 4 vs 290 ± 16 beats/min). At 10 min (of 10 μM) a further fall in heart rate was associated with a fall in aortic flow (42 ± 2 vs 50 ± 1 ml/min) and a rise in aortic pressure (132 ± 4 vs 119 ± 3 mmHg). All the changes produced by ketanserin were sustained for the 40 min observation period. The

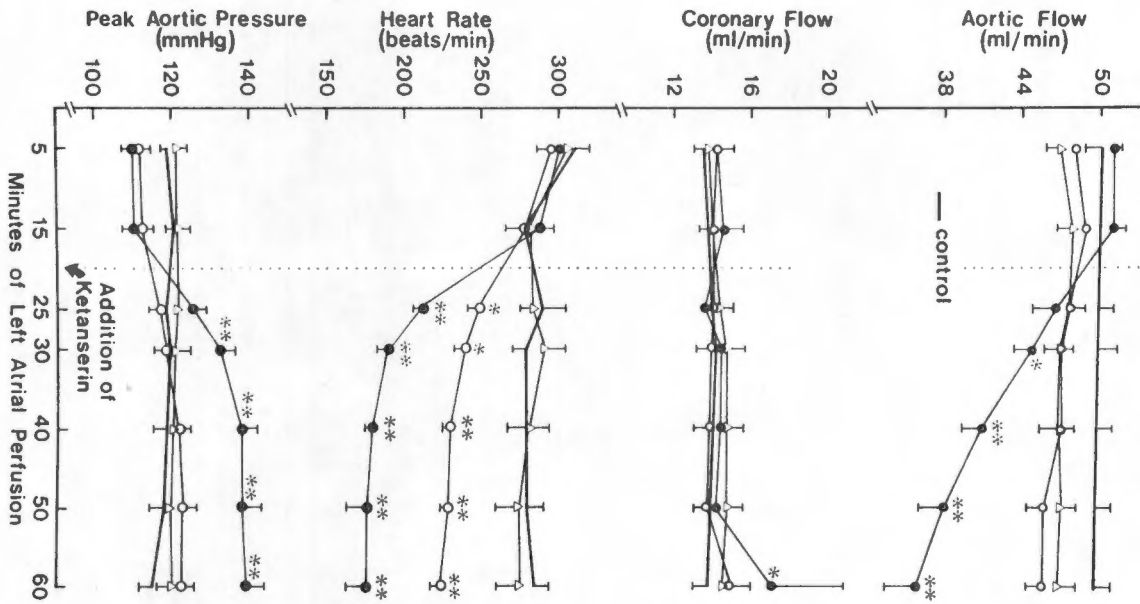


Figure 17

Effect of ketanserin (0.1, 1 and 10 μM) on aortic flow, coronary flow, heart rate and peak aortic pressure of isolated working rat hearts.

Δ = 0.1 μM ; \circ = 1 μM ; \bullet = 10 μM . * $P < 0.05$ versus control;

** $P < 0.01$ versus control. Statistical analysis was by two-way

analysis of variance. Increase of coronary flow due to ketanserin

10 μM was due to one heart which failed completely.

higher coronary flow rate at 40 min of 10 μM ketanserin was due to one heart which failed severely. The other four hearts in that group had normal coronary flow rates at the end of the experiments. Ketanserin in all the concentrations used did not alter heart work, O_2 consumption, high energy compound stores or cyclic AMP after 40 min (Table 5).

DISCUSSION

Ketanserin (0.1 and 1 μM) did not alter mechanical function or the metabolic profile. Ketanserin 10 μM caused heart failure which was not due to change in myocardial cyclic AMP, high energy compounds, heart work or O_2 consumption. Since there was no alteration in left ventricular power production, the heart failure was most probably due to the marked reduction in heart rate. How ketanserin reduces the heart rate is not known.

Table 5

Heart work and metabolic profile

	<u>Control</u>	<u>Ketanserin</u>		
		0.1 μ M	1 μ M	10 μ M
Left ventricular work (mJ/sec/g)	17.4 \pm 0.6	17.6 \pm 0.3	17.6 \pm 0.6	17.2 \pm 1.3
O ₂ consumption (μ l/g/min)	184 \pm 9	175 \pm 7	175 \pm 8	182 \pm 20
ATP (μ M/g/fresh wt)	2.49 \pm 0.10	2.58 \pm 0.07	2.59 \pm 0.10	2.39 \pm 0.10
PCr (μ M/g fresh wt)	3.11 \pm 0.16	3.17 \pm 0.10	2.90 \pm 0.12	2.51 \pm 0.20
cAMP (nM/g fresh wt)	0.40 \pm 0.02	0.40 \pm 0.03	0.38 \pm 0.03	0.38 \pm 0.02

COMMENTS

At this time, the cellular electrophysiological mechanism that initiates and sustains serious cardiac arrhythmias including ventricular fibrillation is not clear. Over a hundred years of research using several models has drawn to attention that automaticity, re-entry or the injury current might initiate arrhythmias. Can more than one of the three mechanisms be involved? Scrutiny of the literature (see Introduction) has revealed the possibility that ectopic ventricular automaticity might explain extrasystoles and ventricular tachycardia. These premature ventricular beats might, by reducing the effective refractory period and by slowing impulse conduction, set the stage for re-entry, which in turn might sustain ventricular fibrillation.

On the basis that automaticity might ultimately initiate ventricular fibrillation, the present study explored ventricular automaticity induced by simulated "reperfusion" or beta-adrenergic stimulation with isoprenaline. Table 6 summarizes the similarities of the afterpotentials that were induced by "reperfusion" or by isoprenaline. The similarity in the afterpotentials evoked by the two conditions suggests that a common mechanism operated. The present experimental findings support the hypothesis that an elevated cytosolic calcium mediated the afterpotentials. However, it must be stressed that the role of calcium is inferred as cytosolic calcium was not directly measured.

It is proposed that ventricular automaticity induced by "reperfusion" or isoprenaline could be an important model to explore the mechanism of arrhythmias and the mechanism of new anti-arrhythmic drugs. Moreover, this phenomenon might have implications for lethal arrhythmias associated with clinical ischemic heart disease, since both reperfusion after transient ischemia and excessive beta-adrenoceptor

Table 6 "Reperfusion" versus isoprenaline-induced automaticity

	<u>"Reperfusion"</u>	<u>Isoprenaline</u>
Arising from afterpotentials	Yes	Yes
Afterpotential amplitude	5 mV	5 mV *
Coupling interval	About 200 msec	About 200 msec
Threshold potential	About -75 mV	About -75 mV
Verapamil	Partially prevented by 5 μ M	Completely prevented by 1 μ M

* Exaggerated by low K^+ (Figure 9)

stimulation might be features of coronary artery disease.

CLINICAL IMPLICATIONS

Sudden Coronary Death

The following short quotation highlights a fearsome expression of coronary artery disease over the ages:

"In the industrially developed countries, sudden cardiac death is the leading cause of death. It was recognized at the dawn of recorded history and even depicted in Egyptian relief scripture from the tomb of a noble of the sixth dynasty approximately 4,500 years ago. Sudden cardiac death has left no age untouched. Sparing neither saint nor sinner, it has burdened man with a sense of uncertainty and fragility. The enormity of this problem demands attention. In the United States, sudden death claims 1,200 lives daily, or approximately one victim every minute. It is the leading cause of death among men aged 20 to 64 years, accounting for 32 per cent of the fatalities in this group" (Lown, 1979).

In the present day, calculation from global data reveals that 20 to 30 sudden cardiac deaths occur per week in most populations of one million (Pisa, 1980). In South Africa, 52 victims are claimed weekly (MRC, 1980). The majority of patients with coronary artery disease are at risk of sudden death (Goldstein, 1974; Oliver, 1982). Conversely, 80 to 90% of sudden deaths occur in patients with underlying coronary artery disease (Spain et al, 1960), which may be silent or manifest as angina or myocardial infarction.

Sudden coronary death has been broadly defined as death occurring within 24 hours of the acute onset of symptoms. However, this

definition is inadequate because the duration of symptoms reflects the pathogenesis. Deaths occurring soon after symptoms are deemed to be caused by arrhythmias. About half of the coronary deaths occurring within 24 hours of the onset of symptoms occur in the first hour (Kannel et al, 1975), and in the latter group, the majority occur instantaneously (Lieberthson et al, 1974; Wikland, 1968). For the purposes of this thesis, sudden coronary death is defined as death occurring within one hour of the onset of acute symptoms.

Presently, the pathogenesis of sudden death is poorly understood but recent evidence suggests that ventricular fibrillation is the terminal event. Over the past three decades specialized coronary care units have emerged for the constant surveillance of patients with acute symptoms of coronary disease. In this way, it was hoped that resuscitation might be effective in reducing sudden death. However, it was soon realized that two-thirds of coronary deaths occur outside hospitals. Also, the out of hospital deaths were more likely to be sudden than hospital deaths (Gordon and Kannel, 1971). The next natural step in the struggle against coronary mortality was the introduction of mobile coronary care units. This intervention revealed that the major mechanism of outside hospital cardiac arrest was ventricular fibrillation (Cobb et al, 1975; Myerburg et al, 1980). It was also found that in the majority of instances, the ventricular fibrillation is unaccompanied by clinical evidence of acute myocardial infarction (Baum et al, 1974). This finding was confirmed in post mortem studies which revealed that only one-third of patients dying within half an hour of symptoms, demonstrate acute myocardial infarction (Lovegrove and Thompson, 1978). In this group, ventricular fibrillation is deemed to be the cause of death. Furthermore, in a major 5 year prospective study, in which civil

servants were screened, 90% of coronary deaths occurring within one hour of onset of symptoms were attributed to ventricular arrhythmias (Hinkle, 1982). Direct evidence for the role of ventricular fibrillation was obtained from coronary care units (Killip and Kimball, 1967; Robinson et al, 1965) and from fortuitous Holter monitoring during sudden death (Bleifer et al, 1974; Gradman et al, 1977; Lahiri et al, 1979; Hinkle et al, 1977; Panidis and Morganroth, 1983; Pratt et al, 1983).

Reduction of sudden coronary death may be sought by three possible approaches: First, control of the underlying coronary artery disease; secondly, by prompt resuscitation of ventricular fibrillation. In most communities the value of this intervention is limited because ventricular fibrillation usually occurs unexpectedly outside hospital; and thirdly, by drug prophylaxis in patients at risk (those having angina, recent acute myocardial infarction, old myocardial infarction, recurrent episodes of tachyarrhythmias or those resuscitated from ventricular fibrillation). Presently, although in some studies anti-arrhythmic drugs reduce the mortality after myocardial ischemia (Lie et al, 1974; Multicentre International Study, 1975; Wilhelmsson, 1974) other reports question the value of anti-arrhythmic therapy (Adgey and Webb, 1979; Clausen et al, 1966; Durandher et al, 1971; Pantridge and Geddes, 1974). In general, due to their doubtful protective value and side-effects, no ideal anti-arrhythmic drug exists. To anticipate new drugs with anti-arrhythmic action, it is necessary to clarify the mechanism of ventricular fibrillation.

Information on the evolution of electrical events immediately before and during ventricular fibrillation is difficult to obtain because death usually occurs instantaneously outside hospital (Lown and Wolf, 1971). Ambulatory electrocardiograms which were fortuitously recorded during

sudden death have revealed that increasing salvos of ventricular extrasystoles and ventricular tachycardia almost always precede ventricular fibrillation (Pratt et al, 1983; Panidis and Morganroth, 1983). In experimental models, coronary artery ligation-induced (Harris and Rojas, 1943) or electrically-induced (Moe et al, 1941) ventricular extrasystoles and tachycardias degenerate into ventricular fibrillation. The ventricular ectopic rhythms appear to originate from sites demonstrating oscillating potentials. These findings support the hypothesis (Harris and Rojas, 1943) that ventricular tachycardia might evoke the electrical inhomogeneity required for re-entry circuits to sustain ventricular fibrillation. The ventricular ectopics might be mediated by oscillating membrane afterpotentials (Cranefield, 1977).

Reperfusion Arrhythmias

In the group in which coronary death is not accompanied by acute myocardial infarction, ventricular fibrillation induced by reperfusion after coronary spasm is a possible mechanism: the arrhythmias associated with coronary artery spasm are well known (Maseri, 1981) and occur when reperfusion is signalled by the reversal of ST-segment changes (Araki et al, 1983; Maseri et al, 1979; Previtali et al, 1983).

Reperfusion ventricular fibrillation might also be of importance during spontaneous thrombolysis (Braunwald, 1983) or thrombolysis therapy (Corr and Witkowski, 1983; Mathey et al, 1981).

Beta-adrenoceptor Mediated Arrhythmias

The following evidence links ventricular arrhythmias to the autonomic nervous system. Excessive beta-adrenoceptor stimulation has been suggested to play a role in sudden death (Lown et al, 1977). Catecholamines induce severe ventricular tachyarrhythmias in children with Adams-Stokes syndrome (Coumel et al, 1978). Local lesions,

remarkably resembling the lesions induced experimentally by catecholamines, are observed in the hearts of victims of instantaneous death (Baroldi, 1975).

IMPLICATIONS FOR THE RESEARCH OF ARRHYTHMIAS

Since it is difficult to investigate arrhythmias in humans, experimental models have included arrhythmias induced by electrical stimulation, coronary artery ligation or by subsequent reperfusion. Reperfusion arrhythmias have been used widely as an experimental model because of their consistent appearance. Also, there is the possibility that reperfusion after transient regional ischemia (coronary artery spasm) might be a mechanism in sudden coronary death. There has been much debate about whether re-entry or automaticity is responsible for reperfusion-induced ventricular fibrillation.

Automatic Focus or Re-entry?

In several in vivo models of regional ischemia, reperfusion-induced ventricular fibrillation was ascribed to either abnormal automaticity or re-entrant excitation. The mechanism involving automaticity was suggested on the basis of a reperfusion-induced increase in idioventricular rate (Penkoske et al, 1978), a finding that could not be subsequently confirmed (Levites et al, 1975; Murdock et al, 1980). The different methods used to block atrioventricular conduction (right vagal nerve stimulation in the former study and formaldehyde infiltration in the latter studies) might explain the conflicting results. The formaldehyde injection might have depressed potential pacemakers in the region of the atrioventricular node (Corr and Witkowski, 1983). In isolated rat hearts, reperfusion tachyarrhythmias were ascribed to automaticity evoked by oscillatory afterdepolarizations on the basis of their similarity to digitalis arrhythmias (Carbonin et al, 1981). The alternative

mechanism of re-entry has been suggested because reperfusion arrhythmias were associated with electrical heterogeneity (Levites et al, 1975; Murdock et al, 1980). However, the dispersion of refractoriness occurring after reperfusion could not be temporally related to the arrhythmias (Naimi et al, 1977). An argument for multifocal activation from the border of ischemic-reperfused region has been made on the basis of multiple epicardial electrogram recordings but this technique cannot distinguish between abnormal pacemakers and microcircuits (Ideker et al, 1981).

In the present study, return to normal medium after the temporary withdrawal of glucose and O_2 induced automaticity that was mediated by afterpotentials. Although the "ischemic" conditions in the present study are not exactly representative of the changes that occur during regional ischemia, the minimum conditions of substrate and O_2 deprivation were capable of provoking the "reperfusion" automaticity. The present findings support the view that automaticity might play a role in reperfusion arrhythmias. However, it must be noted that direct evidence can only be obtained by recording automaticity in a reperfusion model after regional ischemia.

Evidence in Favour of the Idea that Ventricular Automaticity Might Initiate Fibrillation

1. In previous studies (Penkoske et al, 1978; Murdock et al, 1980; Carbonin et al, 1981), the duration of ischemia and time of onset of ventricular fibrillation on subsequent reperfusion, correspond to the duration of hypoxic glucose-free period (25-40 min) and time of onset of automaticity (within seconds) in the present study.
2. In Langendorff perfused hearts, "reperfusion" after hypoxic glucose-free perfusion, induced premature extrasystoles and ventricular

tachycardia which appeared to degenerate into fibrillation. In the Langendorff model, the time of onset of the premature extrasystoles (within seconds) and their coupling interval (about 200 msec), correspond to the time of onset and coupling intervals of the extrasystoles induced by "reperfusion" in the papillary muscle.

3. Excess beta-adrenoceptor stimulation, which is thought to mediate ventricular fibrillation (Lown, 1977), also induced automaticity in the present study.
4. Carminomycin and ketanserin, which prevented ventricular fibrillation during coronary artery ligation and subsequent reperfusion (unpublished), also prevented automaticity.
5. It may be worth speculating that afterpotentials evoke premature extrasystoles and ventricular tachycardia in humans. Salvos of premature extrasystoles and/or ventricular tachycardia almost always precedes ventricular fibrillation in humans (Pratt et al, 1983; Panidis and Morganroth, 1983). However, it must be stressed that great caution must be exercised in extrapolating from the experimental model to man.

SUMMARY

Ventricular automaticity might be induced by "reperfusion" or isoprenaline. The automaticity might arise from membrane afterpotentials which might be triggered by elevated cytosolic calcium. It is proposed that automaticity induced by "reperfusion" or isoprenaline might be an important model to explore the mechanism of arrhythmias and to study new anti-arrhythmic drugs.

Very high concentrations (10 μ M) of carminomycin or ketanserin were required to prevent automaticity in the papillary muscle. This

concentration was in the cardiotoxic range for both carminomycin and ketanserin. It is unlikely that these drugs could have clinical application unless an intervention against the heart failure is found. Nevertheless, the two drugs may be useful to explore further the mechanism of ventricular automaticity.

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