STUDIES ON THE ANTIARRHYTHMIC ACTIONS OF PROSTAGLANDINS

by

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ABSTRACT

The antiarrhythmic actions of prostaglandins were first investigated using arrhythmias associated with cardiac ischemia in the dog and the rat. These studies were followed by investigations of the possible mechanisms of action, using rat heart tissue in intact, isolated, and cell culture preparations.

Preliminary experiments in the dog revealed that prostaglandins E_2 and $F_{1\alpha}$ markedly reduced the number of premature ventricular contractions occurring within the first 25 minutes following coronary artery ligation. Prostaglandin E_2 or $F_{1\alpha}$ did not markedly alter the cardiovascular response to occlusion, making it unlikely that modulation of autonomic reflexes is a central factor in their antiarrhythmic action.

Coronary ligation in the rat was used to compare the antiarrhythmic effectiveness of prostaglandins, lidocaine, and quinidine. Prostaglandins E_2, $F_{2\beta}$, and quinidine were found to be the most effective against arrhythmias occurring within the first 25 minutes following occlusion, reducing the number of PVCs by 40 to 50 per cent. The number of flutter episodes and the number of animals dying from arrhythmias was also markedly decreased by prostaglandins E_2 and $F_{2\beta}$, and by quinidine. Prostaglandins $F_{1\alpha}$ and A_2, and lidocaine had lesser effects. Prostaglandins had only minor effects on blood pressure or heart rate, which were not related to their antiarrhythmic activity. No significant differences were found in the infarct size with prostaglandin treatment.

The effects of prostaglandins E_2, A_2, $F_{1\alpha}$, and $F_{2\beta}$, quinidine, and lidocaine were tested in *in situ* rat heart on electrically-induced flutter threshold and maximum following frequency. Flutter threshold was not changed by any of the prostaglandins tested, although lidocaine increased and quinidine decreased it. Prostaglandins caused a dose-dependent change in maximum following frequency which was usually less than 10 per cent of control. Lidocaine produced a marked increase and quinidine a marked decrease in maximum following frequency. The slight depressive action of prostaglandins does not correlate with their antidysrhythmic actions.

Prostaglandins of the E, A, and F series were found to have only minimal effects on rate and force in isolated rat hearts. However, both PGE_2 and $PGF_{2\beta}$ delayed the loss of contractile force with time at 10^{-7} M. All prostaglandins tested markedly increased coronary flow rate at 10^{-5} M.

The effects on the beating behavior of cultured rat heart cells of fourteen prostaglandins of the A, B, D, E, and F series were investigated in cultured rat heart cells. With the exception of $PGF_{2\alpha}$, which produced a chronotropic response, prostaglandins had limited direct action in cultured rat heart cells.

The effects of ouabain, calcium, potassium, dinitrophenol, and *Cyanea* toxin, together with prostaglandins, lidocaine, and quinidine on cultured rat heart cells were also investigated. Ouabain and calcium increased rate and fibrillatory movements, while potassium and dinitrophenol slowed rate and decreased rhythmic beating. *Cyanea* toxin produced a characteristic

series of arrhythmogenic changes which were also used to test for antiarrhythmic activity in cultured heart cells. Lidocaine and quinidine were effective only against cellular arrhythmias caused by high calcium concentration, and prostaglandins were effective only against dinitrophenol-induced arrhythmias, indicating that there is no over-all "protective" effect of prostaglandins in cell culture.

TABLE OF CONTENTS

TABLE OF CONTENTS continued

LIST OF TABLES

LIST OF FIGURES

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CHAPTER I

INTRODUCTION

A. General chemistry and Metabolism

Prostaglandins are a family of unsaturated hydroxy fatty acids which have a wide spectrum of pharmacological activity. Prostaglandins are best classified as autacoids or "local hormones". They are not stored in the body but are synthesized in response to a particular stimulus. Due to rapid metabolic degradation their effects are mainly confined to their site of production. They have potential therapeutic uses as vasodilators, decongestants, antiarrhythmics, and as agents which prevent blood platelet aggregation. They can also be used in the treatment of asthma and stomach ulcers, as abortifacients, and to induce labor (Colbert, 1973). The experimental investigation of their possible use as ANTIARRHYTHMICS is the concern of this thesis.

Although prostaglandin-like activity had been noticed in seminal fluid as early as 1930 (Kurzrok, 1930), it was not until 1934 that workers in England (Goldblatt, 1935) and Sweden (von Euler, 1936) independently found that the active principle extracted from seminal plasma was an acidic, lipid-soluble material. The term "prostaglandins" was coined by von Euler (von Euler, 1936) in the belief that the biologically active substance was a secretion of the prostate gland. It is

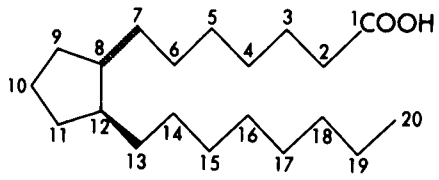
now known that prostaglandins occur in widespread distribution in animal tissues, and that they are produced in response to a wide variety of stimuli. In minute amounts they produce effects on a large variety of physiological processes (Douglas, 1975).

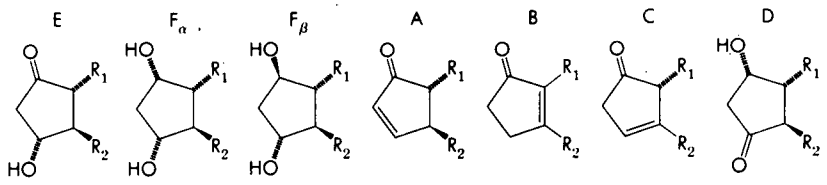
Chemistry

The prostaglandins may be regarded as derivatives of a hypothetical parent structure with the name prostanoic acid. (Figure 1, Douglas, 1975). The different prostaglandins fall into several main classes, known as the E, F, A, B, C, and D series of compounds. This classification is based on the constituents of the cyclopentyl ring (Figure 2; Douglas, 1975). These classes are further subdivided with the subscript 1, 2, or 3 according to the number of double bonds in the side chains. All natural prostaglandins have a trans double bond at the 13, 14 position and a hydroxyl group at C_{15}. Thus prostaglandin E_1 (PGE_1) has only the $C_{13,14}$ double bond, while PGE_2 has an additional cis double bond at $C_{5,6}$ and PGE_3 has a third cis double bond at $C_{17,18}$. These double bonds occur in the same positions in all classes. The E and F series, often referred to as the 'primary prostaglandins', are the most commonly found and also the most intensively studied.

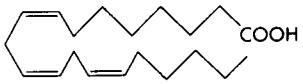
A large number of stereoisomeric forms are possible, based on the several asymmetric centers. The prostaglandins obtained from natural sources have the side chains arranged trans to each other, i.e. they are oriented at opposite sides of the ring (Figure 2; Douglas, 1975). C_9 and C_{11} hydroxyl groups are

Figure 1: The structure of prostanoic acid (top) and several main classes of prostaglandins together with precursor fatty acids. In the stereochemical convention followed, the groups indicated by a hatched line lie behind the plane of the cyclopentane ring, while those indicated by ▬ lie in front of it. (Douglas, 1975)

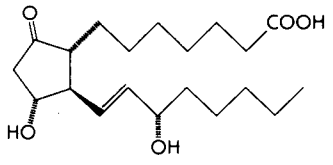




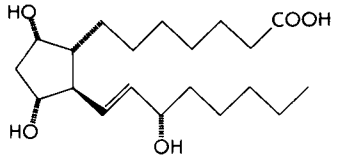
Basic Ring Structures



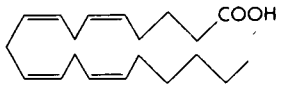
8,11,14-Eicosatrienoic Acid (Dihomo-γ-Linolenic Acid)



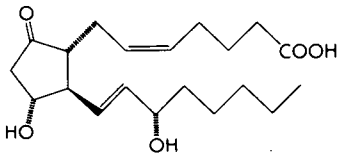
PGE_1



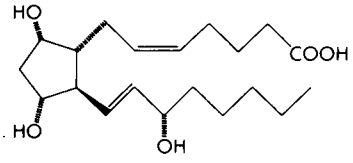
$PGF_{1\alpha}$



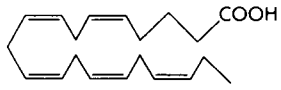
5,8,11,14-Eicosatetraenoic Acid (Arachidonic Acid)



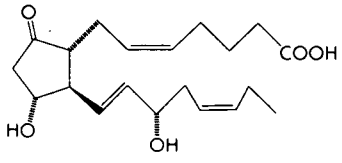
PGE_2



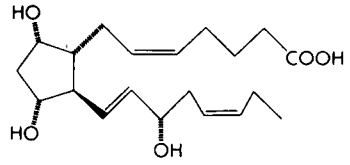
$PGF_{2\alpha}$



5,8,11,14,17-Eicosapentaenoic Acid



PGE_3



$PGF_{3\alpha}$

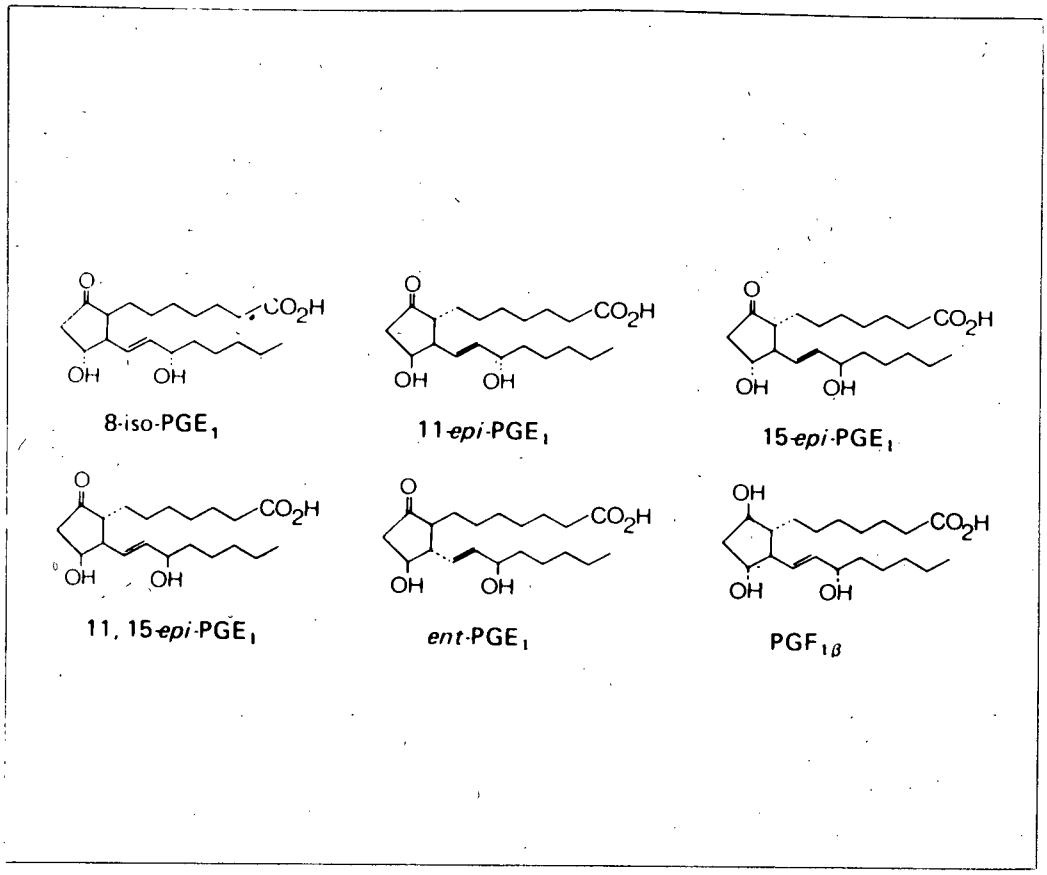
also normally in the alpha configuration, i.e. on the same side of the ring as the carboxylic acid side chain. The C_{15} hydroxyl group is also normally designated as alpha.

Synthetic stereochemical variants of these natural forms may arise in several different ways. (Figure 2) First, the side chains may be cis to one another. These are referred to as 8-iso-prostaglandins. Secondly, one or more hydroxyl groups may have the beta configuration. The C_{11} and C_{15} beta hydroxyls are sometimes referred to by the term epi. The C_{15} hydroxyl group can also be named after the Chan-Ingold-Prelog Convention, i.e. S for alpha and R for beta (Butcher, 1973). Thirdly, all prostaglandins may exist in two optically active forms. Natural prostaglandins are levorotatory and their mirror images, which are dextrorotatory, have their side chains transposed. (The latter are also referred to as the ent forms.) All of these stereochemical variants may be combined with each other.

Reference to the stereochemistry is often omitted in the abbreviated forms, e.g. PGE_1. For the F series, however, the term alpha or beta (e.g. $PGF_{1\alpha}$ or $PGF_{1\beta}$) is added to denote the configuration of the C_9 hydroxyl group. A method based on the structure of prostanoic acid may also be used to describe prostaglandins in terms of their full systematic nomenclature when necessary (Nugteren et al, 1966). For example, the full systematic name of PGE_1 is (-)-11α, 15(S)-dihydroxy-9-oxo-13-trans-prostanoic acid, where the (-) refers to levorotation and the trans refers to the $C_{13,14}$ double bond.

Over the last decade more than two thousand prostaglandin analogs have been prepared (Schneider, 1976). It has been

Figure 2: Several synthetic variants of naturally-occurring prostaglandins. The groups indicated by a dotted line lie behind the plane of the cyclopentane ring. (Caton, 1973)


8-iso-PGE1
11-epi-PGE1
15-epi-PGE1
11, 15-epi-PGE1
ent-PGE1
PGF1β

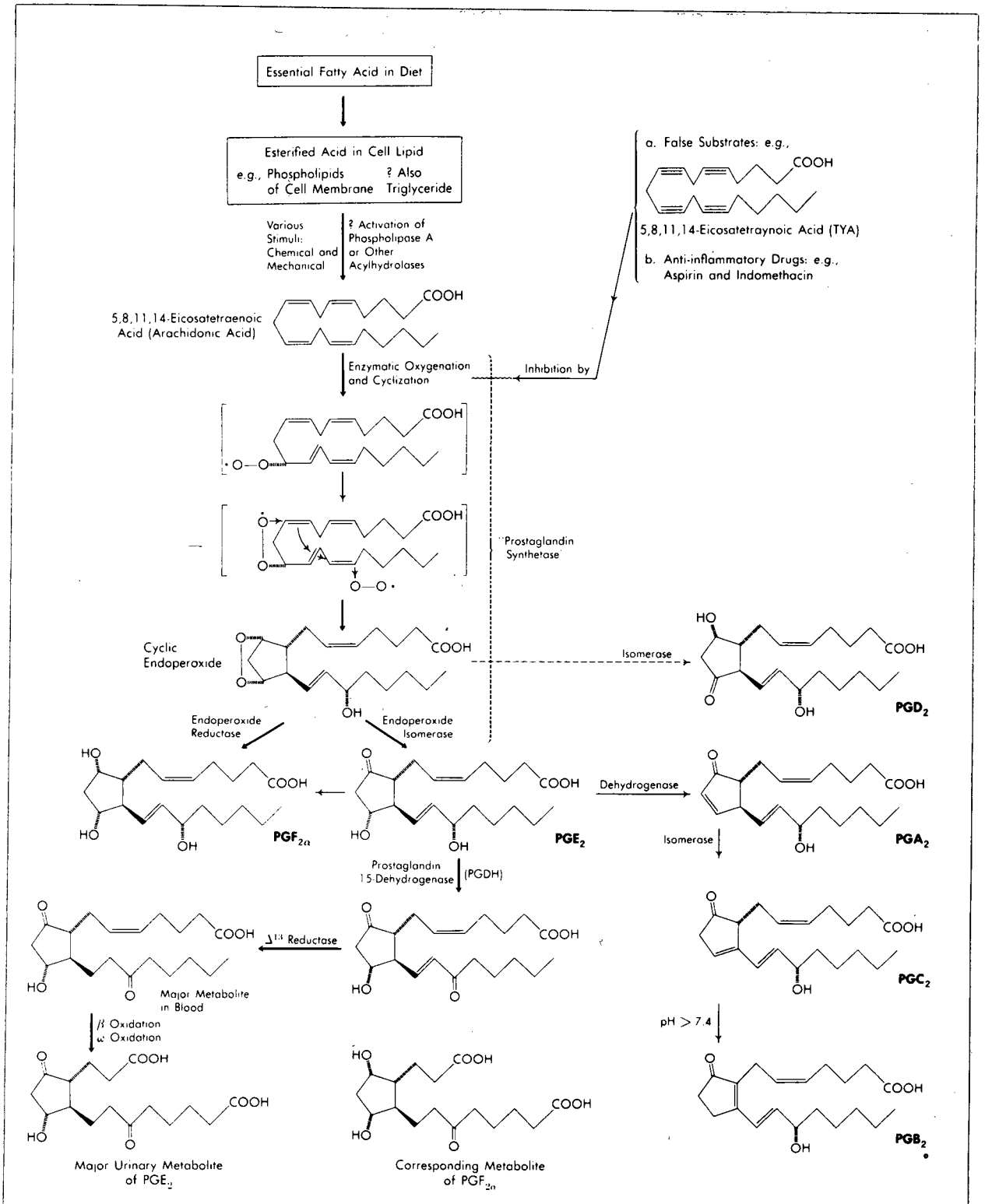
possible to name the great majority of these compounds after the natural group to which they are most closely related.

Biosynthesis and Catabolism

Prostaglandins are not stored in the body but are synthesized from the essential fatty acid precursor in response to widely divergent physical, chemical, hormonal, and other influences (Figure 3; Douglas, 1975). Synthesis depends on phospholipase-catalyzed release of precursor acid from cellular phospholipid stores. Activation of phospholipase A is thought to be the rate-limiting step in prostaglandin production. The primary prostaglandins (E and F series) are synthesized in a stepwise manner by a complex of microsomal enzymes referred to as "prostaglandin synthetase". Oxygenation and cyclization of the precursor acid take place to form a cyclic peroxide derivative. This endoperoxide is either isomerized or reduced to yield PGE or PGF compounds. Prostaglandins A, B, and C arise from PGE by dehydration and isomerization (Samuelsson, 1972; Hamberg and Samuelsson, 1973).

Prostaglandins E and F are fairly stable in blood but are rapidly degraded by tissue-bound enzymes; 80 to 90% of a dose is destroyed during a single passage through the lungs or the liver (Vane, 1969: Piper, 1973). Metabolic pathways are species dependent. In man the major metabolic step for both PGE and PGF compounds is oxidation of the secondary alcohol group at C_{15} by prostaglandin dehydrogenase, followed by reduction of the C_{13} double bond. The resulting 15-dehydro-13, 14-dehydro derivatives are inactive (Samuelsson, 1972; Andersen and Ramwell, 1974). Among the most successful analogs

Figure 3: Synthesis and catabolism of the two principal mammalian prostaglandins, PGE_2 and $PGF_{2\alpha}$ (Douglas, 1975)


Essential Fatty Acid in Diet
Esterified Acid in Cell Lipid
e.g., Phospholipids of Cell Membrane
? Also Triglyceride
Various Stimuli: Chemical and Mechanical
? Activation of Phospholipase A or Other Acylhydrolases
5,8,11,14-Eicosatetraenoic Acid (Arachidonic Acid)
Enzymatic Oxygenation and Cyclization
Inhibition by
a. False Substrates: e.g.,
5,8,11,14-Eicosatetraynoic Acid (TYA)
b. Anti-inflammatory Drugs: e.g., Aspirin and Indomethacin
Prostaglandin Synthetase
Cyclic Endoperoxide
Isomerase
PGD2
Endoperoxide Reductase
Endoperoxide Isomerase
PGF2α
PGE2
Dehydrogenase
PGA2
Isomerase
Prostaglandin 15-Dehydrogenase (PGDH)
Δ13 Reductase
PGC2
Major Metabolite in Blood
β Oxidation
ω Oxidation
pH > 7.4
PGB2
Major Urinary Metabolite of PGE2
Corresponding Metabolite of PGF2α

to be prepared are the 15- and 16-alkyl prostaglandins, which were designed to resist enzymatic C-15- dehydrogenation. Two members of this series, 15(5)-methyl PGE_2 and 16,16-dimethyl PGE_2, have been found to possess potential gastric antisecretory activity in animals (Robert, 1972). PGAs are degraded more slowly, and this series may be the only naturally occurring prostaglandins capable of functioning as circulating hormones (Lee, 1974). New prostanoids are continually being discovered, i.e. the endoperoxides (PGG and PGH) and PGI (Schaaf, 1976). Thromboxanes, other products of endoperoxide metabolism, have also been found to possess marked pharmacological activities (Schaaf, 1976). They are unstable compounds with extremely short half lives, but may nevertheless be responsible for physiological processess. Other analogues of these compounds are being actively investigated.

B. Prostaglandins and the Heart

Investigation of prostaglandins as antiarrhythmics requires detailed physiological, pathological and pharmacological knowledge of their importance in cardiac tissue.

Effects on Coronary Circulation

Pharmacological experiments on intact animals and isolated hearts have shown that prostaglandins of the E and A series have strong vasodilating actions on coronary arteries (Berti et al., 1965; Bloor and Sobel, 1970; Higgins et al., 1971, 1973; Hutton et al., 1973; Nakono and McCurdy, 1968; Nutter and Crumly, 1970; Vergroesen, 1967). In anesthetized dogs prostaglandins E_1 and E_2 have been reported to be more

potent coronary vasodilators than adenosine, exceeding its efficacy five to ten times (Rowe and Afonso, 1974). The duration and magnitude of the coronary vasodilator action of PGE_1 have also been shown to be greater than that of nitroglycerin (Nakano and McCurdy, 1968). The coronary vasodilator effect of PGA_1 has been reported to be much less than that caused by equivalent doses of PGE_1, although the systemic hypotensive effect of PGA_1 exceeded that produced by PGE_1. The increased coronary blood flow and decreased coronary vascular resistance produced by intracoronary administration of prostaglandins of the E and A series in the dog has been shown to occur without any change in heart rate or systemic blood pressure (Nakano and McCurdy, 1967, 1968; Nakano, 1968; Hollenberg et al. 1968; Nutter and Crumley, 1970, 1972). The increase in coronary blood flow with intravenous administration of E or A series prostaglandins appears to be independent of changes in heart rate and systemic arterial pressure (Maxwell, 1967; Nakano, 1968; Higgins et al., 1970, 1971; Bloor and Sobol, 1970) and is not abolished by β-adrenergic blockade or atropine.

Most authors report an increase in coronary blood flow in isolated perfused hearts with prostaglandins. PGE_1 has been reported to enhance coronary blood flow in rat, cat and rabbit heart without significant inotropic or chronotropic effects (Mantegazza, 1965). In the isolated perfused rat heart this effect has been reported to be concentration-dependent between 2 and 10 ng/ml PGE_1, with the maximum recorded coronary flow being 2.5 to 3 times the basal level (Ten Hoor, 1975). In con-

trast, Berti et al., (1965) failed to find any effect of PGE_1 on heart rate and coronary flow in isolated rat heart. Vergroesen et al. (1967), however, reported that PGE_1, PGE_2 and PGA_1 increased coronary flow in isolated rat heart. In the isolated guinea pig heart the increase in coronary flow produced by PGE_1 was not blocked by propranolol, pronethalol, or reserpine pre-treatment. Most authors report that coronary blood flow in intact animals is not affected by prostaglandins of the F series, although they can cause peripheral vasoconstriction (Nakano, 1968; Hollenberg et al., 1968; Nutter and Crumly 1970, 1972; Bloor and Sobol, 1970). In contrast, Kateri et al. (1970) reported that high doses of $PGF_{1\alpha}$ (50 µg/ml) increased coronary flow in the isolated dog heart-lung preparation. The reports for the isolated rat heart are controversial. Vergroesen et al. (1970) reported that $PGF_{1\alpha}$ or $PGF_{1\beta}$ failed to alter coronary flow, while Willebrands and Tasseman (1968) found that $PGF_{1\alpha}$ increased coronary flow.

It has been suggested that prostaglandins may be endogenous mediators involved in coronary vasodilation, This possibility follows the finding of a basal release of prostaglandins when hearts are perfused by the Langendorff technique (Bader and Johnson, 1972; Block et al., 1974; Junstad and Wennmalm, 1972; Neddleman et al., 1975; Wennmalm, 1975). Both PGE_1 and PGE_2 appear to be released at approximately 5 ng/heart/min. Prostaglandin release in increased following hypoxia, mechanical manipulation, and acetylcholine or noradrenaline administration. No increases were found with alterations in pH,

temperature, osmolarity, or changes in the potassium or calcium levels of perfusing solutions. Indomethacin, a prostaglandin synthetase inhibitor, has been shown to block hypoxia-induced increased coronary blood flow in intact anesthetized dogs (Afonso et al., 1974). Prostacyclin (PGI_2) has recently been identified as the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid (Gregory et al., 1977). The physiological importance of such prostaglandin release has not been fully elucidated; however, it is being rapidly resolved that PGI_2 is probably the coronary vasodilator in hypoxic hyperemia. The hypothesis that prostaglandins E_1 and E_2 are the vasodilators in hypoxic hyperemia has been rejected (Block et al., 1975). Nevertheless, the evidence seems to indicate that these pharmacologically active compounds are involved in the local regulation of the coronary vascular bed; they may also play a physiologic role in the cardiac response to ischemia.

Inotropic and Chronotropic Effects

Prostaglandins have been shown to produce cardiac inotropic and chronotropic effects dependent upon experimental conditions and varying qualitatively and quantitatively in different species. When given to intact animals, they increase cardiac output, partially by reflex mechanisms (Malik and McGiff, 1976). For example, in dogs the in vivo increases in myocardial contractile force and heart rate reported in response to prostaglandins of the E, A, and F series appear to be secondary to reflex sympathetic activation possibly

involving cardio-accelerator centers in the medulla (Malik and McGiff, 1976). Variations in inotropic and chronotropic actions of prostaglandins, and species specificity, have also been observed *in vitro*. Prostaglandin E_1 failed to alter the rate of the isolated heart of rabbit, cat, dog and chicken, but produced positive chronotropic and inotropic effects in isolated guinea pig heart which were not blocked by either propranolol or reserpine pre-treatment, thus indicating a direct myocardial effect (Berti *et al.*, 1965; Mantegazza, 1965; Sunahara and Talesnik, 1974; Horton and Main, 1967).

In rat myocardium the amplitude of contraction of the isolated heart was found to be only modestly increased by PGE_1 and PGE_2 (Berti *et al.*, 1965; Vergroesen *et al.*, 1967; Vergroesen and de Boer, 1968), with a greater effect in the spontaneously beating atria of normal and genetically hypertensive rats (Levy, 1973). In the former studies no increased chronotropic action was noted, while in the latter experiments (Levy, 1973), PGE_2 increased rate; it is not known whether the inotropic and chronotropic effects on rat myocardium are due to direct or indirect actions or combinations of both.

It has been demonstrated in isolated rat and frog hearts that PGE_1, which normally only modestly influences myocardial contractility, will restore normal contraction if the heart function is depressed by an increased K^+/Ca^{2+} ratio of the perfusion fluid, by propranolol, by barbiturates or by excess Mg^{+2} (de Boer *et al.*, 1973; Vergroesen and de Boer, 1968). Such results may indicate a direct membrane effect of prostaglandins.

Furthermore, some of the apparently controversial results previously discussed may not be due to differences in species, but to differences in experimental conditions.

Effects of Prostaglandins on Cyclic AMP in Cardiac Tissue

The mechanism by which prostaglandins produce rate and force effects is separate from the adrenergic system, although the E and A series prostaglandins increase cyclic AMP levels in homogenates of guinea pig hearts (Klein and Levey, 1971; Sobol and Robinson, 1969). PGE_1 has also been shown to increase cyclic AMP levels in rat heart (Curnow and Nuttal, 1971) and to increase adenylate cyclase activity in guinea pig and rat heart (Sobol and Robinson, 1969). Prostaglandins of the F series have been found to be inactive in particulate membrane preparations, but active in solubilized preparations (Levey and Klein, 1973). Beta-blocking agents are not effective in preventing the activation of adenylate cyclase by prostaglandins E and A (Klein and Levey, 1971). Sen et al. (1976) have suggested that PGE_2 may modulate the cardiac cAMP level and that the latter plays an important role in the adaptive regulation of the coronary flow. Whether elevation of cyclic AMP is responsible for the rate and force effects of prostaglandins in the heart remains to be completely resolved, but there is no well-defined, obligatory association between all prostaglandins and cyclic AMP anymore than there is an association between all adrenergic agonists and cyclic AMP.

Effect of Prostaglandins on Myocardial Metabolism

Prostaglandins of the E series have marked effects on lipid and carbohydrate metabolism whereas those of the A series

are inactive and those of the F series are only minimally effective. Prostaglandin E_1 has been reported to be a potent inhibitor of lipolysis (Steinberg et al., 1964; Carlson, 1965) induced by catecholamines (Steinberg, 1963), sympathetic nerve stimulation (Berti and Usardi, 1964), and by a wide variety of hormones (Steinberg et al. , 1963, 1964; Mandel and Kuehl, 1967; Fain, 1967). This action of PGE_1 was found, however, to be dose dependent with small doses stimulating and large doses inhibiting lipolysis.

In isolated perfused rat hearts PGE_1 and $PGF_{1\alpha}$ have been found to stimulate oxygen consumption, glucose uptake in hearts using glucose as a substrate, and uptake of palmitic acid in hearts given the fatty acid as a substrate (Willebrands and Tasseron, 1968). Maxwell (1967) similarly reported a rise in oxygen uptake and coronary blood flow which was accompanied by decreased glucose and free fatty acid levels and increased lactate concentrations in response to intracoronary administration of PGE_1 in anaesthetized dogs. In contrast to the effects of PGE_1, $PGF_{2\alpha}$ did not produce a significant effect upon either arterial glucose, myocardial glucose, or free fatty acid extraction, while it increased both lactate and pyruvate levels in arterial and in coronary sinus blood (Maxwell, 1969).

Effect of Prostaglandins on Cardiac Neurotransmission

Prostaglandins are thought by some to modulate autonomic neurotransmission. Of the various types of prostaglandins synthesized in cardiovascular tissues in response to various

stimuli (Ramwell and Shaw, 1970; Horton, 1973; Hedqvist, 1973; Crowshaw and McGiff, 1973), those of the E series appear to be the most likely physiological modulators of adrenergic transmission (Hedqvist, 1973). Exogenously administered PGE_1 and PGE_2 (10^{-8} to 10^{-6}M) inhibit the release of noradrenaline from the sympathetic nerve endings of rabbit heart (Hedqvist and Wennmalm, 1971; Wennmalm, 1976). PGE compounds, however, do not alter the basal efflux of norepinephrine in isolated rabbit heart (Hedqvist et al., 1970) which suggests that PGE compounds inhibit outflow of norepinephrine from adrenergic nerves indirectly by interfering with some step(s) in the release mechanism. PGE inhibition of noradrenaline release is frequency-dependent and is most marked at lower impulse frequencies (50% reduction in release at 2 to 5 Hz.). Interestingly, sympathetic nerve stimulation elicits release of endogenous PGE (Wennmalm and Hedqvist, 1971; Wennmalm, 1976) while inhibition of prostaglandin synthesis with indomethacin results in an increase of noradrenaline release with nerve stimulation. These results may indicate that the process of transmitter release is normally modulated by endogenously formed PGE (Samuelsson and Wennmalm, 1971).

Most of the available evidence indicates that PGA and PGF compounds are less likely to be physiological modulators of adrenergic transmission for they are synthesized in cardiovascular tissues in relatively small amounts as compared to prostaglandins of the E series (Karim, 1967; Karim et al., 1967, 1968; Horton, 1973; Crowshaw and McGiff, 1973;

Papanicalaou et al., 1974). The effect of series A prostaglandins on adrenergic neurotransmission has not been studied in the heart, but in the isolated perfused rabbit kidney PGA_2 was 10 to 50 times less active than PGE_1 or PGE_2 in inhibiting responses to sympathetic nerve stimulation. Prostaglandins of the F series have been found to either be ineffective or to exert a slight facilitory effect on adrenergic transmission in the cardiovascular system. $PGF_{2\alpha}$ failed to alter the contractile responses or the release of noradrenaline produced by sympathetic nerve stimulation in rabbit, cat, and dog heart (Hedqvist and Wennmalm, 1971; Brody and Kadowitz, 1974).

PGE_1 has also been reported to inhibit parasympathetic neurotransmission in the isolated rabbit heart. Wennmalm and Hedqvist (1971) reported that PGE_1 inhibited the chronotropic response to exogenous acetylcholine, which would indicate a presynaptic site of action.

Junstad and Wennmalm (1974) demonstrated that infusion of acetylcholine or vagal nerve stimulation at low frequency released a PGE-like substance from the isolated heart and that this release was blocked by atropine. Acetylcholine, however, is markedly less potent than noradrenaline in stimulating PGE release from the heart (Wennmalm, 1976). It therefore seems doubtful that a physiological inhibition occurs with the parasympathetic nervous system. In contrast to the results in rabbit heart no presynaptic action at the cholinergic neuromuscular junction has been found in guinea pig heart (Park et al., 1973; Betting and Salzman, 1974; Hadhazy et al., 1973).

These observations indicate that the modulatory action of prostaglandins on cholinergic transmission may be species dependent.

Cardiac Electrophysiological Actions of Prostaglandins

Low concentrations of PGE_1, PGE_2 (5 ng/ml) and $PGF_{2\alpha}$ (1 µg/ml) have been reported to cause an increase in the maximal rate of depolarization (V_{max}) and in some cases also of the overshoot of the transmembrane action potential in the auricles of the cat, rabbit, guinea pig and in the ventricles of the rat, with the magnitude of effect being species dependent (Kecskemeti, Keleman, and Knoll, 1973, 1974, 1976). Higher doses of prostaglandins (20 ng/ml for PGEs; 10 µg/ml for $PGF_{2\alpha}$), however, decreased Vmax and overshoot. This dose-dependent nature of the effect of PGE_1 and $PGF_{2\alpha}$ on the cardiac action potential is complex for at still higher concentrations (0.1 µg/ml) PGE_1 and PGE_2 decreased the resting potential in cat and guinea pig auricles by a maximum of 15 and 17% respectively (Kecskemeti, Keleman, and Knoll, 1976). In the presence of carbaminoyl choline, which increases potassium permeability of atrial fibers, a high dose of PGE_2 did not decrease the resting potential. This result was interpreted to mean that the depolarizing effect of high concentrations of PGEs was due to changes in the potassium permeability of the cardiac cell.

The biphasic effect of prostaglandin E_2 and $F_{2\alpha}$ on maximum rise-rate, together with a slight increase in resting membrane potential at low concentrations has also been reported

by other workers (Forster et al., 1974). They found no myocardial depressant "quinidine-like" effects on the isolated atrium and papillary muscle of the guinea pig at antiarrhythmic concentrations. Small changes, usually not exceeding 10%, could be detected in the electrical threshold, left atrial conduction time, and maximum driving frequency. Prostaglandin E_2 had little effect on the fibrillation threshold of the cat heart; however, $PGF_{2\alpha}$ produced a moderate increase in fibrillation threshold at high doses. Prostaglandins of the E series have been reported either to have no effect or to shorten the action potential duration (Kecskemeti et al., 1976). Prostaglandins $F_{2\alpha}$, however, has been found to significantly prolong action potential duration by 30 to 60 per cent in ventricular strips from guinea pigs and in rat papillary muscle, respectively (Mentz et al., 1976; January and Schotelius, 1974).

C. ANTIARRHYTHMIC EFFECTS

Almost simultaneously, and independently, Zijlstra et al. (1972) and Mest et al. (1972, 1973) reported the first preliminary investigations of the antiarrhythmic effects of prostaglandins. The study by Zijlstra et al. was not planned, but was suggested by the occurrence of arrhythmias in some experiments being conducted on the cardiovascular effects of PGE_1 in dogs. It was observed that intravenous injection (0.5 to 4 μg/Kg) or infusion (0.5 to 4 μg/Kg/min) of PGE_1 effectively suppressed bigeminy occuring during the course of thiobarbitone anesthesia. In addition, ventricular tachycardia

due to acute myocardial ischemia was changed to sinus rhythm by a single intravenous injection of 2 μg/Kg PGE_1. The authors suggested that PGE_1 might suppress cardiac arrhythmias through its moderating influence on the sympathetic-parasympathetic balance, which would influence impulse conduction in the heart (Hedqvist and Brundin, 1969; Hedqvist, 1970; Wennmalm and Hedqvist, 1970; Wennmalm, 1971).

Mest et al. (1972, 1973) and Forster et al. (1973) published further findings on preliminary investigations into the antiarrhythmic effects of PGE_1, PGE_2, and $PGF_{2\alpha}$ in mini-pigs, dogs, and cats. PGE_1 was found to protect the mini-pig from ventricular flutter produced by coronary artery ligation. In cats with similar ligations $PGF_{2\alpha}$ resulted in longer post-infarct survival times, together with better maintenance of cardiac output. It was suggested that such protection might be the result of maintenance of lysosomal integrity in the ischemic tissue and prevention of the formation of a cardiotoxic peptide. Support for this suggestion has been provided by further study in dogs (Glenn et al., 1975) which showed that $PGF_{2\alpha}$ produced higher aortic flow rates, rates of development of ventricular pressure (dp/dt) and mean arterial blood pressures post infarct, while reducing lysosomal enzyme release. Methylprednisolone produced effects similar to that of $PGF_{2\alpha}$, and both agents were presumed to act by preserving cellular integrity. Such a mechanism has also been suggested by experiments with a stable free-radical form of PGB_1, PGB_x, which has been reported to preserve oxidative

phosphorylation in degenerated mitochondria, and at the same time protect Rhesus monkeys from the effects of coronary artery ligation, so that the mean control survival time of five minutes is prolonged to ninety minutes (Angelakos, et al., 1975).

A number of comparative studies have now been carried out with PGs A_1, A_2, E_1, E_2, $F_{2\alpha}$, $F_{2\beta}$, and the precursors arachidonic acid and linoleic acid using several other experimental dysrhythmias, such as those produced by aconitine, calcium chloride, barium chloride, ouabain, and electrical stimulation (Chiba et al., 1972; Kelliher and Glenn, 1973; Mest et al., 1972, 1973, 1975, 1976; Forster et al., 1973, 1974; Mentz and Forster 1974a, 1974b; Bayer et al., 1976; Mann et al., 1973). The calcium chloride and aconitine arrhythmias were induced in rats (Forster et al., 1973; Mentz and Forster 1974a, 1974b). The strongest effect against calcium induced arrhythmias was shown by $PGF_{2\alpha}$ with a bolus injection of 5 µg/Kg I.V. This protected a maximum of 84 per cent of animals, for 10 minutes, from lethal ventricular fibrillations. Ajmaline, the standard antiarrhythmic drug, was effective in 50 per cent of the animals. A good antiarrhythmic effect was also shown with PGA_2, followed in decreasing effectiveness by PGE_1, E_2, A_1, and $F_{2\beta}$. Prostaglandin $F_{2\alpha}$ was also the most effective against aconitine-induced arrhythmias, followed by PGE_2, A_2, E_1, A_1, and $F_{2\beta}$.

Barium chloride arrhythmias were induced in unanesthetized rabbits and a normalization of the ECG for at least one minute was taken as indication of an antiarrhyth-

mic effect (Forster et al., 1973; Mentz and Forster, 1974; Mest et al., 1974). Prostaglandins E_2 and A_1 at 1 µg/Kg/min infusion showed the strongest effect with normalization in 60 per cent and improvement in 40 per cent of the animals. Similar effects, but at higher doses, were obtained with PGA_2, $F_{2\alpha}$ and E_1. Ajmaline was also effective in 60 per cent of the animals, but at a dose one thousand times higher than that of the prostaglandins.

In ouabain arrhythmias in cats, PGE_2 was most effective, causing 60 per cent of the animals to revert to temporary sinus rhythm (Mest et al., 1976; Kelliher and Glenn, 1973). Similar improvement was obtained with PGE_1, with lesser effects seen for $PGF_{2\alpha}$, A_2, and E_1. Ajmaline was also effective in approximately 50 per cent of the animals, but at doses 100 times higher than that of the prostaglandins.

Prostaglandins have not been found to be particularly effective against arrhythmias induced by electrical stimulation (Bayer et al., 1976; Forster et al., 1974). As mentioned earlier they have most often been found to produce only small (and not statistically significant) changes in conduction velocity and functional refractory period. Prostaglandin $F_{2\alpha}$ produces a significant increase in the duration of the cardiac action potential, but the PGEs have no such effect (Forster, 1976; Mentz, 1976). A possibly related finding is the ability of $PGF_{2\alpha}$ but not PGE_2, to increase the fibrillation threshold in isolated atria from rats (Forster et al., 1974). There is, however, no correlation between this action and other antiarrhythmic activities (Forster, 1976). In summary, electrical changes

observed with prostaglandins are varied and distinctly weaker than those seen after the application of classical antiarrhythmic agents, which may point to a mode of action different from that of quinidine-like antiarrhythmic drugs.

The prostaglandin precursor fatty acids, linoleic acid and arachidonic acid, have also been tested and found to be effective against barium chloride, ouabain, and aconitine arrhythmias when infused at 0.5 to 1 mg/Kg/min (Mest and Forster, 1973; Mest et al., 1976; Forster et al., 1976). Indomethacin, an inhibitor of prostaglandin synthetase, abolished the antiarrhythmic effect of the precursors, while prostaglandin effects were not influenced. It therefore appears that prostaglandins biosynthesized from the precursors, rather than the precursors themselves, are responsible for the antiarrhythmic activity (Forster, 1976).

Clinical Evidence

Short clinical trials with $PGF_{2\alpha}$ have demonstrated its effectiveness in some dysrhythmias (Mann et al., 1973; Mann, 1976). Positive results were obtained with ventricular arrhythmias in patients suffering from myocardial infarction and possibly from the toxic administration of cardiac glycosides. Paroxysmal atrial tachycardia did not respond. In contrast to the results obtained with other antiarrhythmic drugs, such as ajmaline, the administration of $PGF_{2\alpha}$ did not result in any essential changes in the diastolic stimulus threshold. This was interpreted as support for the conclusion that the mechanism of antiarrhythmic action of $PGF_{2\alpha}$ cannot represent a non-specific, quinidine-like, antiarrhythmic effect.

Although prostaglandins are antiarrhythmic, it is of interest to note that $PGF_{2\alpha}$ in at least one report has been cited as responsible for inducing arrhythmias (Koss and Nakano, 1974). Transient episodes of sinus bradycardia and ventricular bigeminy were observed with the intravenous administration of prostaglandin $F_{2\alpha}$ (5 to 15 µg/Kg) in anesthetized cats. This arrhythmogenic effect of $PGF_{2\alpha}$ was abolished following bilateral vagotomy, which would indicate the arrhythmias were most likely due to marked stimulation of vagal tone in this species. The $PGF_{2\alpha}$ dose used in this experiment, however, was above the maximal antiarrhythmic dose of 1 to 2 µg/Kg/min, in this species (Mest et al., 1974).

Purpose of Experiments to be Described

From the literature, several conclusions may be drawn:

1.) Prostaglandins have a complex, and species-dependent, cardiac pharmacology affecting coronary circulation, contractility, cyclic AMP levels, myocardial metabolism, neurotransmission, and cardiac membrane potentials.

2.) Prostaglandins are effective antiarrhythmic agents which are endogenously formed from precursors in the heart.

3.) All prostaglandins tested have an antiarrhythmic effect, although relative potencies vary with the prostaglandin, species, and type of arrhythmia.

4.) Prostaglandins appear to be effective against arrhythmias induced by damage or ionic disturbance. They have little effect against non-damaging disturbances such as electrical stimulation.

5.) The mechanism of the antiarrhythmic action of prostaglandins is not likely to be due to a general myocardial depressant (quinidine-like) effect.

Although the above conclusions may be drawn from the literature, the evaluation of prostaglandins antiarrhythmic activity requires that the following question be answered:

1) which prostaglandins are antiarrhythmic,

2) against which arrhythmias and

3) by what mechanisms

The answer to such a question should allow evaluation for suitability and the choice of the best prostaglandin for future clinical trial. The treatment of arrhythmias is still far from perfect. There is need, for example, for an antiarrhythmic capable of being used to protect the recently infarcted patient prior to full medical care. Furthermore, the extensive pharmacological spectrum of prostaglandin activity needs the fullest investigation in order to define the various activities with a view to the maximum utilization of each. Only the fullest analysis of the pharmacological activities in adrenergic amines has allowed the development of highly selective drugs.

With the above firmly in mind the experiments described in this work were initiated to answer, at least in part, all the three facets of the initial question. In view of the previously considered antiarrhythmic efficacy of prostaglandins and their ability to reduce the deleterious effects of coronary ischemia (Zijlstra et al., 1972; Mest et al., 1972, 1973; Forster et al., 1973; Glenn et al., 1975; Angelakos, et al.,

1975) it was decided to test the effectiveness of prostaglandins against the various arrhythmias associated with cardiac ischemia. Initial results were obtained in the dog and then reconfirmed in the rat, where a more thorough comparison of different prostaglandins was performed. Rat heart tissue in intact, isolated, and cell culture preparations was used to investigate possible mechanisms of action. Secondary considerations of choice of experimental model, selection of specific prostaglandins and selection of doses are more fully discussed in the methods section.

This work therefore I) first describes the activity of prostaglandins on arrhythmias induced in vivo in A) dogs and B) rats by coronary artery ligation together with C) the effects of prostaglandins on the electrical characteristics of in vivo rat hearts. II) This activity is then compared with an analysis of the ability of prostaglandins to prevent contractility losses in isolated rat hearts as well as their pharmacological effects in this preparation. III) The possible protective actions of prostaglandins is also described for abnormalities of beating induced in cultured neonatal rat heart cells by various physiological and pharmacological interventions.

Dog experiments were primarily conducted by M. J. Walker and C. Harvie with my cooperation. Experiments on isolated rat hearts were performed with the help of T. Au and G. Collins.

CHAPTER II

MATERIALS AND METHODS

Experimental methods are described in order of the list outlined in the Introduction.

EXPERIMENTAL SECTION I

Production of Arrhythmias in Dogs and Rats

A. Coronary Occlusion in Dogs

Acute Coronary Artery Ligation for Early and Occlusion Release Arrhythmias

In order to confirm the reports of prostaglandin antiarrhythmic activity we first tested two prostaglandins, E_2 and $F_{1\alpha}$, against arrhythmias induced in anesthetized dogs by coronary artery ligation. The two prostaglandins were chosen for testing because the former is a vasodilator and the latter is a vasoconstrictor. The dog model was chosen because it is a standard antiarrhythmic test preparation and because the ischemic model most closely resembles the clinical situation following a myocardial infarction. A problem with this preparation is the large degree of variability in results obtained with different animals. We felt, however, that if prostaglandins were to have any potential therapeutic value, it should be possible to demonstrate their effectiveness in this system. From the literature we determined the antiarrhythmic dose of prostaglandins to fall between 1 and 8 µg/Kg/min (Mest et al., 1974). On this basis the test dose of 1 µg/Kg/min was chosen.

Studies were carried out in 18 mongrel dogs of either

sex weighing between 22.2 and 28.6 Kg. Anesthesia was induced with I.V. sodium pentobarbital (30 mg/Kg) and maintained with 1 per cent halothane, 40 per cent nitrous oxide and 59 per cent oxygen with respiratory assistance from a Bird Mark 8 respirator. The heart was exposed through a left thoracotomy in the 5th intercostal space and suspended in a pericardial cradle. The left anterior descending coronary artery was dissected free and prepared for ligation with the tie in place, but not tightened, approximately 2 to 3 cm from the left atrial margin.

Aortic pressures were recorded by means of a catheter introduced retrogradely from the femoral artery and connected to an appropriately calibrated P23Db Statham pressure transducer. Electrocardiograms (ECG) were taken from a precordial lead and recorded on a Grass model PCPb polygraph with EKG Tachograph pre-amplifier model 17P4P. Freshly prepared prostaglandins were infused by way of a catheterized femoral vein.

Experimental Protocol

One hour after operation animals were monitored for 30 minutes of infusion. Five minutes after the start of the infusion the coronary artery ligature was tightened and the chest closed. The ligature was kept tightened for 25 minutes before its rapid release. Infusions were continued for 15 minutes after release, but if fibrillation occurred, the animal was electrically defibrillated.

Determination of Arrhythmias and Analysis of Data

Acute Dogs

Premature ventricular contractions were counted from continuous records. Each PVC was recognized from the ECG

and corresponding anomalies in the pressure record. Pressures were measured as mean systolic or diastolic over the measurement period.

Statistics

The Student 't' test for unpaired data was used to test for differences between the drug treatments. A probability of less than 0.05 was chosen as the criterion of significance.

B. Acute Coronary Occlusion in Rats

In order to confirm and expand our studies on the antiarrhythmic actions of prostaglandins we also performed coronary ligation experiments in rats. This allowed us to use cheaper animals and simpler surgical techniques to screen a larger number of drugs. Prostaglandins E_2, $F_{1\alpha}$, A_2, and $F_{2\beta}$ as well as lidocaine and quinidine were tested in this preparation. Prostaglandin E_2 and A_2 are vasodilators. Prostaglandin A_2 is more slowly metabolized than E_2 (Lee, 1974). Prostaglandin $F_{1\alpha}$ as mentioned previously is a vasoconstrictor, and $PGF_{2\beta}$ has little smooth muscle activity. The standard antiarrhythmic drugs lidocaine and quinidine were additional controls. A second consideration in using rats was that it allowed for testing of antiarrhythmic activity in a second species. A potential problem was that this was a new model for antiarrhythmic testing. Comparison of control animals and those treated with PGE_2 and $F_{1\alpha}$ which we had previously tested in dogs allowed for comparison between the rat and dog models. A prostaglandin dose of 2 µg/Kg/min was chosen as this was within the previously reported antiarrhythmic dose range. Quinidine at a bolus dose of 3 mg/Kg was also taken

from the literature (Moe and Abildskov, 1975), and lidocaine 6 µg/Kg/min was the highest dose which could be infused throughout the test period without seriously depressing blood pressure.

Male Wistar rats (300 to 400 g) were used in this experiment. The procedure of left coronary occlusion was a modification of techniques described by John and Olsen (1954) and Selye et al., (1960). The animal was anesthetized with pentabarbital sodium (60 mg/Kg I.P.), placed on a heated (37° C) pad, and immobilized with tape. A tracheotomy was performed and a blunt 15 guage needle was secured in the trachea. The animal was allowed to respire itself while cannulae were inserted into the carotid artery and femoral vein. Aortic pressures were recorded from the carotid artery using a calibrated P23Dc Stratham pressure transducer. Infusions of test drugs were made into the femoral vein. Three needle limb leads were used to monitor the ECG which was recorded on a Grass model PCPb polygraph with EKG Tachograph pre-amplifier model 7P4F. An incision was then made through the skin at the base of the sternum, using large blunt scissors. The skin was loosened from the underlying muscle mass using blunt dissection to the base of the neck. The skin was then cut from the base of the sternum to the neck and peeled back, revealing the chest musculature. A scalpel was used to make an incision through the chest muscles to the ribs, parallel to and approximately 5 mm to the left of the sternum. Bleeding from the internal epigastric artery (which courses just to the left of the sternum) can thus be avoided. Artificial respiration was

applied at this time using room air with a Palmer small animal respirator having a stroke volume of 4 ml and a rate of 30 strokes per minute. Fine pointed scissors were used to cut through the ribs and remaining tissue in a line 5 mm from and parallel to the left of the sternum exposing the heart. Retractors were used to hold the chest walls back, and blunt forceps were used to open the pericardium. Control measurements of ECG, rate, and blood pressure were taken and infusions of test drugs then started. Five minutes after the start of the infusion, ligation of the left coronary artery was performed.

Ligation of the Left Coronary Artery

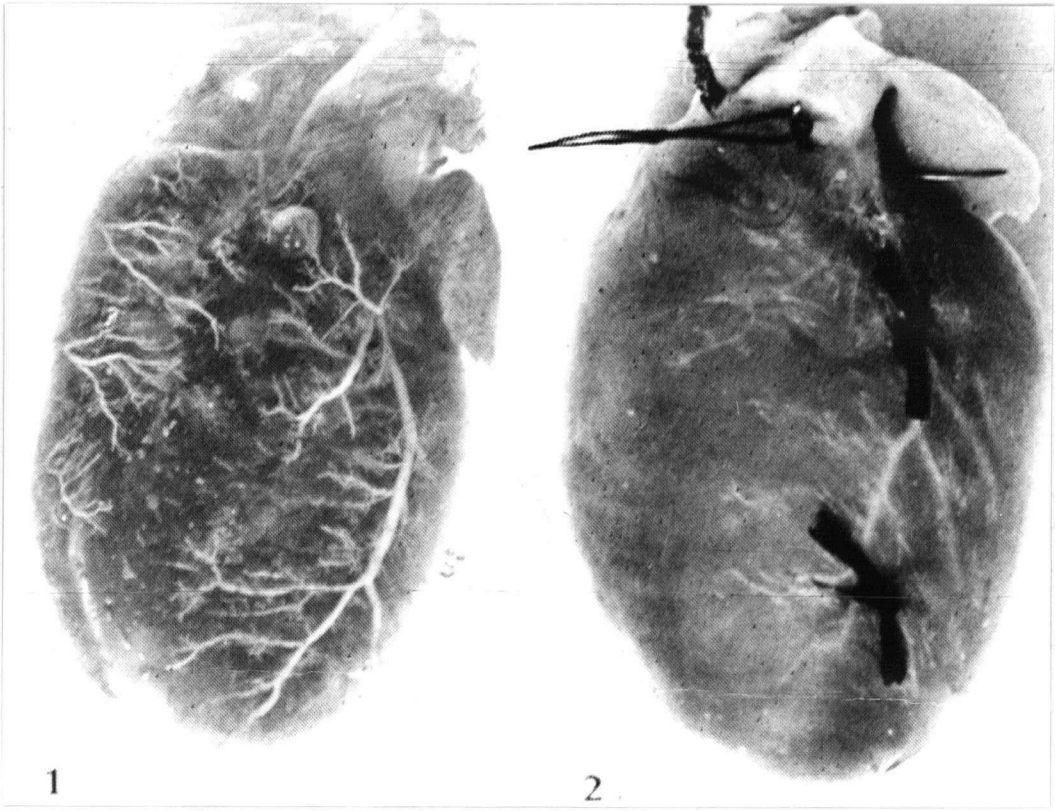
The left coronary artery is predominant and supplies the left ventricle. There is no true circumflex artery in the rat. The coronary arteries lie beneath the epicardium and can be seen at operation in the intact beating heart as tiny red streaks beneath the surface of the heart. In the rat the main left coronary artery can be ligated at a point just beneath the left auricular appendage. Occasionally, branching will have already begun under the left atria. In this situation it is necessary to ligate several branches at the same time in order to obtain a good-sized area of infarct. A small curved needle (corneal needle 3/8 circle, 9 mm, atraumatic) carrying a thread (black braided, non-slipping silk, size 50) held in straight hemostats was used for ligation.

In the experiments, the left atria was lifted with blunt forceps; since the coronary artery and its branches were visible the needle was simply passed beneath them and the ligature tied (Figure 4). Virtually the entire anterior lateral

Figure 4: Ligation of the left coronary artery in rat

1.: Anterior aspect of the heart in a rat whose coronary arteries have been injected with lead oxide in latex. The superficial myocardial layers have been partially cleared in methyl benzoate to make the intramuscular arteries more readily visible. The left coronary artery originates between the pulmonary cone and the left auricular appendage (right side of picture). After sending a few major branches towards the pulmonary cone, it descends almost directly towards the apex, along the curved surface of the left ventricle. The main trunk of the right coronary artery is not visible, but its perpendicular lateral branches can be seen to advance (from the left side of the picture) towards the anterior part of the heart, within the wall of the right ventricle.

2.: Heart of a rat in which the arteries have again been made visible by injection of colored material, but the myocardium has been left opaque to show its surface markings. A curved needle has been introduced between the pulmonary cone and the insertion of the left auricular appendage, to demonstrate the procedure to be followed for the occlusion of the left coronary artery near its origin. Additional ligatures (such as are used for the production of smaller infarcts) have been placed at the lower points along the course of this vessel. Only the ligation near the origin was used in the present series of experimants to produce arrhythmias. (Selye et al., 1960).



1 2

wall of the left ventricle then became ischemic. Arrhythmias usually occurred between 4 and 11 minutes after ligation and were easily detected by changes in the ECG and blood pressure.

The size of the infarct produced was measured after the experiment by perfusing the hearts with Krebs solution. Blood quickly washed out of all areas except the infarct which was then cut out and weighed as the per cent of whole heart weight.

Infusions

All prostaglandin infusions were given as 2 μg/Kg/min in a volume of 0.1 ml saline/ min. Lidocaine was given as an infusion 6 μg/Kg/min in a volume of 0.1 ml saline/min and quinidine 3 mg/Kg in a volume of 0.3 ml was given as a slow IV injection.

Determination of Arrhythmias in Rats

In initial experiments on rats not given any type of antiarrhythmic a common series of arrhythmic events following ligation was seen. Initially sinus beats with occasional PVCs occurred. This was followed by bursts of ventricular flutter of varying duration and then, sometimes, ventricular fibrillation which did not always end in death. To quantitate these arrhythmias a number of measurements were taken. These included:

1. the number of PVCs in the 25 minute occlusion period
2. the number of PVC salvos (with one salvo being 3 or more consecutive PVCs)
3. the number of flutter episodes

4. the time spent in flutter
5. the time for flutter to first occur
6. the time for fibrillation to first occur

On the basis of these measurements a subjective scoring system for arrhythmias was constructed as shown below.

Arrhythmia		Subjective Score
PVC	49 or less	1
PVC	50 to 74	2
PVC	75 to 99	3
PVC	100 or more	4
Flutter after 10'		5
Flutter between 5-10'		6
Flutter total less than 1'		7
Flutter total greater than 1'		8
Flutter between 0-5'		9
Flutter before 1'		10
Flutter after 1'		11
Fibrillation after 10'		12
Fibrillation between 5-10'		13
Fibrillation before 5'		14

Experimental Design

All experiments were done "blind" and all data were evaluated using a "blind" (drug treatment unknown to the scorer) technique. After all drug treatments were evaluated the code used was broken and the scores for the treatments using the same drug were averaged. Five animals were included in each drug treatment group, and each animal received only one antiarrhythmic test agent.

Statistics

Dunnett's 't' test for unpaired data was used to test for differences between the various drug treatments and

control. Chi square test was used to test for significance of the proportion of animals dying. A probability of less than 0.05 was chosen as the criterion of significance.

C. Production of arrhythmias by Electrical Stimulation in the Rat: Flutter Threshold Current

Intact and isolated rat hearts were used to investigate possible mechanisms of prostaglandin antiarrhythmic activity. We determined the effect of prostaglandins E_2, A_2, $F_{1\alpha}$, $F_{2\beta}$, $F_{2\alpha}$, lidocaine, and quinidine on flutter threshold and maximum following frequency. Flutter threshold is defined as the current necessary to produce flutter and a drop in blood pressure when the heart is being driven at a supramaximal rate. Maximum following frequency is the maximum rate the heart can beat when being driven by an electrical stimulator using a supramaximal pulse current. $PGF_{2\alpha}$, a vasoconstrictor, was added to allow for comparison with other reports of prostaglandin effects on electrical activity. Flutter threshold measurements allowed us to determine the effectiveness of prostaglandins against a non-damaging type of arrhythmia, and maximum following frequency measurements allowed us to determine the quinidine-like depressive action of prostaglandins.

Male Wistar rats (300 to 400 g) were prepared similarly to those used in the coronary ligation experiments but without actual coronary ligation. Instead, two punctate silver electrodes were inserted into the heart tissue, one on the right anterior ventricular surface and the other in the apical region. The wires were attached to a Grass SD9 stimulator

and an oscilloscope which had been especially calibrated to measure current, and the electrical flutter threshold was then measured by determining the threshold intensity of serial shocks required to evoke flutter of the ventricles. The heart was stimulated by serial rectangular pulses of 20 cycles/sec and 1 msec duration, and the strength of the stimulus was gradually increased. Extrasystoles were seen first followed by tachycardia, flutter, and finally by fibrillation. The appearance of ventricular flutter was easily recognized by a sudden fall in blood pressure, and the current required to produce flutter with each drug treatment was measured. Periods of stimulation of approximately 4 seconds duration were required for each determination.

Maximum Following Frequency

The maximum following frequency was determined by a similar procedure except that a supramaximal current of 1.0 mA was used and the frequency of stimulation was rapidly increased until the heart was unable to follow. This was easily determined as the blood pressure, which had been suppressed by tachycardia and flutter with increasing rate, gives a series of sharp pulses at the point where the heart begins to skip beats. The maximum following frequency is reciprocally related to the effective refractory period (the shortest interval between two stimuli to which the heart responds with contraction).

EXPERIMENTAL SECTION II

Isolated Rat Hearts and Contractility Losses

It was of interest to evaluate the cardiac pharmacology

of the prostaglandins used so conventional studies were performed on unpaced Langendorff perfused hearts. As there is a decrease in contractility with time of hearts perfused by this technique it was of interest to see whether prostaglandins would have a 'protective' effect on this contractility loss.

The Langendorff rat heart model was selected mainly for convenience. We perceived a number of deficiencies of this model. All Langendorff hearts are in a condition of failure with edema due to the physiological solution. In addition only the ventricles are filled with solution. Measurements of coronary flow by simply collecting the outflow dripping off the hearts is also subject to considerable error. However, the use of five hearts for each determination tended to cancel the error and allowed sufficient accuracy for our purpose as we were looking for marked pharmacological effects on the myocardium which might explain prostaglandin antiarrhythmic activity. The same four prostaglandins - A_2, E_2, $F_{1\alpha}$, and $F_{2\beta}$-which had been used with the in vivo rat experiments were used for comparison, and prostaglandin infusions of 10^{-9}, 10^{-7}, and 10^{-5} M were used to allow for dose-effect comparisons.

Method

Male Wistar rats (250 to 350 g) were killed by a blow to the base of the skull and quickly bled from the neck. The heart was removed and immediately placed in a dissecting dish containing oxygenated Krebs solution at 20° C. Prior to mounting on the perfusion apparatus, which was specially constructed to allow five hearts to be perfused at the same time by the Langendorff (1895) technique, a small hook was placed in the

apex of the ventricles so that each heart could be attached at will to an inverted Grass force displacement transducer by means of a second hook and piece of string. A Harvard isometric tension clamp allowed tension on the ventricles to be adjusted. No longer than 10 to 15 minutes were required to attach all five hearts to the cannulae. Hearts were perfused at a constant 90 mm mercury perfusion pressure at 33° C. The purpose of the reduced temperature was to allow for a more gradual 'run down' curve. A mixture of oxygen (95 per cent) and carbon dioxide (5 per cent) was bubbled through the solutions. A modified Krebs (1950) solution was used exclusively in all experiments. The solution was prepared by dissolving the following amounts of reagents in one liter of distilled water (all weights are expressed as grams of the anhydrous compound): NaCl, 6.92; KCl, 0.35; $CaCl_2$, 0.28; $MgSO_4$, 0.15; $NaHCO_3$, 2.1; KH_2PO_4, 0.16; Glucose, 2.0.

For the first hour, hearts were perfused with Krebs solution alone and measurements of heart rate and force developed at 25 g, diastolic tension, and flow rates were taken every 10 minutes. For the next 20 minutes the hearts were perfused with Krebs solution containing various prostaglandins at different concentrations; measurements were taken every five minutes. During the last hour, the hearts were again perfused with only Krebs solution and measurements taken every 10 minutes. Control experiments used only Krebs solution.

Statistical Analysis

Statistical analysis were performed by assuming that

changes with time were linear. Linear regression lines were calculated for the variables over the periods 20-60', 60-80' (infusion), and 80-140' and significant changes calculated by standard statistical tests for slopes and intercepts.

EXPERIMENTAL SECTION III

Experiments on Cultured Heart Cells

We had three motives in choosing cultured myoblasts as a test model: 1) we wished to investigate the direct cellular cardiac pharmacology of prostaglandins; 2) we hoped to develope a screen for antiarrhythmic activity using cultured heart cells; and 3) we felt that by choozing suitable arrhythmogenic agents it should be possible to produce arrhythmias whose ionic and biochemical causes were understood, thus allowing insight into the mechanism of the antiarrhythmic activity of prostaglandins. Cultured heart cells would appear to be a particularly useful preparation in that only direct actions need be considered, the effects of metabolic poisons are readily quantifiable, and it has been reported that antidysrhythmic actions can be demonstrated in such cultures (Giraidier, 1971; Hyde, 1972; Goshima, 1976). In addition the physiology of cultured heart cells closely resembles that of intact tissue (Wollenberg, 1970). A potential difficulty with this model is related to the fact that only single cells were studied. This system lacks the factor of cell to cell communication and therefore the model most closely resembles ectopic foci. Fourteen prostaglandins of the A, B, D, E, and F series were chosen for investigation of their cellular pharmacology. We wished to test as wide a selection as possible in

this relatively simple system. For testing against arrhythmias induced by the different arrhythmogenic agents it was necessary to reduce the number of prostaglandins in order to have a manageable experiment. Prostaglandins A_2, E_2, $F_{1\alpha}$, and $F_{2\beta}$ were used to allow comparison with the in vivo rat experiments.

Heart Cell Culture Preparation

Heart cell cultures were prepared by a modification of a method described by Harary and Farley (1963). One mm cubes of ventricular tissue (one heart for each flask to be prepared) from 5-day-old Wistar rats were digested at 37° C with 0.15% trypsin (Nutritional Biochemicals Corporation) for 20 minute periods. The resulting cell suspensions were collected in fetal bovine serum (Microbiological Associates) at 4° C and three such digestates were pooled for centrifugation at 220 x g for 3 minutes.

Washed pellets of cells were resuspended in a tissue culture medium of the following composition; 20 ml F-10 (Colorado Serum Co.); 4 ml fetal bovine serum (Microbiological Associates), and 1 ml antibiotic solution (6.2 mg penicillin G + 10 mg streptomycin sulfate in Puck's saline A) diluted to 100 ml with Hank's basic salt solution (without KCl or $NaHCO_3$). Aliquots of the suspension were incubated in Falcon Tissue culture flasks for 2 hours to remove fibroblasts (which adhered rapidly in this period) before being transferred to fresh flasks for the growth of a primary myoblast-enriched culture. Culture media were changed at one and three days and experiments were performed on single cells 4-5 days in culture.

Measurements of Myoblast Beating Activity

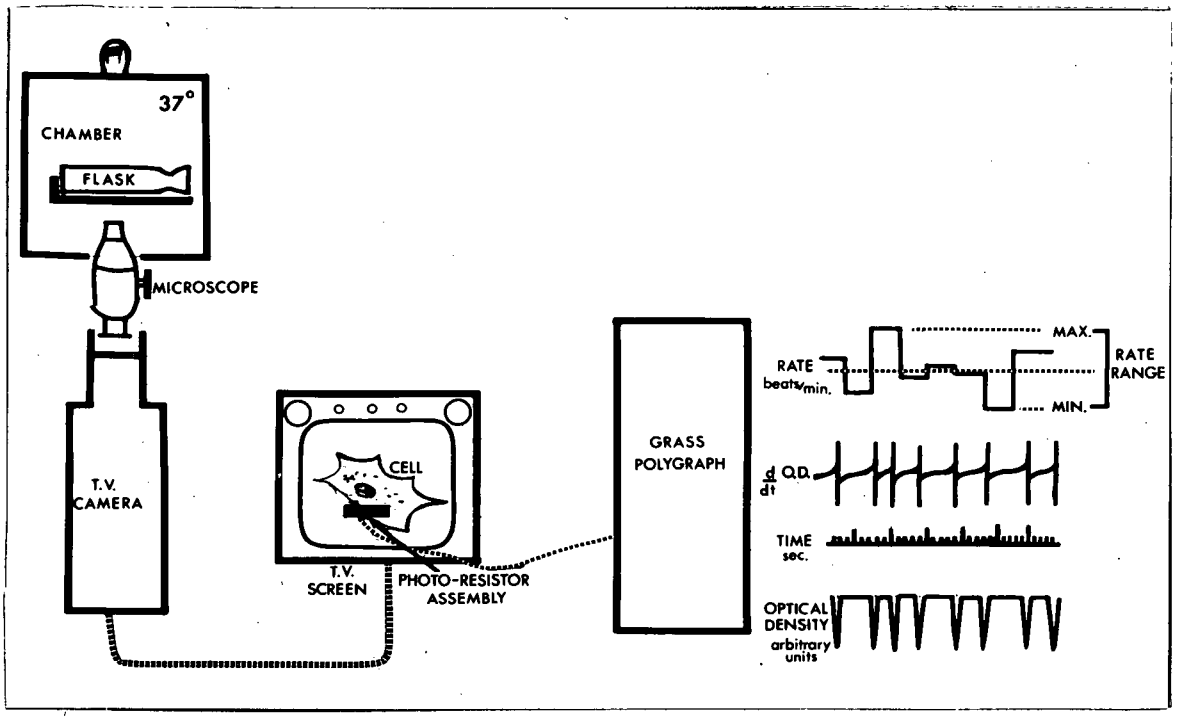
The beating activity of single myoblast cells was monitored by means of a photo-optical device similar to that described by Schanne (1971). A closed circuit television system on a phase contrast microscope was employed to obtain cell images on a 12-inch monitor while a pair of photo-resistors placed on the monitor screen measured the variation in optical density accompanying each cell beat (see Figure 5). Output was displayed on a Grass Polygraph and processed (Grass tachograph) to give beating rate, rate variation (rate range), and the first differential of optical density changes (Tetronix Type O differential amplifier). Typical records from a group of five cells are shown in Figure 6. Although there was variation between individual cells, group mean values from different cultures and flasks were very constant.

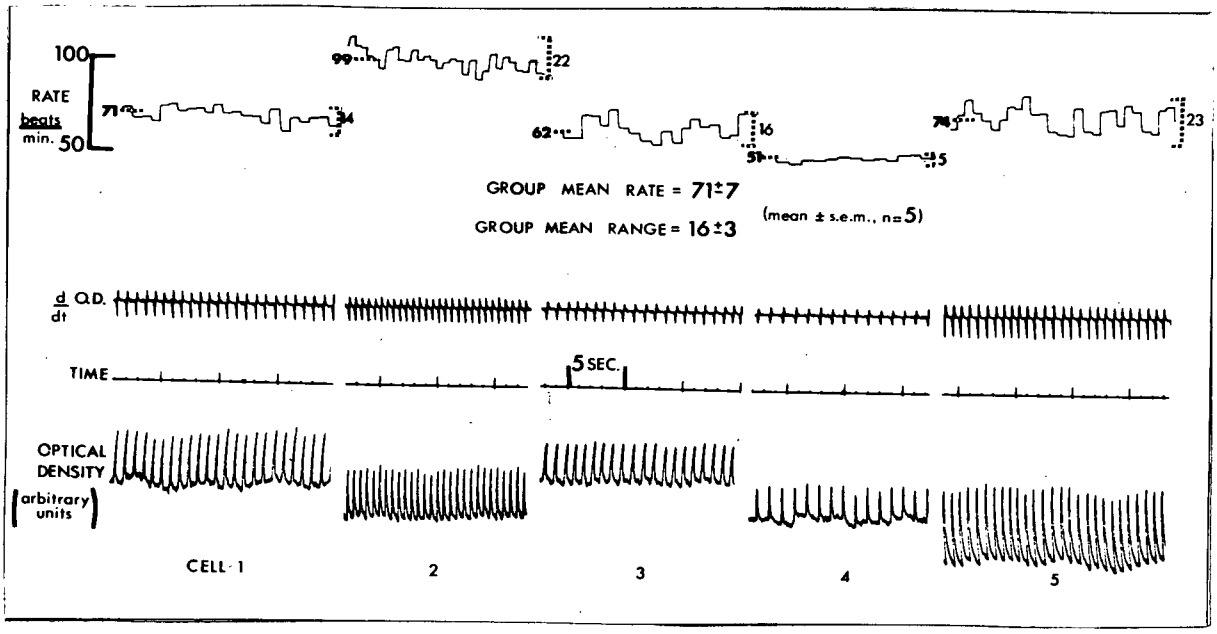
The effect of arrhythmogenic agents, drugs and mixtures of such were assessed by a random "blind" observation technique. Drugs (nature unknown to the observer) were added at time zero and individual cells were each examined for 20 seconds after 10 and 35 minute exposure to drug. For each drug concentration six cells, randomly selected, were examined at each time in each of three different cultures and all values were pooled. Appropriate 't' tests for unpaired, pooled data were used to test for statistical significance.

In contrast to the studies performed on random samples of individual cells, it was also possible to conduct experiments on the same selected cell. A calibrated tissue flask

Figure 5: Diagram of apparatus for measuring beating activity in single cultured heart cells. A. Photoresistors mounted on a T.V. monitor screen detect changes in optical density with beating activity. Changes are electrically processed to give the: I., rate of beating, II., range of beating, III. optical density, and IV., the first derivative of optical density with time (d OD/dt).

Figure 6: Typical records of changes in optical density, the corresponding derivative of optical density, and the tachograph measured beat-to-beat rate from five single myoblasts. The figures on the rate record give the values of rate and rate range, for that cell. The group means were found to be very consistent between cells, flasks, and cultures.


37°
CHAMBER
FLASK
MICROSCOPE
T.V. CAMERA
CELL
T.V. SCREEN
PHOTO-RESISTOR ASSEMBLY
GRASS POLYGRAPH
MAX.
RATE beats/min.
MIN.
RATE RANGE
d/dt O.D.
TIME sec.
OPTICAL DENSITY arbitrary units


100
RATE beats/min.
50
99
22
71
34
62
16
58
5
78
23
GROUP MEAN RATE = 71±7
GROUP MEAN RANGE = 16±3
(mean ± s.e.m., n=5)
d/dt O.D.
TIME
5 SEC.
OPTICAL DENSITY (arbitrary units)
CELL 1
2
3
4
5

holder allowed a particular microscope field to be selected when required and this, together with a sketch of cell shapes to aid identification, allowed the behavior of a number of single cells to be followed in time, so that individuals in a group of cells could be sampled repeatedly for any dose of drug or length of time. This method was used to obtain the complete chronotropic log dose-response curve for $PGF_{2\alpha}$ (Figure 23).

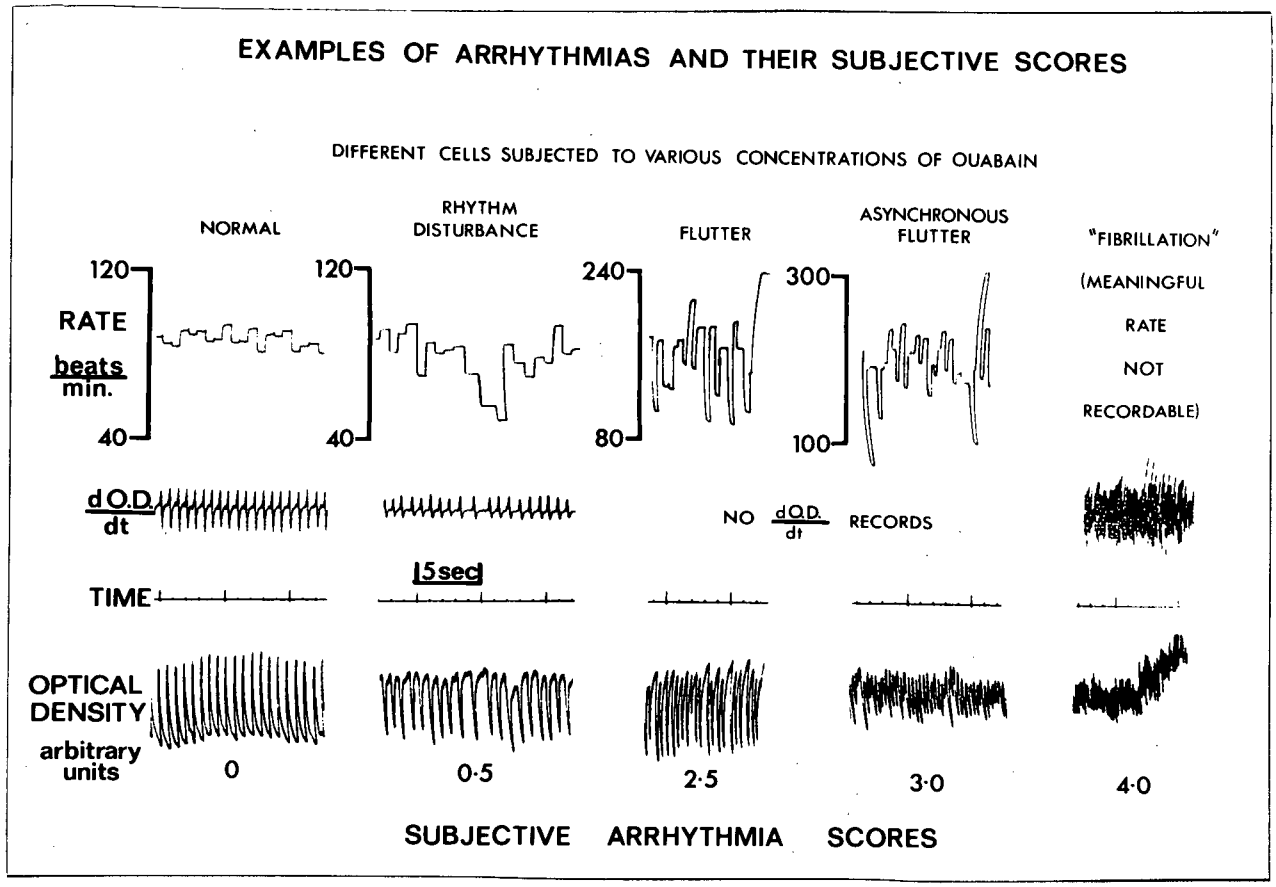
Quantitation of Arrhythmias in Single Cells

Two methods of quantifying arrhythmias in single myoblasts were used, one subjective and one objective. Both types of arrhythmia quantitation studies were performed on random samples of individual cells.

Subjective Measurements

A qualitative account of arrhythmias in heart cell cultures has previously been described by Goshima (1976). In the present experiments the severity of arrhythmias was quantitated by using a scale of arrhythmias graded 0-4. A completely rhythmic cell scored 0. Intermediate stages were recognized; for example, a slight irregularity in beat was given a score of 0.5; a failure to maintain rate, 1.0; fluttering (extremely rapid synchronous but weak rate), 3.0; and fibrillation (rapid asynchronous contractions) were scored 4.0. The scale was based on observations of cells progressing from normal behavior to death after treatment with ouabain and Cyanea toxin (Walker, Martinez and Godin, 1977). Examples are shown in Figure 7. The percentage of cells beating arrhythmically was also recorded from random

Figure 7; Quantitation of arrhythmias in single cultured heart cells. The severity of arrhythmias was measured using a subjective scale graded from 0 to 4. The difference between the highest and lowest beat-to-beat rate recorded by the tachograph is shown along with changes in rate range.


EXAMPLES OF ARRHYTHMIAS AND THEIR SUBJECTIVE SCORES
DIFFERENT CELLS SUBJECTED TO VARIOUS CONCENTRATIONS OF OUABAIN
NORMAL
RHYTHM DISTURBANCE
FLUTTER
ASYNCHRONOUS FLUTTER
"FIBRILLATION" (MEANINGFUL RATE NOT RECORDABLE)
120
40
120
40
240
80
300
100
RATE
beats/min.
dO.D./dt
NO dO.D./dt RECORDS
TIME
15sec
OPTICAL DENSITY
arbitrary units
0
0·5
2·5
3·0
4·0
SUBJECTIVE ARRHYTHMIA SCORES

samples of ten cells.

Objective Measurements

Arrhythmogenic effects were assessed objectively in terms of the percentage of cells beating, the beating rate, and rate range. The percentage of cells beating was determined in random samples of 100 cells. The rate was the mean of the tachograph readings during a 20 second sample period. Deviations from the normal rate were judged arrhythmogenic when produced by an arrhythmogenic agent. Rate range, the difference between the highest and lowest beat-to-beat rate recorded by the tachograph over a 20 second period, was also used as an objective measurement of single cell arrhythmias. Rate range measures the ability of cells to maintain a constant rate, and may, therefore, give an index of the arrhythmic or antiarrhythmic properties of a drug. Rate range tended to increase as a function of rate. Consequently, when large changes in rate occurred the term $(\frac{\text{rate range}}{\text{rate}})$ was used as an index of beating behavior. This had the effect of nullifying the changes due to rate alone.

Assessment of Antiarrhythmic Actions in Cultured Heart Cells

To assess the antiarrhythmic and/ or "cyto-protective" effects of prostaglandins, lidocaine, and quinidine, arrhythmogenic agents were added for set times at appropriate concentrations. The ability of the various drugs to protect from or increase the effects of arrhythmogenic agents was assessed by adding the drug with the agent. Generally, if the drug under test exaggerated the effects of the arrhythmogenic agent it was considered to potentiate, while a lessening of the

effects of the arrhythmogenic agent was amelioration. Obviously if the drug increased the number of cells beating normally or reduced the number beating arrhythmically this would be an antiarrhythmic action. Other ameliorations were not so obviously judged antiarrhythmic. For example, dinitrophenol (DNP) slowed the rate of cells, and if this slowing was reduced by a prostaglandin (i.e. the prostaglandin increased the cell's beating rate) this was considered an antiarrhythmic action. However, with Ca^{++} which increased rate, if the prostaglandin further increased rate then the prostaglandin's action would be arrhythmogenic. Thus the same action of a prostaglandin alone, was not, a priori, considered arrhythmogenic or antiarrhythmic.

Cyanea Toxin-containing Material

Persistent and predictable changes in cell beating behavior were also produced by Cyanea toxin, a cardiotoxin obtained from the jellyfish Cyanea capillata, previously described by Walker (1977a). This toxin was used to produce the most severe types of arrhythmias encountered in cultured myoblasts. One batch of Cyanea capillata tenacles obtained from Hong Kong coastal waters, was subjected to maceration and controlled sieving in order to obtain isolated nematocysts free of tentacles and mesoglial tissue (Walker, 1977a). Nematocysts were lyophilized and stored at -10° C. Ten mg portions of the lyophilized nematocysts were suspended in distilled water for homogenization at 5000 revolutions per minute for 3 min at 4° C, and the volume then increased to 5 ml. The homogenate was centrifuged at 13,500 g for 30 min

to give a faintly opalescent supernatant whose protein concentration was obtained by the method of Lowery et al. (1951). This solution was the concentrated crude soluble toxin-containing material. Lethality of the toxin to mice was assayed as previously described (Walker, 1977a).

Drug Details for All Experiments

The following prostaglandins were used A_1, A_2, B_1, B_2, D_1, D_2, E_1, 2-decarboxy-E_1, E_2, (15S)-15 CH_3-E_2, $F_{1\alpha}$, $F_{1\beta}$, $F_{2\alpha}$, and $F_{2\beta}$. All prostaglandins were kindly donated by The Upjohn Company, Kalamazoo, Michigan. Prostaglandins were dissolved in pure ethanol at a concentration of 3.5 mg/ml as a stock solution and stored at -10° C. Final media concentrations were obtained by adding dilutions of these stock solutions to the appropriate media. Control experiments contained ethanol at the same dilution as with the prostaglandin experiments. Other drugs used in these experiments were adrenaline, lidocaine, and quinidine (Sigma Chemical Co.), and ouabain (Schwartz-Mann Co.). All other chemicals were of analytical grade. They were used as they were received without further purification.

CALCULATIONS, STATISTICAL METHODS, AND EXPERIMENTAL DESIGN

Standard statistical parametric tests such as Student's t were used where possible, together with standard linear regression analysis. Dunnett's t test (Dunnett, 1955) was used for comparing several treatments with control. Multiple range and multiple F tests (Duncan, 1955) were used to assess the anti-arrhythmic and or "cytoprotective" effects of prostaglandins, lidocaine, and quinidine in the presence of arrhythmogenic agents in cell culture. Occasionally, suitable non-parametric

tests such as chi square had to be used. A probability of less than 0.05 was chosen as the criterion of significance in all cases.

Where possible all experiments were performed double blind with the drug under consideration, the arrhythmogenic agent, etc. being unknown at the time of the experiment or the analysis of records. Experiments were conducted according to rigid coded protocols.

CHAPTER III

RESULTS

The results of different experimental sections are considered in order. To recapitulate, the sections are as follows.

Section I. The effect of prostaglandins on arrhythmias in A) the dog and B) the rat and C) the effect of prostaglandins on electrical vulnerability of the in vivo rat heart.

Section II. The effect of prostaglandins on isolated perfused rat hearts.

Section III. The effect of prostaglandins on A) the beating behavior of cultured rat heart cells and B) abnormalities of beating in cultured rat heart cells induced by a variety of arrhythmogenic agents.

EXPERIMENTAL SECTION I.

A) The Effects of Prostaglandins During Coronary Occlusion in Dogs

In order to demonstrate possible antiarrhythmic actions of prostaglandins in a standard experimental model an initial experiment was performed in dogs against arrhythmias induced by coronary artery ligation. The variable measured in this experiment was the incidence of premature ventricular contractions (PVCs). Quantitation of this variable was difficult, due to the non-normal distribution of PVCs occurring as the result of occlusion; even log PVCs were not normally dis-

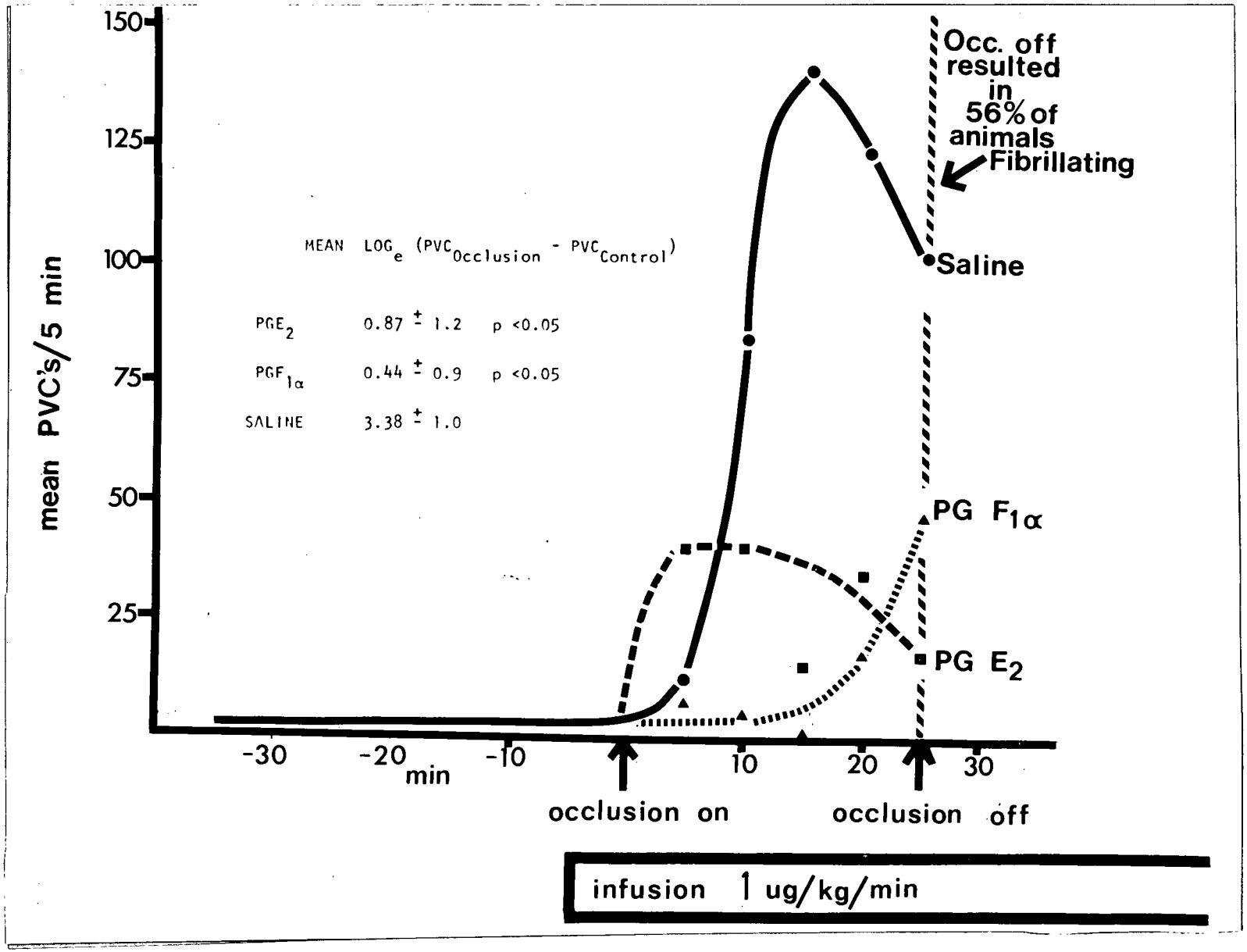
tributed, thus making statistical analysis of these small samples difficult. However, by plotting the mean frequency of PVCs for each group (that is control, E_2, and $F_{1\alpha}$--see Figure 8) it can be seen that both prostaglandins reduce the incidence of arrhythmias associated with occlusion although the usual statistical tests do not show differences between individual points. The figures inserted within the table (Figure 8) are the mean log to the base e PVCs ($PVC_{occlusion} - PVC_{control}$). No significant differences were noted for blood pressure or rate within or between drug treatment groups.

B) The Effect of Prostaglandins, Lidocaine, and Quinidine on Arrhythmias Produced by Coronary Artery Ligation in Rat

As indicated in Methods a number of variables were measured in order to assess the possible antiarrhythmic effects of prostaglandins, lidocaine, and quinidine against arrhythmias associated with coronary artery ligation in the rat. Some of these variables were not of course independent but no standard measure of antiarrhythmic activity in this situation is available.

The effect of prostaglandins, lidocaine, and quinidine on arrhythmias produced by left coronary artery ligation in rats is tabulated Table I and also shown in Figures 9 through 13 for clarity. The average number of premature ventricular contractions (PVCs) experienced by each rat after coronary artery ligation was reduced by all agents tested (Figure 9). Prostaglandin E_2, A_2, and $F_{2\beta}$ reduced the number of PVCs to approximately 40 to 50 per cent of control values. Lidocaine and quinidine showed approximately a 20 per cent reduction;

Figure 8: The effect of left coronary artery occlusion on the mean frequency of premature ventricular contractions with time for control, prostaglandin E_2, and prostaglandin $F_{1\alpha}$-treated dogs. Each point represents the mean of six animals. Figures inserted within the table are the Student 't' test of the mean log to the base e PVCs ($PVC_{occlusion} - PVC_{control}$) for prostaglandin versus saline.


150
125
100
75
50
25
mean PVC's/5 min
MEAN LOG$_e$ (PVC$_{Occlusion}$ - PVC$_{Control}$)
PGE$_2$ 0.87 ± 1.2 p <0.05
PGF$_{1\alpha}$ 0.44 ± 0.9 p <0.05
SALINE 3.38 ± 1.0
Occ. off resulted in 56% of animals Fibrillating
Saline
PG F$_{1\alpha}$
PG E$_2$
-30
-20
min
-10
10
20
30
occlusion on
occlusion off
infusion 1 ug/kg/min

the reduction with $F_{1\alpha}$ was 10 per cent. The number of PVC salvos (groups of 2 to 4 PVCs in rapid succession) was not taken for lidocaine and quinidine (Table I).

The number of flutter episodes was reduced by PGE_2, $PGF_{2\beta}$, and quinidine (Table I). Prostaglandin $F_{1\alpha}$ may have increased the number of flutter episodes. The average duration of each flutter episode was reduced by all drug treatments (Figure 10). Quinidine and PGE_2 were the most effective, followed in order by $PGF_{2\beta}$, lidocaine, PGA_2, and $PGF_{1\alpha}$. The time-to-flutter (Table I) did not prove to be a useful criterion as too few samples were available with the more effective antiarrhythmic agents for meaningful comparison. In general it required approximately 5 to 6 minutes after ligation before the first flutter episode appeared.

The effect of prostaglandins, lidocaine, and quinidine on the infarct size versus whole heart weight is shown in Figure 11. No significant differences were found although there was a tendency for the size of the infarct to be smaller with PGE_2 and lidocaine.

The number of animals dying from arrhythmias within the 25-minute test period following occlusion is shown in Figure 12. Out of the five test animals for each drug, none died with PGE_2; one each died with $PGF_{2\beta}$ and quinidine; two each died with PGA_2, $PGF_{1\alpha}$, and lidocaine; four died with control treatment.

The effect of the different drug treatments on the average subjective score for severity of arrhythmias is shown in Figure 13. Using this scale, PGE_2 reduced the severity

TABLE I

The effect of prostaglandins, lidocaine, and quinidine on arrhythmias produced by left coronary artery ligation in rat. Standard errors of the mean are given unless too few samples were available for calculation. Statistics were calculated with Dunnett's t test or Fishers's exact chi square test. n=5 animals for each drug treatment. Each animal received only one drug. *= significant at 0.05 level, **= significant at 0.01 level

	CONTROL	A_2	E_2	$F_{1\alpha}$	$F_{2\beta}$	Lidocaine	Quinidine
Number of PVC (25 min total)	131±81	61±27*	50±20*	110±49	67±15*	98±30	91±23
Number of PVC Salvos	3.6±1.8	4.6±2.4	3.0±2.2	4.0±1.2	3.2±1.0	-----	-----
Flutter Episodes	1.2±0.2	1.8±0.7*	0.4±0.4**	2.0±0.6*	0.6±0.4*	1.2 (n=4)	0.2 (n=1)
Flutter Duration Total/ heart (sec)	100±35	42±21**	2±2**	89±32	8±6**	21±6.6**	0
Time to Flutter (min)	5.5±0.4 (n-5)	4.8±1.3 (n-4)	7.5 (n=2)	5.6±1.3 (n=5)	6.8 (n=2)	6.2±0.9 (n=4)	4.0 (n=1)
Infarct Size VS Whole Heart Wgt (per cent)	27.8±1.9	28.0±2.8	22.0±3.6	24.4±3.3	27.0±4.2	22.8±2.5	24.6±5.5
Death	4/5 animals	2/5	0/5*	2/5	1/5	2/5	1/5
Subjective Severity of Arrhythmias	11.8±1.2	7.0±2.3**	4.2±1.2**	7.6±0.2**	5.0±2.3**	8.8±1.8*	5.2±2.2**

Figure 9: The effects of prostaglandins, lidocaine, and quinidine on the average number of premature ventricular contractions (PVCs) experienced by five rats over a 25-minute period following acute left coronary artery ligation. Each bar represents the mean of five animals ± S.E.M.

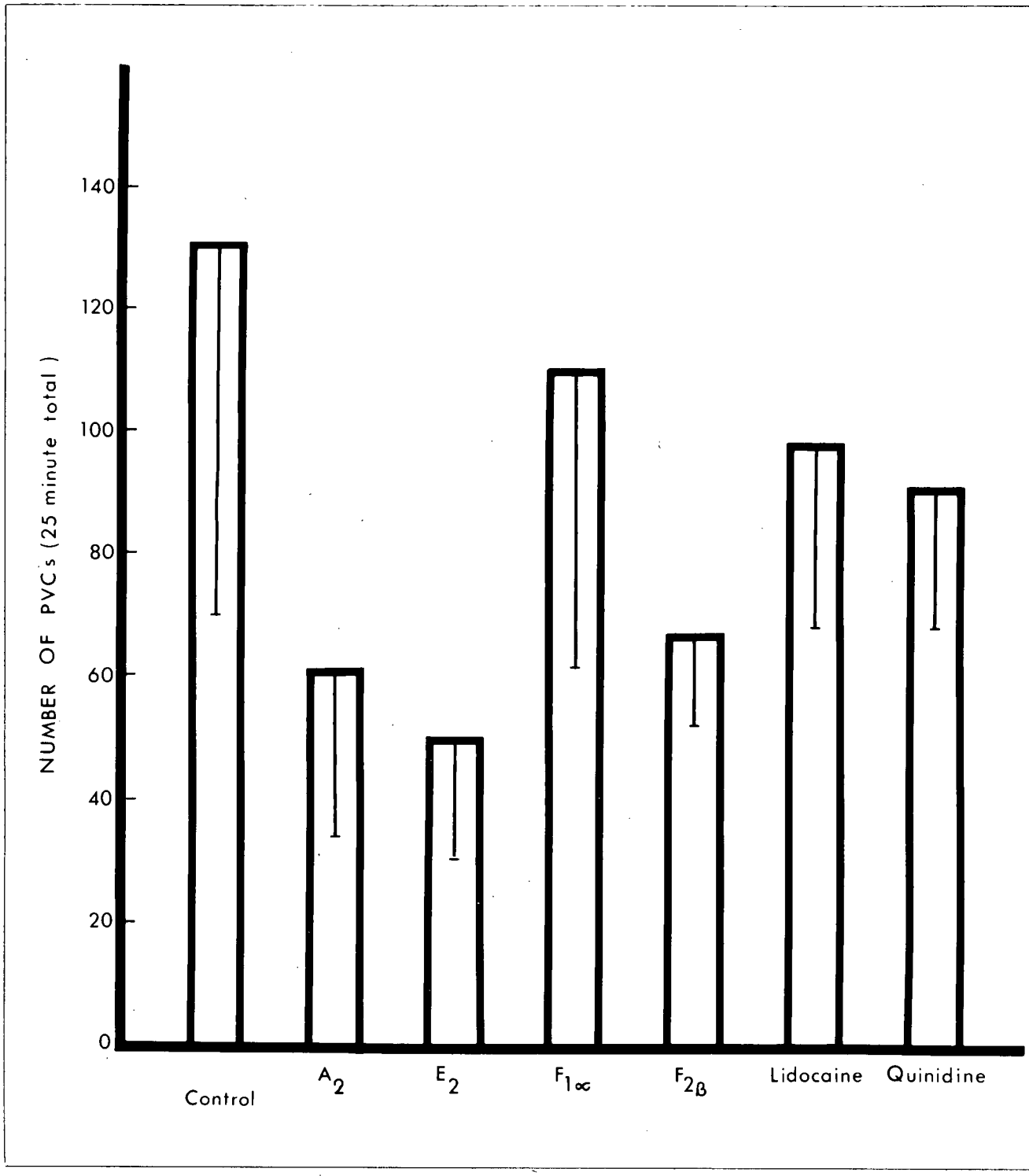

NUMBER OF PVCs (25 minute total)
140
120
100
80
60
40
20
0
Control
A2
E2
F1∝
F2β
Lidocaine
Quinidine

Figure 10: The effect of prostaglandins, lidocaine, and quinidine on the average duration of each flutter episode experienced by rats in a 25-minute period following acute left coronary artery ligation. Each bar represents the mean of five animals ± S.E.M.

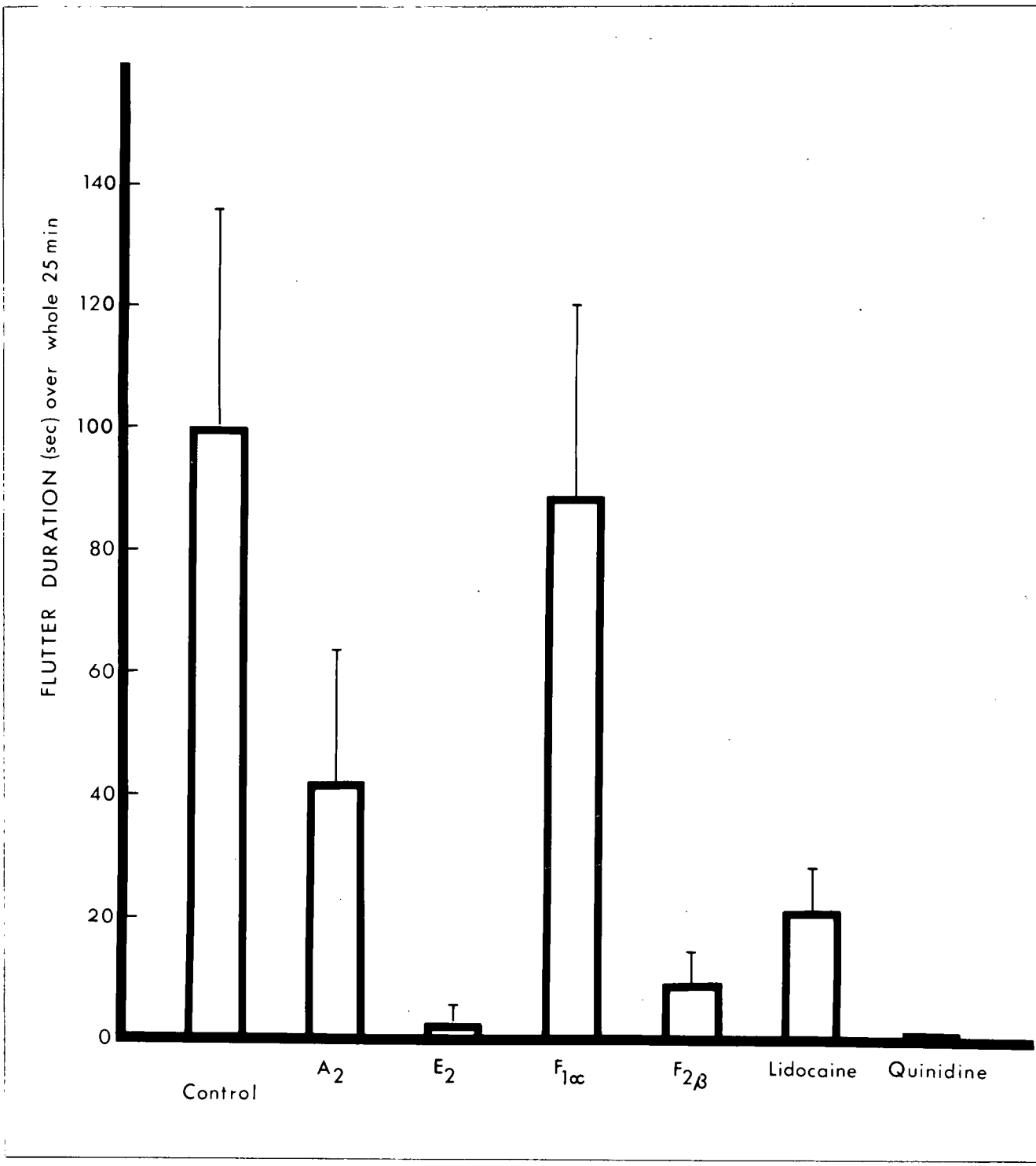

FLUTTER DURATION (sec) over whole 25 min
140
120
100
80
60
40
20
0
Control
A2
E2
F1α
F2β
Lidocaine
Quinidine

Figure 11: The effect of prostaglandins, lidocaine, and quinidine on the average size of the infarct produced (as the percentage of whole heart weight) following acute left coronary artery ligation in rats. Each bar represents the mean of five hearts ± S.E.M.

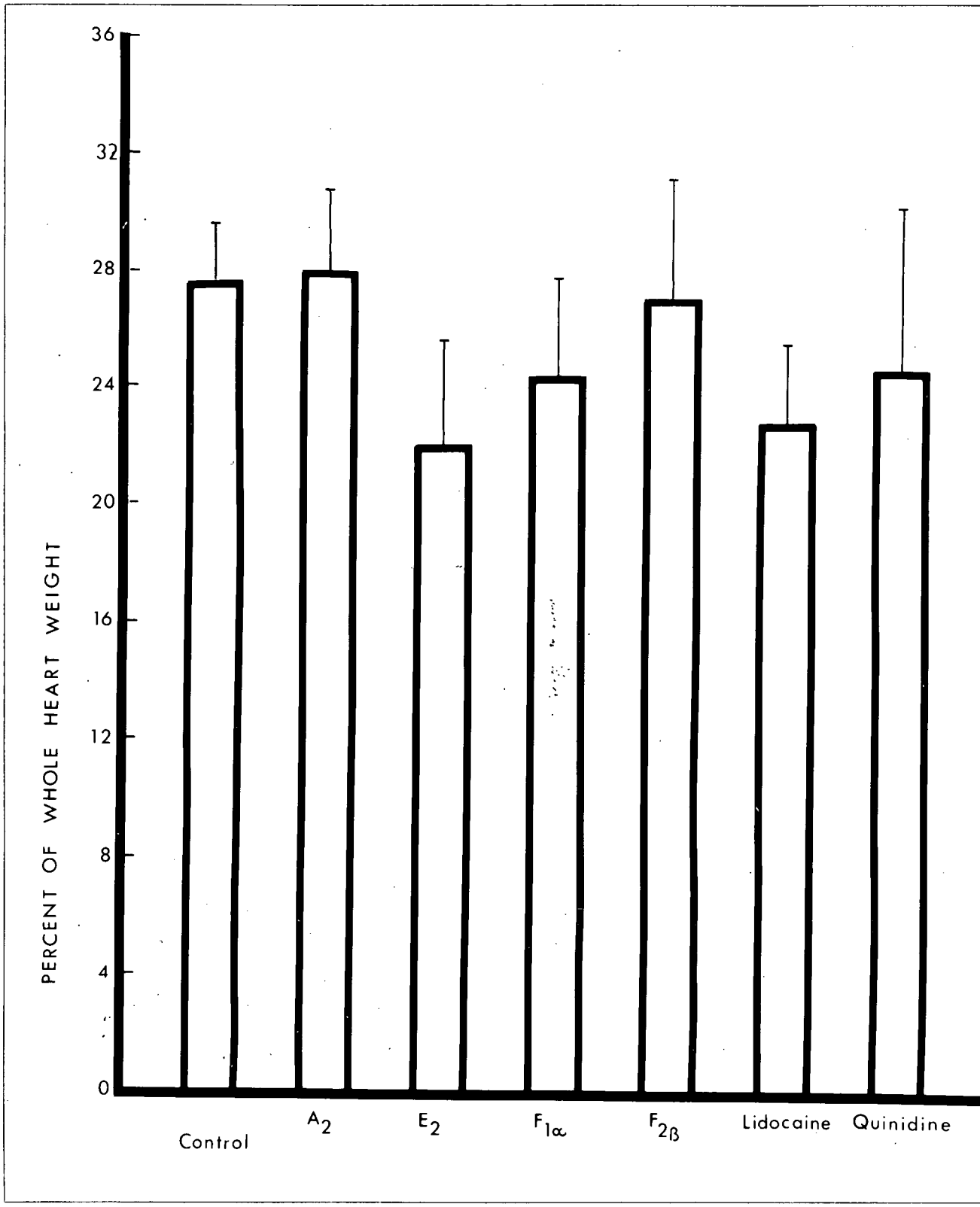

PERCENT OF WHOLE HEART WEIGHT
36
32
28
24
20
16
12
8
4
0
Control
A2
E2
F1α
F2β
Lidocaine
Quinidine

Figure 12: The effect of prostaglandins, lidocaine, and quinidine on the number of animals dying from arrhythmias within the 25-minute test period following acute left coronary artery occlusion. Each group contained five rats.

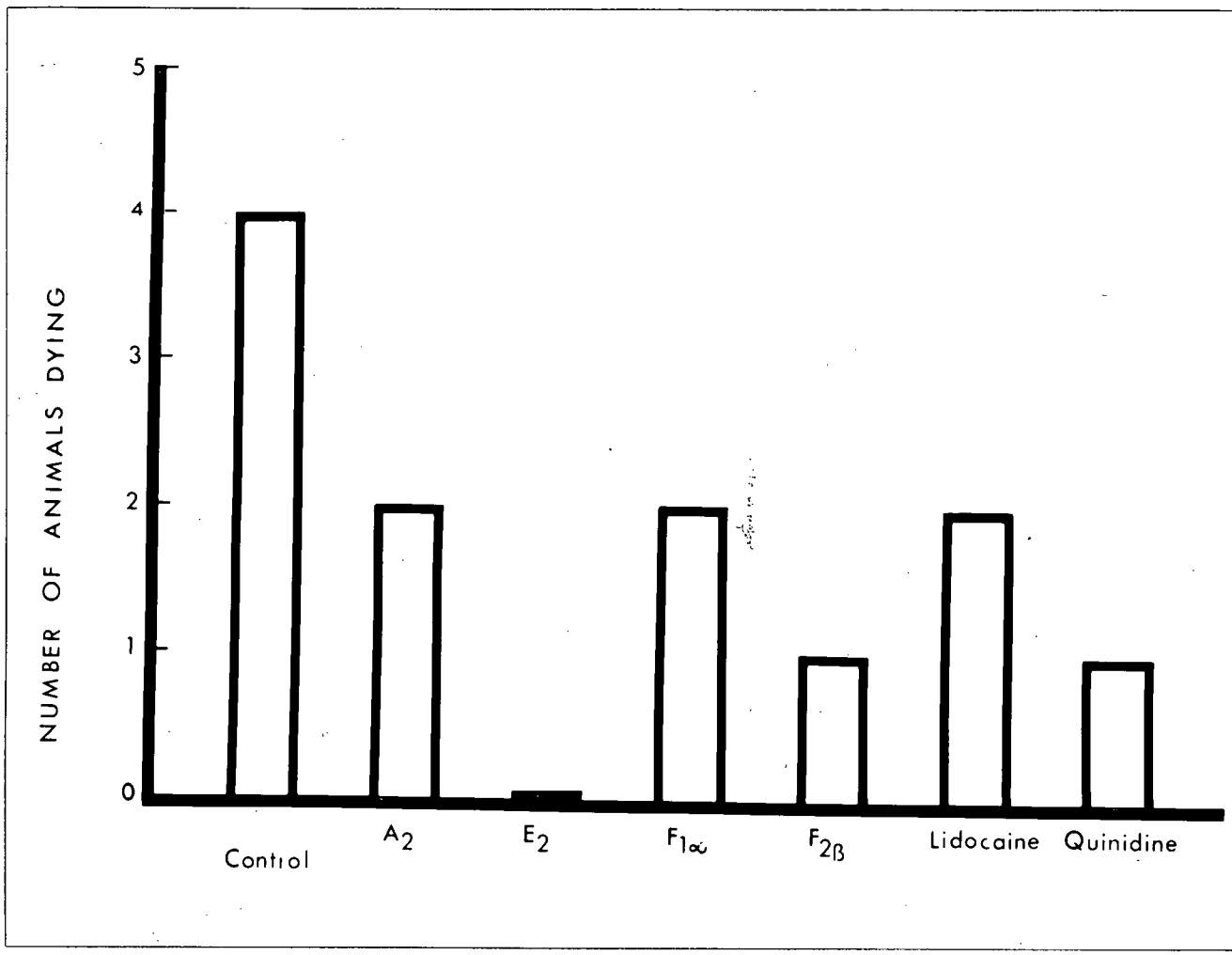
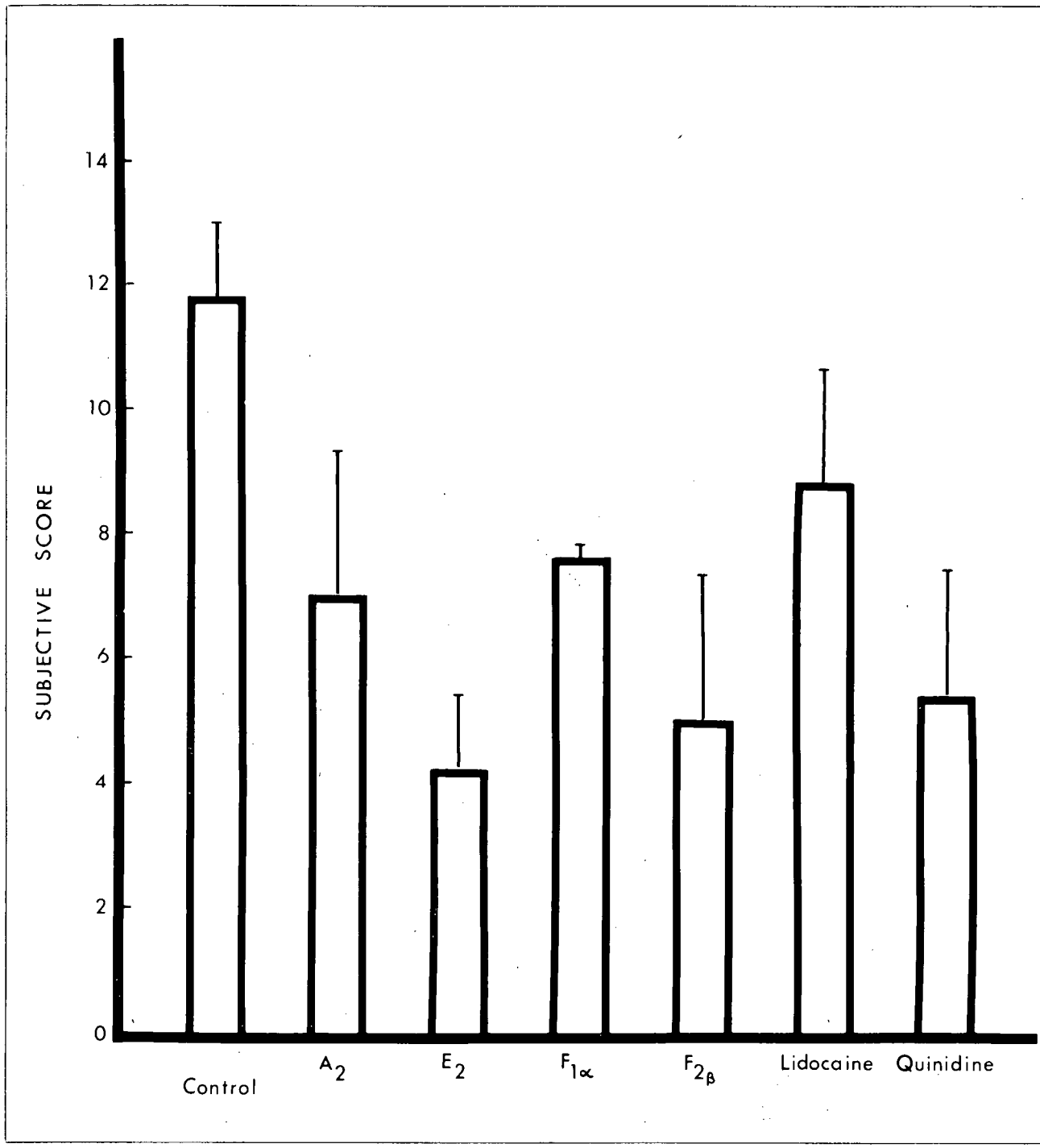

NUMBER OF ANIMALS DYING
5
4
3
2
1
0
Control
A2
E2
F1∝
F2β
Lidocaine
Quinidine

Figure 13: The effect of prostaglandins, lidocaine, and quinidine on the average subjective score for severity of arrhythmias following acute left coronary artery occlusion in rats. Each bar represents the mean of five rats ± S.E.M.


SUBJECTIVE SCORE
0
2
4
6
8
10
12
14
Control
A_2
E_2
$F_{1\alpha}$
$F_{2\beta}$
Lidocaine
Quinidine

of arrhythmias to 37 per cent of control. $PGF_{2\beta}$ and quinidine were nearly as effective as PGE_2. PGA_2, $PGF_{1\alpha}$, and lidocaine were less effective (60 - 75 per cent of control) but were still significantly better than control.

Changes in blood pressure during coronary artery occlusion in rats are shown in Figure 14. Blood pressure was reduced by surgery alone in all cases. PGE_2, PGA_2, and quinidine further reduced blood pressure, although the pressure did not stay down with PGA_2. $PGF_{1\alpha}$, $PGF_{2\beta}$, and lidocaine had little effect on blood pressure. $PGF_{1\alpha}$ may have caused an increase in blood pressure 10 minutes after the start of the infusion.

Changes in heart rate during left coronary artery occlusion in rats are shown in Figure 15. The heart rate tended to increase following surgery in a probable reflex response to the fall in blood pressure (Figure 14). None of the drug treatments produced marked effects on heart rate. $PGF_{1\alpha}$ may have increased rate at 10 minutes after start of the infusion, however, this result is based on only the two surviving animals and is therefore of limited reliability.

C) Changes in Flutter Threshold and Maximum Following Frequency

Flutter threshold (the current required to produce flutter) was not found to be changed by any of the prostaglandins tested (Table II). Lidocaine produced a decrease in flutter threshold at the 6 μg/Kg/min dose after 10 minutes. Higher doses of lidocaine produced a fall in blood pressure with time and could not be used. Quinidine increased the flutter thres-

Figure 14: The effect of prostaglandins, lidocaine, and quinidine on blood pressure during left coronary artery ligation in rats. Both systolic and diastolic pressures are shown. Each bar represents the mean of five rats ± S.E.M. Values are shown at control time before surgery (C), immediately post-surgery (PS), at the start of the infusion of drug (I), 5 min after start of infusion (5'), and 10 min after start of infusion (10'). Standard errors are shown unless too few samples were available for calculation. No value is shown with infusion for quinidine which was given as a bolus. (See TABLE A in appendix for tabulated data.)

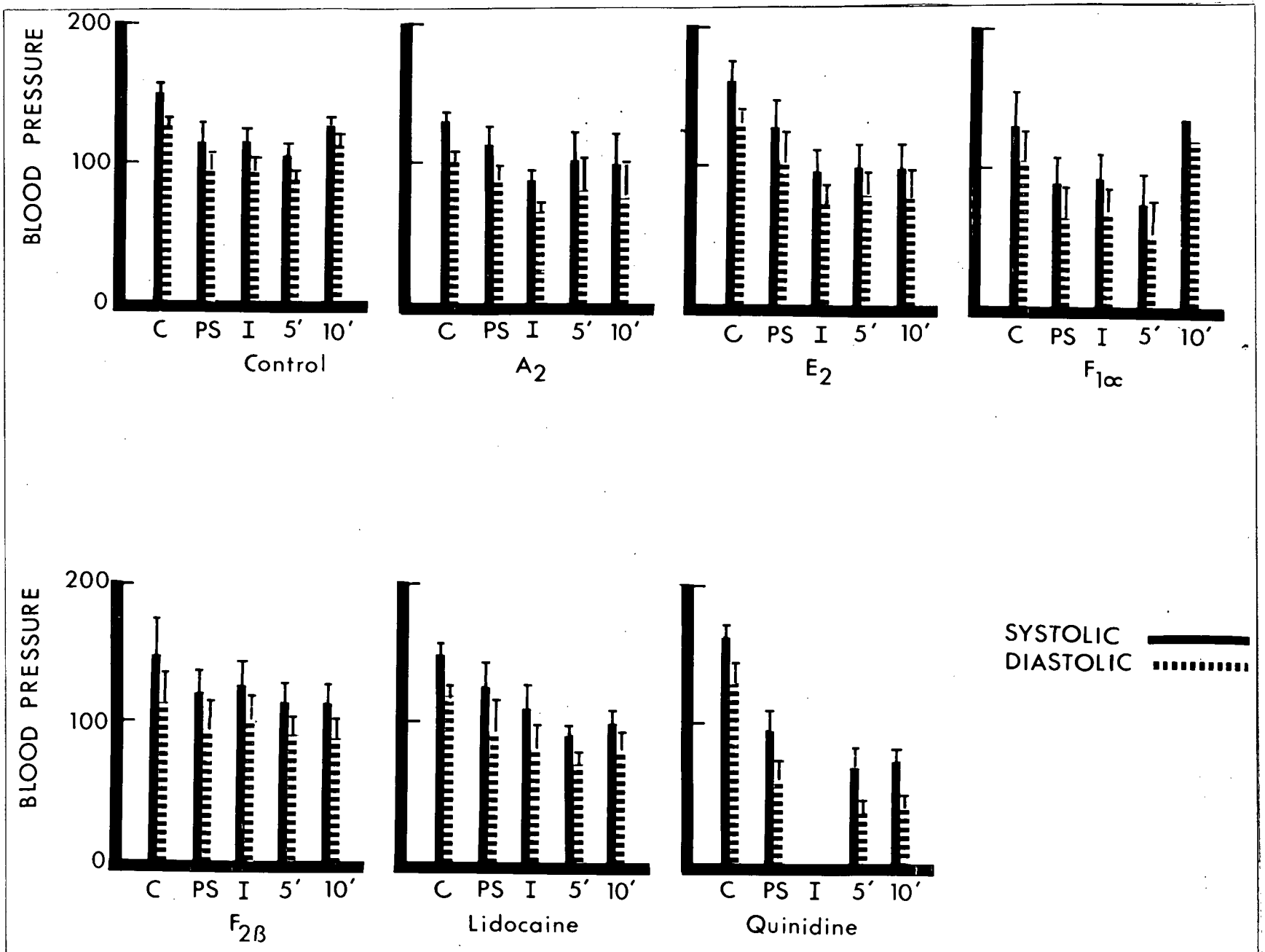

BLOOD PRESSURE
200
100
0
C PS I 5′ 10′
Control
A2
E2
F1α
BLOOD PRESSURE
F2β
Lidocaine
Quinidine
SYSTOLIC
DIASTOLIC

Figure 15: The effect of prostaglandins, lidocaine, and quinidine on heart rate during left coronary artery occlusion in rats. Each bar represents the mean of five rats ± S.E.M. (See Figure 16 for abbreviations) Standard errors are shown unless too few samples were available for calculation. No value is shown with infusion for quinidine which was given as a bolus. (See TABLE A in the appendix for tabulated data)

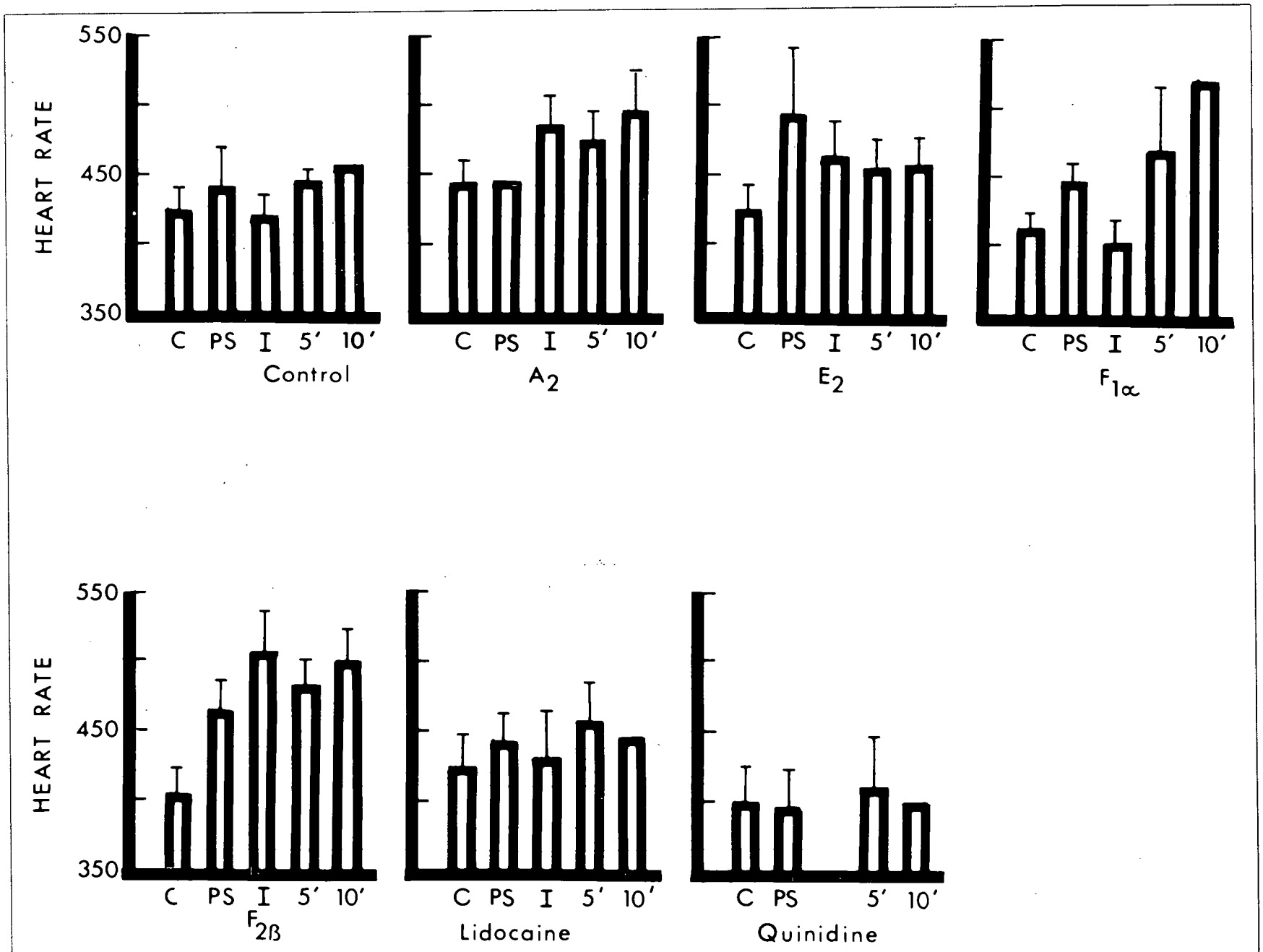

HEART RATE
550
450
350
C PS I 5′ 10′
Control
A2
E2
F1α
F2β
Lidocaine
Quinidine

TABLE II

The effects of prostaglandins, lidocaine, and quinidine on electrically stimulated Flutter Threshold and Maximum Following Frequency in in vivo rat heart.

Mean Control Maximum Following Frequency = 14.42 ± 0.28 Hz (n=36)

Dose: mg/Kg/min		1	4	8
PROSTAGLANDIN		Difference from initial control value		
A_2	Hz	-0.45	-0.8	-1.60
	mA	+0.02	+0.005	+0.003
E_2	Hz	+0.02	-0.5	-1.1
	mA	0	+0.005	0
$F_{1\alpha}$	Hz	-0.03	-1.2	-0.83
	mA	+o.o28	-0.025	-0.007
$F_{2\beta}$	Hz	-0.07	+0.2	+0.17
	mA	+0.04	-0.06	0
$F_{2\alpha}$	Hz	+0.50	-0.6	-1.5
	mA	+0.08	-0.02	-0.005

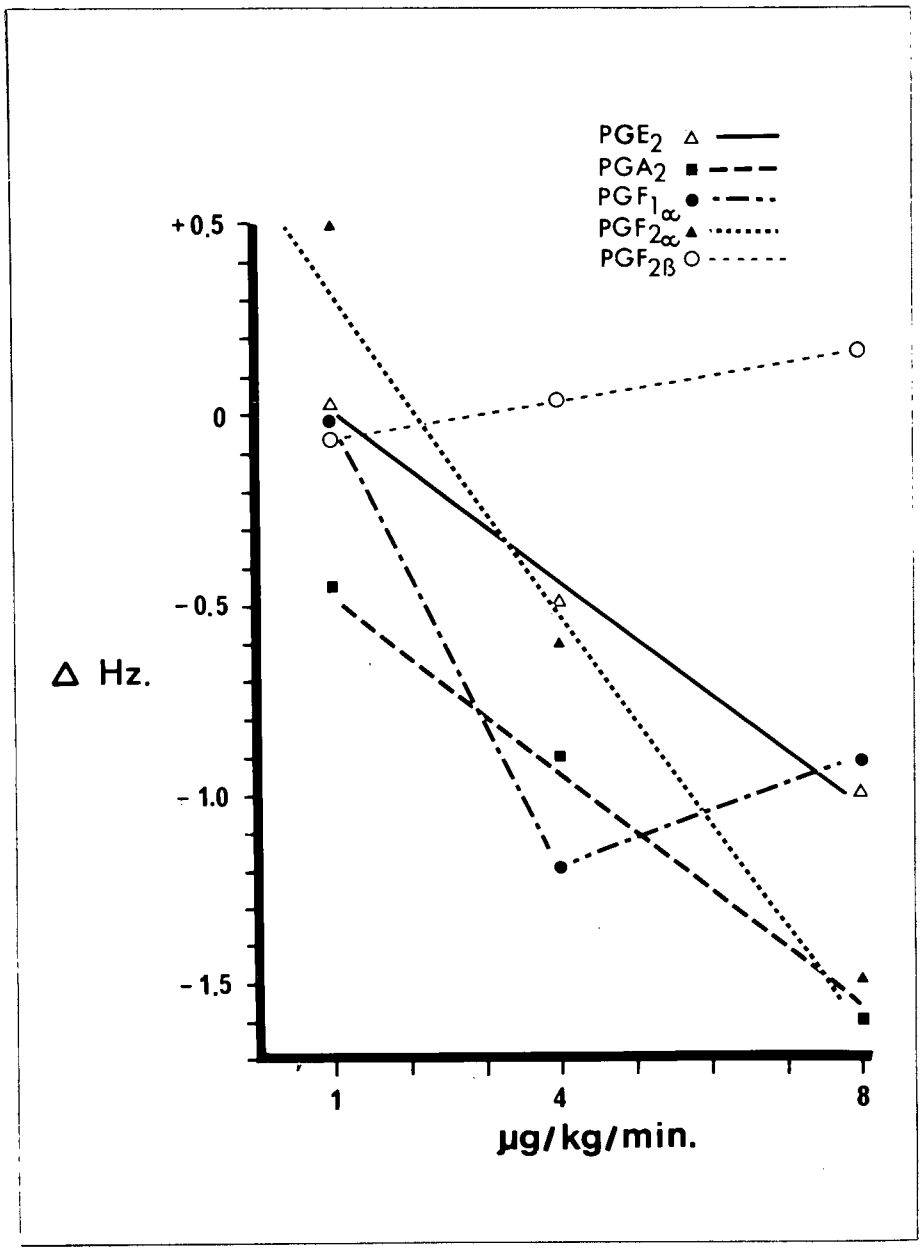
(All values determined 5 min after start of infusion)

		Time after start of infusion (min)		
		5	10	25
LIDOCAINE				
3 µg/Kg/min	Hz	+0.1	-0.2	+0.1
	mA	-0.001	+0.04	+0.02
6 µg/Kg/min	Hz	+7.3	+7.4	+7.2
	mA	-0.014	-0.9	-0.9

		Time after injection (min)
		5
QUINIDINE		
3 mg/Kg	Hz	-2.7
	mA	+0.30
6 mg/Kg	Hz	-4.5
	mA	+1.3

(n = 3 to 5 for all determinations)

Figure 16: Maximum following frequency changes with prostaglandin infusion. Mean control maximum following frequency was 14.42 ± 0.28 Hz (n=36). Data is shown as the difference from the initial control. Each point represents the mean of three to five animals.


PGE2
PGA2
PGF1α
PGF2α
PGF2β
+0.5
0
-0.5
-1.0
-1.5
Δ Hz.
1
4
8
µg/kg/min.

hold at both 3 and 6 μg/Kg doses.

Maximum following frequency was found to change in a dose-dependent manner depending on the prostaglandin; however, this change was usually less than ten per cent of the control (Figure 16, Table II). Mean control maximum following frequency was 14.42 ± 0.28 Hz (n=36). Linear decreases in following frequency were found with prostaglandins E_2, A_2, and $F_{2\alpha}$ (Figure 16). $PGF_{2\beta}$ produced a small increase in maximum following frequency, and $PGF_{1\alpha}$ had a biphasic effect with an initial decrease followed by an increase. Lidocaine at the 6 μg/Kg/min dose produced a marked increase and quinidine at 3 and 6 mg/Kg produced a marked decrease in maximum following frequency (Table II).

EXPERIMENTAL SECTION II

The Effect of Prostaglandins on Isolated Rat Heart

Figures 17 - 22 show the effect of prostaglandin infusion of isolated rat hearts on rate, force, and coronary flow. Prostaglandins (10^{-9}, 10^{-7}, and 10^{-5} M) were infused between minutes 60 and 80 of each experiment. Generally 10^{-9} M prostaglandins did not produce a detectable change in rate, force or flow. The results of prostaglandin infusion at higher doses (10^{-7} M and 10^{-5} M) are shown in figures 17 through 22. Table III contains the statistical testing of these results. At 10^{-7} M prostaglandins did not appear to have any marked effect on heart rate, which was subject to considerable variation (Figure 17). At the end of prostaglandin infusion there was an immediate fall in rate seen in all hearts which had been exposed to prostaglandins. At 10^{-7} M all prostaglandins kept the force

up during infusion (Figure 18). Statistical analysis (see method section or Table III) showed this to be significant. It is interesting that there was a tendency for prostaglandins E_2 and $F_{2\beta}$, but not prostaglandins A_2, $F_{1\alpha}$, or control, to delay the decline in maximum force production after the end of prostaglandin perfusion. Before exposure to prostaglandins the curves for these hearts had been lowest, but following prostaglandin treatment they became highest. Prostaglandin E_2 produced a small increase while prostaglandin $F_{2\beta}$ produced a small decrease in flow at 10^{-7} M perfusion (Figure 19). This effect did not persist after the end of the prostaglandin infusion period. At 10^{-5} M only prostaglandin A_2 markedly increased rate (Figure 20). None of the other prostaglandins produced any significant effect on this parameter during infusion, which was, again, subject to considerable variation. Following infusion of PGE_2, $F_{1\alpha}$, and $F_{2\beta}$, there was a fall in heart rate. It should be noted that there was no change in rate with time for control hearts in any of these experiments. No marked change in maximum force production was seen with prostaglandins at 10^{-5} M (Figure 21). This was true both during and following prostaglandin infusion. All prostaglandins markedly increased flow at 10^{-5} M (Figure 22). This increase in flow may have been partially maintained for a short time following prostaglandin infusion, but the increase did not persist.

TABLE III

Statistical testing of the results of prostaglandin infusions on rate, force and flow in perfused hearts. Levels of significance were $p<0.05$ for slopes and/or intercepts with regressions.

Prostaglandin concentration	RATE				FORCE				FLOW			
	A_2	E_2	$F_{1\alpha}$	$F_{2\beta}$	A_2	E_2	$F_{1\alpha}$	$F_{2\beta}$	A_2	E_2	$F_{1\alpha}$	$F_{2\beta}$
10^{-9}M Infusion	=	=	=	=	=	=	=	=	=	=	=	=
10^{-7}M Infusion	+	=	=	=	+	+	+	+	=	+	=	-
10^{-7}M Post-Inf.	-	-	-	-	=	+	=	+	=	=	=	=
10^{-5}M Infusion	+	=	=	=	=	=	=	=	+	+	+	+
10^{-5}M Post-Inf.	=	-	-	-	=	=	=	=	=	=	=	=

The effect of prostaglandin infusion on rate, force and flow was tested statistically by comparing drug effects against within-group controls, and against over-all controls. As heart rate did not change with time, data from the figures was grouped with control, infusion, and post- infusion groups and appropriate t-tests performed. As flow and force fell with time, linear regressions of response with time over the periods 20-60 min, 65-80 min (infusion), and 85-150 minutes (post-infusion) were appropriately tested.

KEY: = indicates no effect + indicates an increase for rate and maintained or increased effect for force and flow - indicates a decrease

Figure 17: The effect of prostaglandin (10^{-7}M) perfusion of isolated rat heart on rate. Prostaglandins were perfused for 20 minutes between minutes 60 and 80 of the perfusion. Each point represents the mean of five hearts.

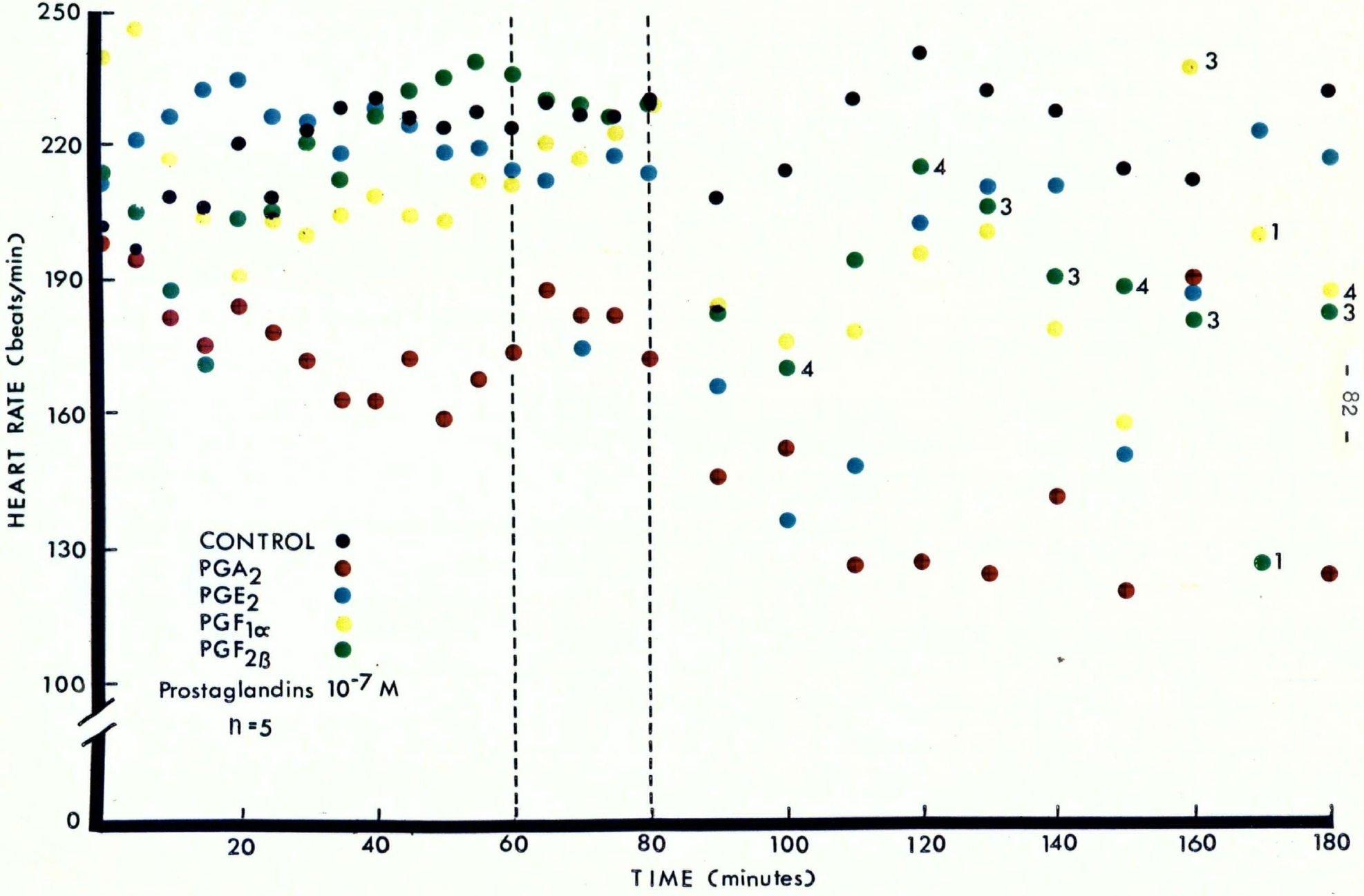

250
220
190
160
130
100
0
HEART RATE (beats/min)
CONTROL
PGA2
PGE2
PGF1α
PGF2β
Prostaglandins 10-7 M
n=5
20
40
60
80
100
120
140
160
180
TIME (minutes)
1
3
4

Figure 18: The effect of prostaglandin (10^{-7}M) perfusion of isolated rat heart on maximal force of contraction. Each heart was tested with 25 g loading tension. Prostaglandins were perfused for 20 minutes between minutes 60 to 80 of the perfusion period. Each point represents the mean of five hearts.

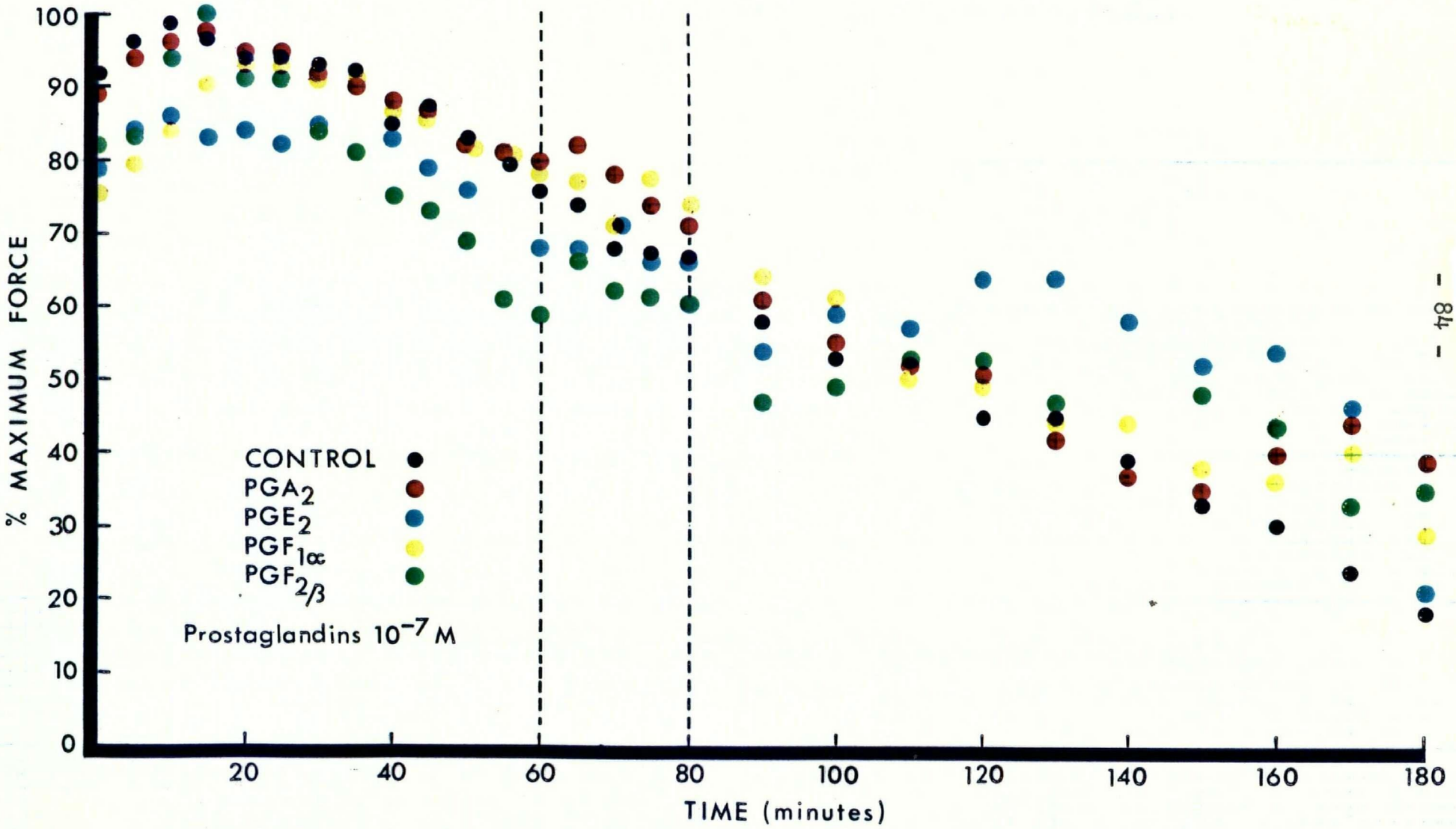

100
90
80
70
60
50
40
30
20
10
0
% MAXIMUM FORCE
CONTROL
PGA2
PGE2
PGF1α
PGF2β
Prostaglandins 10−7 M
20
40
60
80
100
120
140
160
180
TIME (minutes)

Figure 19: The effect of prostaglandin (10^{-7}M) perfusion of isolated rat heart on coronary flow rate. Prostaglandins were perfused for 20 minutes between minutes 60 to 80 of the perfusion period. Each point represents the mean of five hearts.

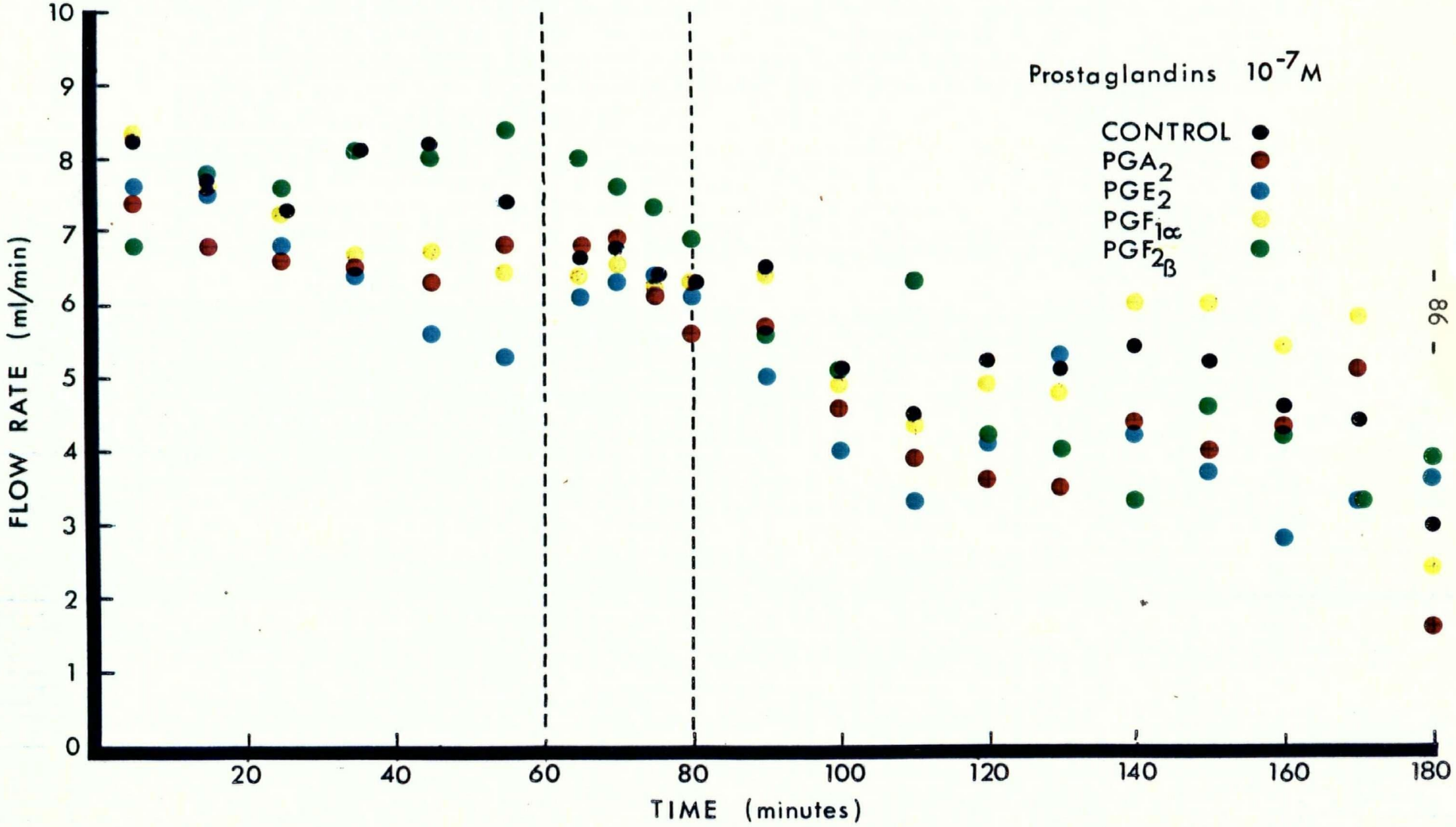

Prostaglandins 10^{-7}M
CONTROL
PGA_2
PGE_2
$PGF_{1\alpha}$
$PGF_{2\beta}$
FLOW RATE (ml/min)
TIME (minutes)
0
1
2
3
4
5
6
7
8
9
10
20
40
60
80
100
120
140
160
180

Figure 20: The effect of prostaglandin (10^{-5}M) perfusion of isolated rat heart on rate. Prostaglandins were perfused for 20 minutes between minutes 60 to 80 of the perfusion period. Each point represents the mean of five hearts.

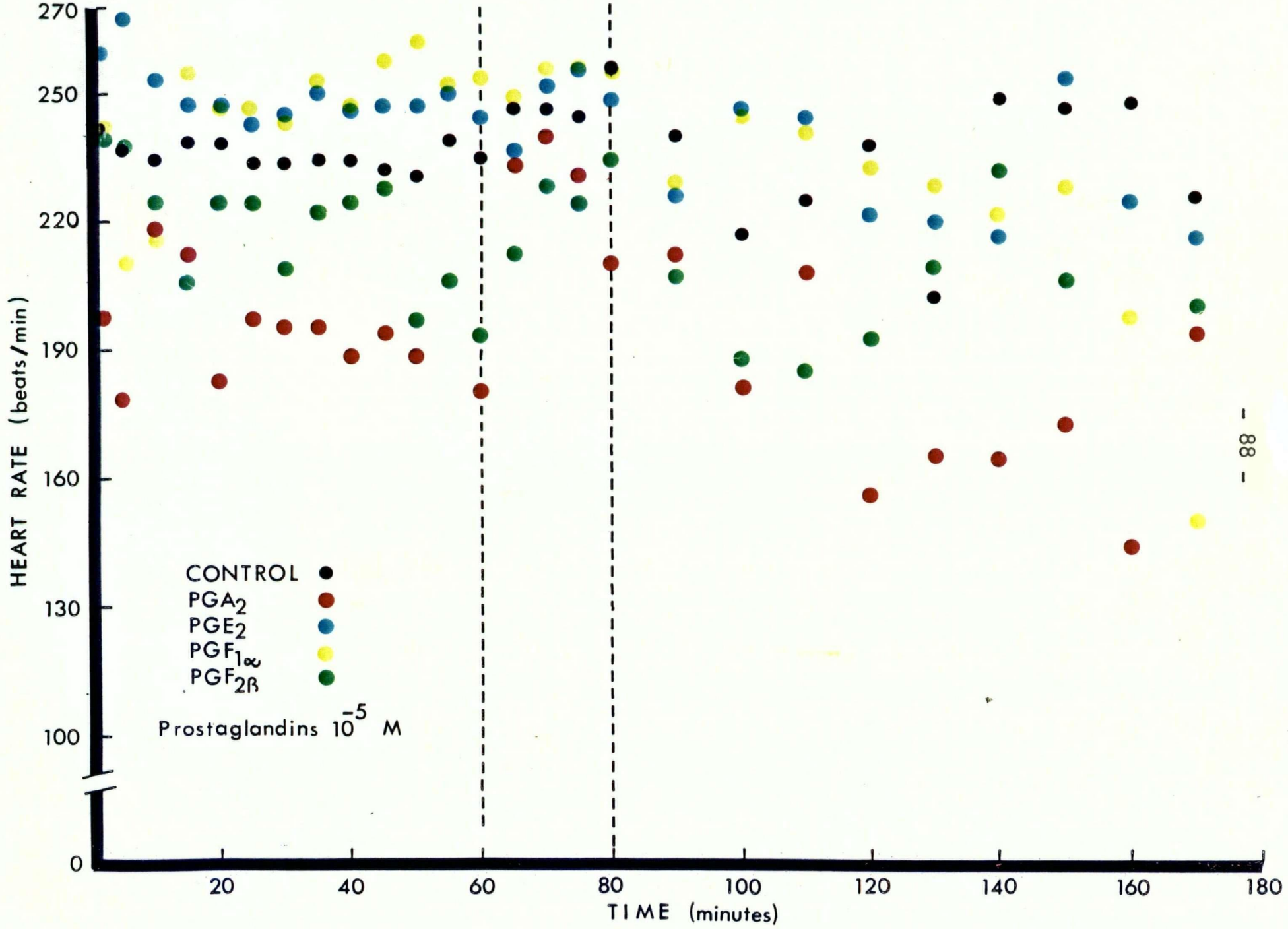

270
250
220
190
160
130
100
0
HEART RATE (beats/min)
CONTROL
PGA2
PGE2
PGF1α
PGF2β
Prostaglandins 10-5 M
20
40
60
80
100
120
140
160
180
TIME (minutes)

Figure 21: The effect of prostaglandin (10^{-5}M) perfusion of isolated rat heart on maximal force of contraction. Each heart was tested with 25 g loading tension. Prostaglandins were perfused for 20 minutes between minutes 60 to 80 of the perfusion period. Each point represents the mean of five hearts.

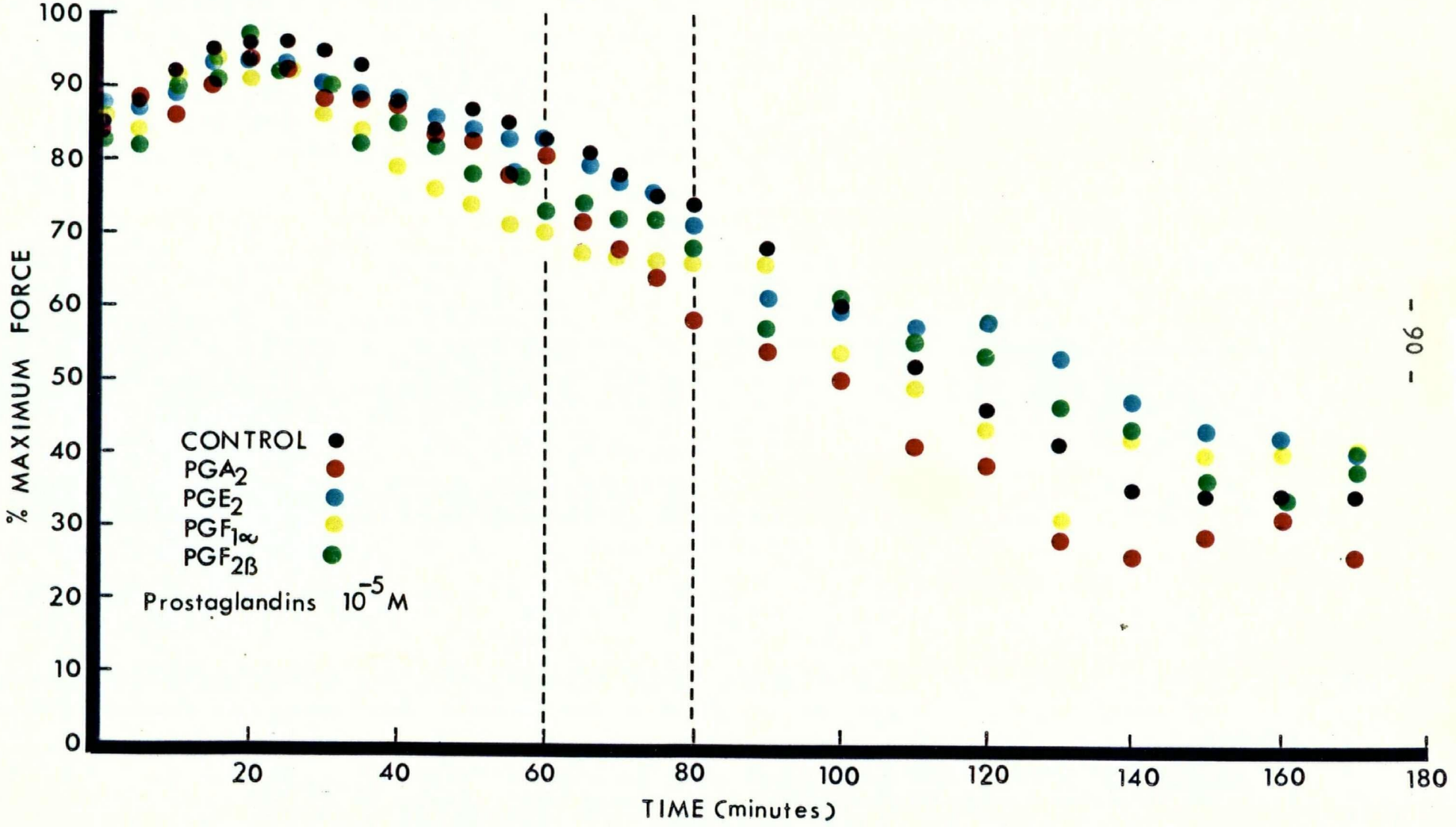
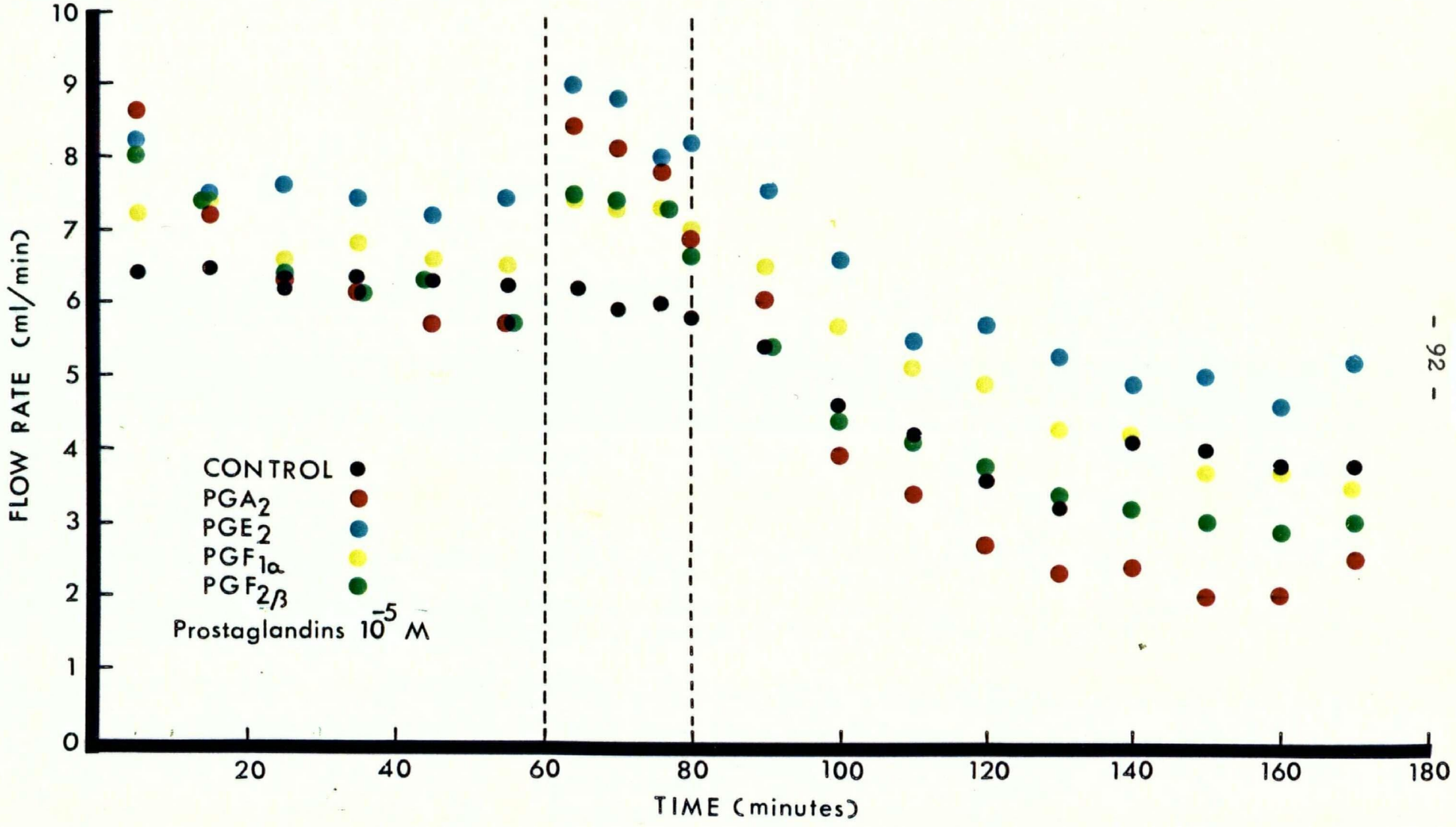

100
90
80
70
60
50
40
30
20
10
0
% MAXIMUM FORCE
CONTROL
PGA2
PGE2
PGF1∝
PGF2ß
Prostaglandins 10-5 M
20
40
60
80
100
120
140
160
180
TIME (minutes)

Figure 22: The effect of prostaglandin (10^{-5}M) perfusion of isolated rat heart on coronary flow rate. Prostaglandins were perfused for 20 minutes between minutes 60 to 80 of the perfusion period. Each point represents the mean of five hearts.


FLOW RATE (ml/min)
10
9
8
7
6
5
4
3
2
1
0
20
40
60
80
100
120
140
160
180
TIME (minutes)
CONTROL
PGA2
PGE2
PGF1α
PGF2β
Prostaglandins 10-5 M

EXPERIMENTAL SECTION III

A) The Effects of Prostaglandins Alone on the Beating Activity of Cultured Heart Cells

Table IV shows the effect of the prostaglandins as well as adrenaline, ouabain, and ethanol alone, on the beating rate of single cells. Ethanol (5 or 10 µl/5 ml of tissue culture media) produced a small increase in beating rate and, due to this small but consistent effect, ethanol alone was used as the control for prostaglandins whereas saline was the control for ouabain and adrenaline. As no changes with exposure time to drug were detected, the results from 10 and 35 minute exposure times were combined. Only prostaglandin $F_{2\alpha}$ produced a marked, statistically significant increase in rate, although others may have produced minimal effects (e.g. D_2 or $F_{1\alpha}$). The reproducibility of the technique and procedures is shown with the repeated experiments using 10^{-5} M prostaglandins A_2, E_2, $F_{1\alpha}$, and $F_{2\beta}$. Values equal to the first experiment were obtained in every case.

Prostaglandin $F_{2\beta}$ seemed to decrease rate compared with ethanol controls although this decrease was not statistically significant. Other prostaglandins may have minimally decreased rate. Adrenaline produced a marked positive chronotropic effect as did ouabain when they were compared with their saline controls.

Figure 23 illustrates the full dose-response curve for the most active chronotropic prostaglandin, $F_{2\alpha}$. The response curve was plotted in two ways: 1) as the mean of rate responses to various concentrations of $F_{2\alpha}$ and 2) as the mean of

TABLE IV

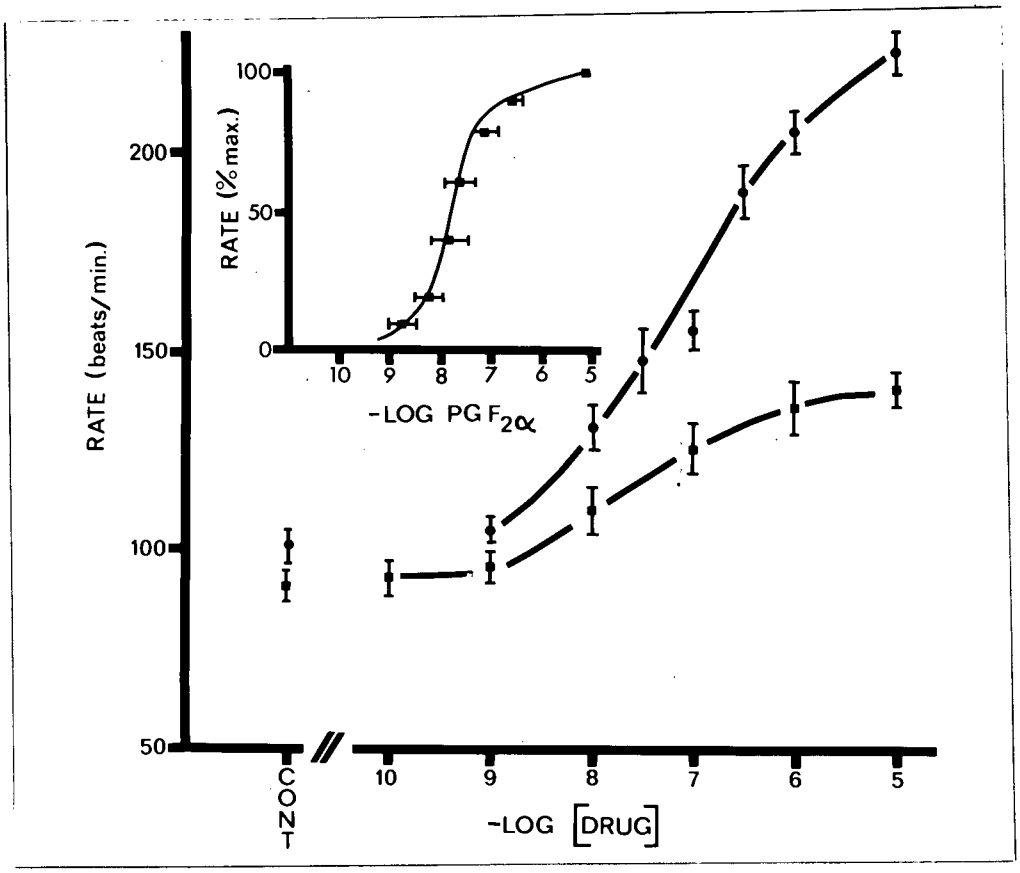
Effect of prostaglandins on the beating rate of single cultured heart cells.

Prostaglandin	10^{-7} M	10^{-5} M	Repeat 10^{-5} M
Ethanol Alone	86 ± 7	92 ± 8	
A_1	76 ± 5	90 ± 4	
A_2	71 ± 4	83 ± 5	77
B_1	80 ± 4	96 ± 6	
B_2	81 ± 7	83 ± 5	
D_1	86 ± 5	100 ± 5	
D_2	96 ± 5	109 ± 5	
E_1	63 ± 4 *	87 ± 5	
2-decarboxy-E_1	81 ± 6	92 ± 7	
E_2	81 ± 5	101 ± 5	99
(15S)-15 CH_3-E_2	83 ± 7	95 ± 7	
$F_{1\alpha}$	86 ± 7	103 ± 7	96
$F_{1\beta}$	85 ± 4	90 ± 5	
$F_{2\alpha}$	122 ± 6 *	130 ± 4 *	
$F_{2\beta}$	69 ± 6	75 ± 5	79
	(beats /minute)		
Saline Control	66 ± 6		
Adrenaline	97 ± 11 **	162 ± 8 ***	
Ouabain	91 ± 7 ***	103 ± 16 *	

* = $p<0.05$ ** = < 0.01 *** = < 0.005

5-6 individual cells per flask from 3-4 different cultures were examined and rates calculated from records. Values for 10 and 35 min exposure to drug did not change; these time periods were therefore pooled. Each value is the mean ± S.E.M. (n=30-36). The repeat column for 10^{-5}M shows mean results obtained in an independent experiment performed, in exactly the same manner, several months after the main experiment. Statistics were calculated with Dunnett's t test.

Figure 23: Dose-response curve to $PGF_{2\alpha}$ and dl-adrenaline. Response curves to both drugs were expressed as the mean responses (beats/min) to the various concentrations at equilibrium. The results for $PGF_{2\alpha}$ were individually recalculated so that the concentration necessary to produce a particular percentile response could be interpolated. Means of such concentrations were then taken (insert). ●------● = dl-adrenaline mean responses; ■----■ = $PGF_{2\alpha}$ mean responses. Each point is the mean of 12 cells ± S.E.M.


RATE (beats/min.)
200
150
100
50
CONT
10
9
8
7
6
5
-LOG [DRUG]
RATE (%max.)
100
50
0
10
9
8
7
6
5
-LOG PG F2α

concentrations required to produce a particular response in individual cells. As expected, the latter plot gave a much more accurate dose-response curve, with a steeper slope, and best represents the response to this prostaglandin (Ariens, 1964). The response to $PGF_{2\alpha}$ can be compared with that of the meaned dose-response curve to dl-adrenaline (also shown in Figure 23). The catecholamine response reached much higher levels and did not reach a maximum even at 10^{-5} M concentration.

The effects of the prostaglandins on rate range (beats/minute) are summarized in Table V. Rate range is an index of the ability of cells to maintain a constant rate as a stable cell can be expected to maintain a more regular rate than an unstable one. Rate range has been found to increase with a number of arrhythmogenic agents (Martinez and Walker, 1977). No changes in this variable were detected with ethanol controls and therefore all prostaglandin values were statistically tested against the appropriate pre-drug controls. None of the prostaglandins statistically significantly changed rate range. Only adrenaline produced a statistically significant increase in this parameter, although $PGF_{2\beta}$ did show a tendency to reduce rate range.

Prostaglandin effects on optical density are shown in Table VI. The optical density of the cell image changes during a contraction cycle as areas of greater or lesser density pass before the photoresistor tubes. Changes in optical density records in cultured heart cells have been reported as corresponding to changes in the force of contrac-

TABLE V

Effect of prostaglandins on the beating rate range of single cultured heart cells.

Prostaglandin	Pre-Drug Control	10^{-7} M (beats/min)	10^{-5} M (10 & 35 min comb)
A_1	28 ± 3	38 ± 5	27 ± 3
A_2	33 ± 5	28 ± 4	23 ± 3
B_1	40 ± 7	39 ± 6	37 ± 7
B_2	36 ± 6	40 ± 6	35 ± 6
D_1	32 ± 5	30 ± 4	31 ± 4
D_2	34 ± 5	36 ± 5	35 ± 4
E_1	25 ± 3	34 ± 4	32 ± 3
2-decarboxy-E_1	37 ± 5	32 ± 3	46 ± 5
E_2	28 ± 5	30 ± 4	22 ± 3
(15S)-15 CH_3-E_2	39 ± 3	35 ± 4	31 ± 4
$F_{1\alpha}$	35 ± 7	38 ± 5	40 ± 5
$F_{1\beta}$	25 ± 3	31 ± 3	28 ± 4
$F_{2\alpha}$	37 ± 5	34 ± 5	30 ± 6
$F_{2\beta}$	33 ± 6	25 ± 4	20 ± 3
Ethanol Control	33 ± 6	41 ± 6	37 ± 6
Adrenaline	32 ± 1	49 ± 8 **	50 ± 7 **
Ouabain	30 ± 5	35 ± 4	31 ± 4

** = $p < 0.01$

Samples were obtained as for TABLE IV and the 10 and 35 minute values pooled. Range is the maximum rate minus the minimum rate occurring in a 20 sec sampling period. Each value was statistically compared with its pre-drug control using the Student t test.

TABLE VI

The effect of prostaglandins on the optical density change with beats in single cultured heart cells.

Sampling of the optical density changes with each beat was the same as that for rate. As optical density changes (in arbritrary units) changes with prostaglandin exposure time, values for 10 and 35 minutes are not pooled and only 10 minute values given. Each value was statistically compared with its pre-drug control using the Student t test.

Prostaglandin	Pre-Drug Control	10^{-7}M	10^{-5}M
Ethanol Alone	22 ± 3	21 ± 3	20 ± 4
A_1	20 ± 7	21 ± 3	20 ± 4
A_2	18 ± 4	18 ± 7	13 ± 2
B_1	26 ± 4	27 ± 6	15 ± 2
B_2	26 ± 5	18 ± 3	13 ± 2
D_1	18 ± 3	16 ± 3	16 ± 3
D_2	16 ± 2	15 ± 2	14 ± 3
E_1	14 ± 2	17 ± 3	17 ± 5
2-decarboxy-E_1	15 ± 2	11 ± 3	8 ± 2 **
E_2	27 ± 6	18 ± 5 *	13 ± 2 *
(15S)-15 CH_3-E_2	15 ± 5	11 ± 2 **	14 ± 2 *
$F_{1\alpha}$	30 ± 4	21 ± 3 *	17 ± 2 ***
$F_{1\beta}$	21 ± 3	20 ± 3	14 ± 3 *
$F_{2\alpha}$	17 ± 3	15 ± 2	16 ± 2
$F_{2\beta}$	12 ± 2	19 ± 3 *	12 ± 2
Adrenaline	16 ± 3	30 ± 6 *	14 ± 2
Ouabain	13 ± 2	18 ± 4	13 ± 3

* = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.005$

tion, although such a proposition has still to be fully evaluated (Butcher and Kolb, 1962; Okarma and Kalman, 1971). Nevertheless, density could be measured with some precision (as witnessed by the small S.E.M.s) and it changed in the presence of prostaglandins.

In the presence of 10^{-7} M prostaglandins, an overall tendency for changes in optical density to decrease with exposure time was noted; therefore, the results from 10 minutes only are given. Values fell with 10^{-7} M prostaglandins over the 35 minute drug exposure time (35 minute value lower or equal to 10 minute value in 11 of 14 cases). Optical density changes with 10^{-5} M prostaglandins did not fall with exposure time. Prostaglandins A_1, B_1, 2-decarboxy-E_1, E_2, (15S)-15 CH_3-E_2, $F_{1\alpha}$, and $F_{1\beta}$ all decreased the optical density record. Only the negative chronotropic prostaglandin, $F_{2\beta}$, produced a statistically significant increase in optical density change. Adrenaline increased the optical density record at 10^{-7} M, and the increase returned to control levels at 10^{-5} M, possibly as a result of the marked positive chronotropic response seen at high concentrations. No significant change was seen with ethanol controls, whereas possible changes with ouabain are clouded by the increasing dysrhythmias seen at higher concentrations (Martinez and Walker, 1977).

The optical density changes were also electronically differentiated to give first derivative (i.e. dOD/dt) for, if optical density gave an index of inotropism it was expected that this second parameter would give a second index analogous to dp/dt. The derivative associated with contraction, and not

TABLE VII

Effect of prostaglandins on the time derivative $\frac{d \text{ optical density}}{dt}$ of optical density changes

Prostaglandin		Pre-Drug Control	10^{-7} M (10 and 35 min comb)	10^{-5} M (10 and 35 min comb)
Ethanol Alone		12 ± 3	9 ± 2	12 ± 3
A_1	r	13 ± 5	8 ± 2	5 ± 1 *
A_2		14 ± 3	14 ± 4	14 ± 3
B_1	r	7 ± 3	9 ± 2	8 ± 2
B_2	r	13 ± 5	7 ± 2	4 ± 1 *
D_1		10 ± 3	5 ± 1 *	9 ± 2
D_2		8 ± 3	11 ± 4	10 ± 3
E_1		13 ± 6	6 ± 2	9 ± 3
2-decarboxy-E_1	r	8 ± 1	13 ± 4	8 ± 1
E_2	r	15 ± 5	16 ± 4	7 ± 3 *
(15S)-CH_3-E_2	r	12 ± 2	7 ± 1 **	5 ± 1 ***
$F_{1\alpha}$	r	14 ± 3	14 ± 4	11 ± 2
$F_{1\beta}$	r	11 ± 4	8 ± 2	11 ± 3
$F_{2\alpha}$		11 ± 3	11 ± 3	7 ± 2
$F_{2\beta}$	i	6 ± 2	17 ± 5 *	10 ± 2
Adrenaline		11 ± 5	17 ± 6	13 ± 4
Ouabain		8 ± 2	13 ± 3	10 ± 3

* = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.005$

r = PGs reducing optical density
i = PGs increasing optical density

Since no effect of exposure time was noted results from 10 and 35 min combined are given for both concentrations tested. Each value was statistically compared with its pre-drug control using the Student t test.

TABLE VIII

Effect of prostaglandins on the percentage of single cultured heart cells spontaneously beating.

The percentage of cells beating was determined from five samples, of ten cells each, in three different cultures. The two concentrations of prostaglandins were added and the percentage of cells beating assessed after 10 minutes exposure. Figures are the mean ± S.E.M. of differences from control percentages of cells beating ($n = 10-16$). Statistics were calculated with Dunnett's t test.

Prostaglandin	10^{-7} M (Percentage diff. from pre-drug)	10^{-5} M
Ethanol Alone	-3.0 ± 4.0	-10.0 ± 4.5
A_1	-4.5 ± 5.0	-7.0 ± 9.0
A_2	-3.5 ± 6.5	-8.5 ± 8.9
B_1	-0.5 ± 9.0	-6.5 ± 9.0
B_2	-6.5 ± 6.4	+4.5 ± 7.4
D_1	+5.0 ± 4.9	-5.0 ± 7.0
D_2	-12.0 ± 5.0	-11.0 ± 7.0
E_1	+0.8 ± 5.1	-6.3 ± 7.4
2-decarboxy-E_1	-3.0 ± 4.5	-12.0 ± 3.8
E_2	-4.6 ± 5.0	-17.7 ± 3.0
(15S)-15 CH_3-E_2	-6.7 ± 6.5	-6.7 ± 5.5
$F_{1\alpha}$	-7.2 ± 4.0	-32.0 ± 6.0 *
$F_{1\beta}$	0 ± 8.8	-3.0 ± 5.5
$F_{2\alpha}$	-15.5 ± 5.6	-33.0 ± 5.5 *
$F_{2\beta}$	+4.0 ± 6.5	-10.0 ± 7.0

* = $p < 0.05$

that associated with relaxation, was measured and the effects of the drugs are given in Table VII. No effect of exposure time was noted; results from 10 and 35 minutes combined are therefore given. As with optical density changes, there was an overall tendency for the prostaglandins to reduce this index at both concentrations. Excluding $PGF_{2\beta}$, the 10^{-7} M values were equal to or lower than controls in 9 out of 13 cases, as compared with 11 out of 13 cases with 10^{-5} M. Some of the prostaglandins producing marked decreases in optical density changes also statistically significantly reduced the first derivative (PGs A_1, B_2, E_2, (15s)-15 CH_3-E_2). As with changes in optical density, only prostaglandin $F_{2\beta}$ statistically significantly increased the derivative. Both adrenaline and ouabain showed tendencies to increase the derivative of optical density, but this was not statistically significant. The ouabain results were complicated by increasing dysrhythmias.

During experiments with the prostaglandins, it was noted that some appeared to reduce the percentage of spontaneously beating cells. The effect of the various prostaglandins on the per cent of cells beating spontaneously is given in Table VIII. At 10^{-5} M, most of the prostaglandins had no effect, except for prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ which reduced the percentage of cells beating spontaneously. Reductions were dose-dependent, as is shown by a comparison of the 10^{-7} M with the 10^{-5} M results. Ethanol alone produced a small, but consistent, effect and was therefore used as the control for all prostaglandins.

B) The Effect of Prostaglandins on Abnormalities of Beating in Cultured Heart Cells Induced by a Variety of Arrhythmogenic Agents

Introduction

To test for a possible "cyto-protective" action of prostaglandins, experiments were devised to determine whether or not prostaglandins protected against abnormalities of beating behavior induced in cultured neonatal rat heart cells by a variety of arrhythmogenic agents. The effect of each of the agents on beating behavior had to be determined initially in order to choose suitable times and doses for the cyto-protection studies.

The Action of Ouabain, Ionic Manipulation, Dinitrophenol, and Anoxia on Cultured Heart Cells

Ouabain

The effect of ouabain (5×10^{-5} M) on rate and rate range/rate versus time is shown in Figure 24. Rate increased to 200 per cent at 2 minutes, and to a sustained maximum of 310 per cent after 10 minutes. Rate range/rate (an objective measure of arrhythmic activity) first decreased to 80 per cent of control at 1 minute, then increased to 150 per cent of control at 4 minutes and 200 per cent of control at 10 minutes. Equilibrium effects were obtained within 10-14 minutes.

Figures 25 and 26 show the effects of different concentrations of ouabain on the objective (rate, rate range/rate, per cent beating) and the subjective (per cent arrhythmic, mean score for arrhythmias) indices of arrhythmias in single cells.

Figure 24: The effect of ouabain 5×10^{-5} M on rate and rate range/rate versus time. The initial control measurement was taken as 100%. Mean values for 7 cells are shown.

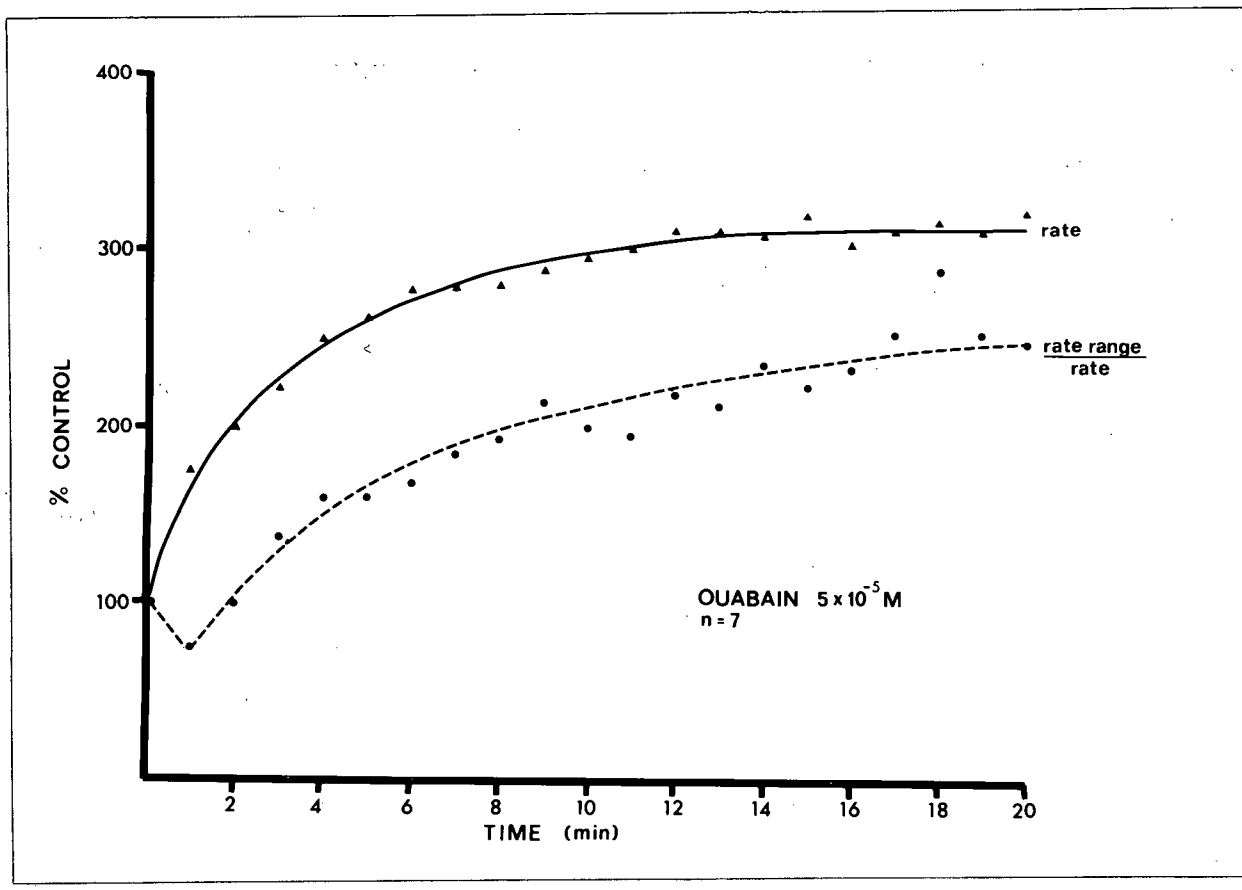
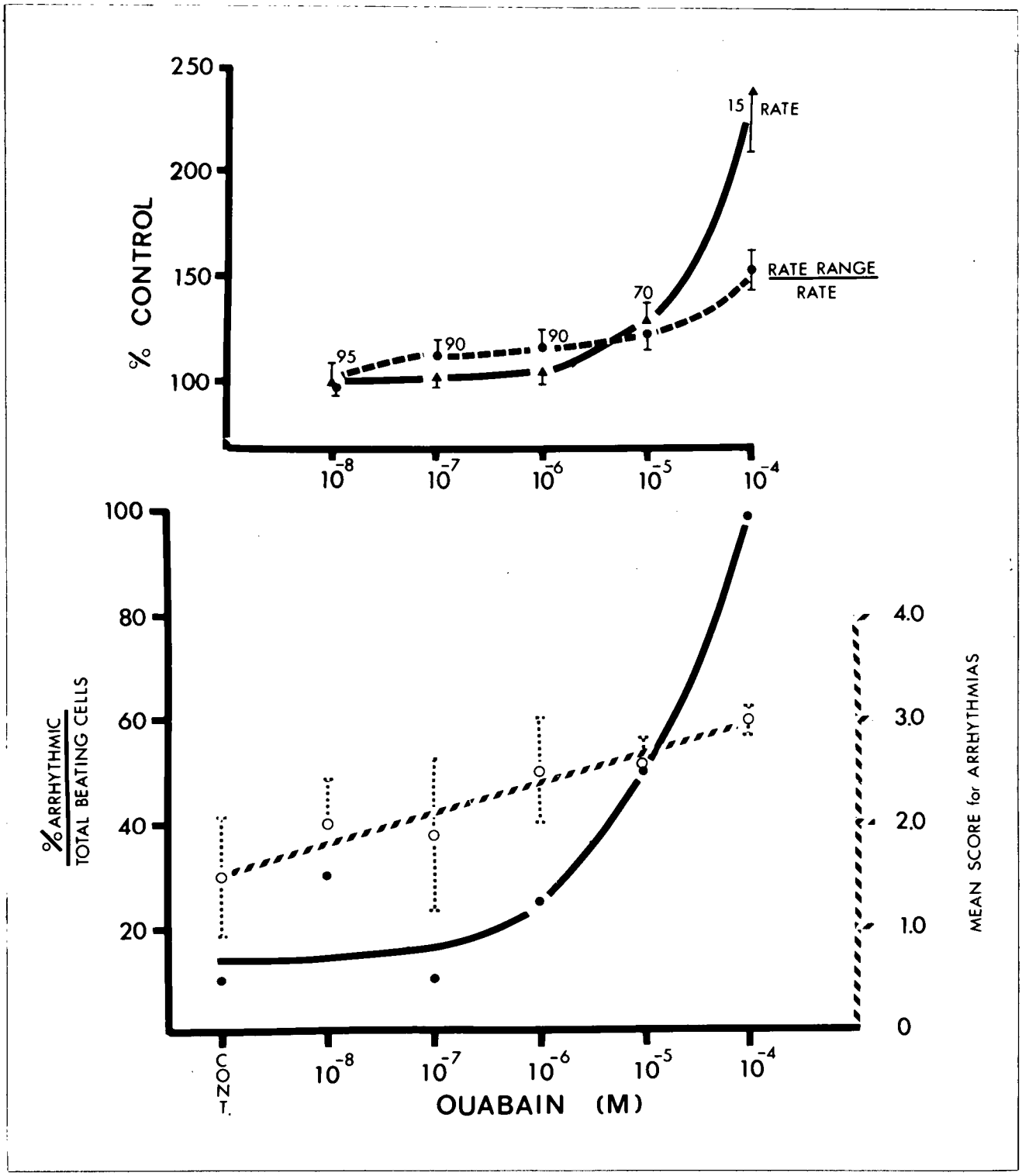

400
300
200
100
% CONTROL
rate
rate range / rate
OUABAIN 5 x 10⁻⁵ M
n = 7
2
4
6
8
10
12
14
16
18
20
TIME (min)

Figure 25: The effect of ouabain on rate and rate range/rate in cultured heart cells. The initial control measurement was taken as 100%. The number of cells beating out of 100 sampled is shown by the small numbers next to the data points. Mean values for 10 cells ± S.E.M. are shown.

Figure 26: The effect of ouabain on the percent of cells arrhythmic/total cells beating, and the mean subjective score of arrhythmias. Mean values for 10 cells ± S.E.M. are shown.


250
200
150
100
% CONTROL
15
RATE
RATE RANGE
RATE
70
95
90
90
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
100
80
60
40
20
% ARRHYTHMIC
TOTAL BEATING CELLS
4.0
3.0
2.0
1.0
0
MEAN SCORE for ARRHYTHMIAS
CONT.
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
OUABAIN (M)

All values were determined 10 minutes after the addition of each drug dose. Increasing concentrations increased all indices, but with different dose-response relationships. Rate increased with doses between 10^{-7} and 10^{-4} M to reach 225 per cent of control at 10^{-4} M (Figure 25).

The number of cells beating out of 100 sampled decreased rapidly above 10^{-5} M. The per cent of cells beating arrhythmically increased rapidly between 10^{-7} and 10^{-4} M, to a maximum of 100 per cent. The mean score for severity of arrhythmias increased linearly throughout the dose range (Figure 26). From these data an arrhythmogenic concentration of 30 uM was chosen for studying the effects of prostaglandins on the induced beating abnormalities.

Calcium

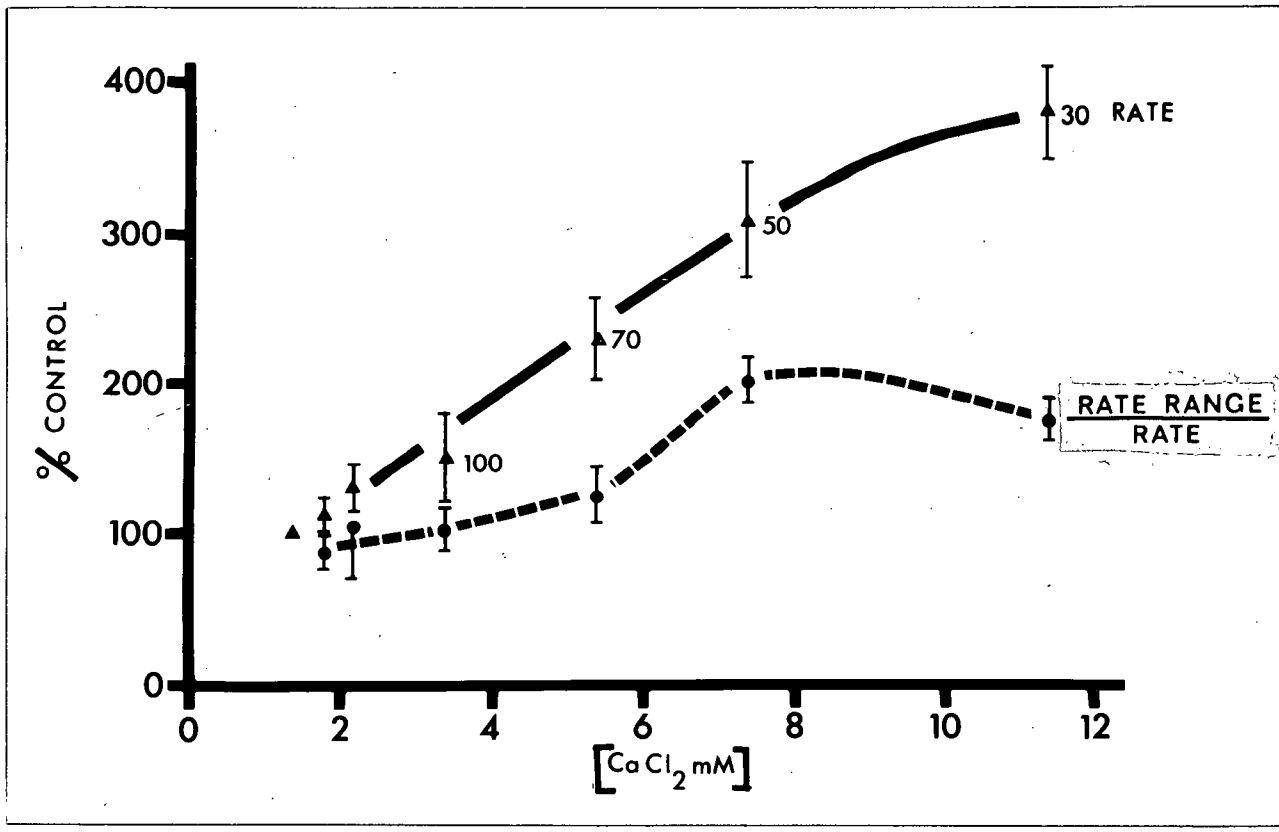
Calcium chloride (2 - 12 mM) markedly increased rate (to 380% $\pm$ 30 S.E.M. of control at 12 mM); rate range/rate increased to a maximum of 200 per cent of control at 8 mM all at 10 min, Fig 27). The percentage of arrhythmic cells increased from 15 per cent at 2 mM to 100 per cent at 12 mM, while mean arrhythmia score increased from 0.5 $\pm$ 0.2 at 2 mM to 3.0 $\pm$ 0.2 at 12 mM (Figure 28). From this data a concentration of 6.0 mM Ca^{++} was chosen for the prostaglandin study.

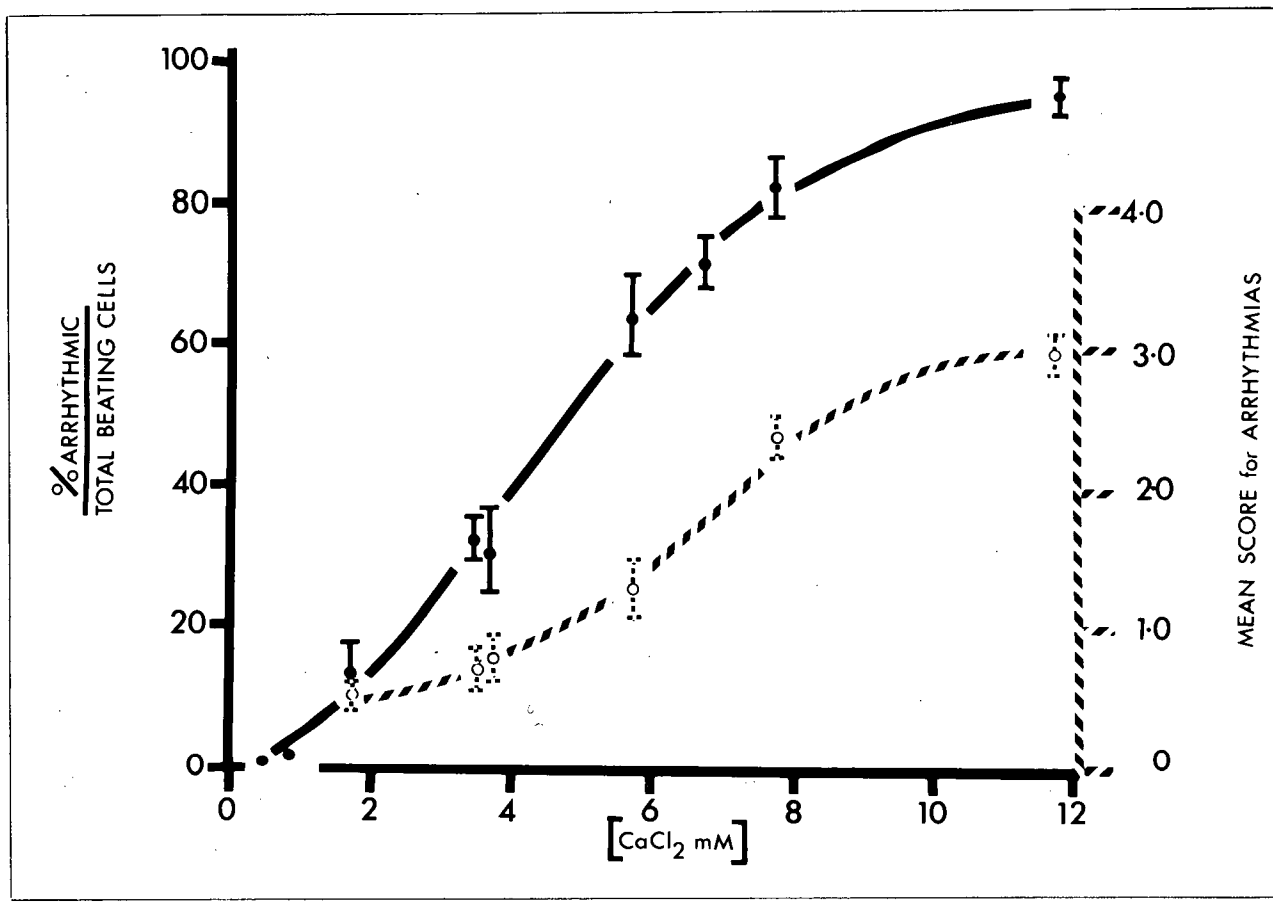
Potassium

Elevation of K^+ decreased rate (35% $\pm$ 7 of control at 10 mM) and then increased it (80% of control at 24 mM) (Figure 29). Rate range/rate increased to 300 per cent of control at 10 mM and then fell to 160 per cent at 28 mM K^+. Subjective measurements of arrhythmias on the scale used for Ca^{++} and

Figure 27: The effect of calcium on rate and rate range/rate in cultured heart cells. The initial control measurement was taken as 100%. The number of cells beating out of 100 sampled is shown by the small numbers next to the data points. Mean values for 10 cells ± S.E.M. are shown.

Figure 28: The effect of calcium on the per cent of cells arrhythmic/total cells beating, and the mean subjective score for arrhythmias. Mean values for 10 cells ± S.E.M. are shown.


400
300
200
100
0
% CONTROL
0
2
4
6
8
10
12
[Ca Cl2 mM]
30 RATE
50
70
100
RATE RANGE
RATE


100
80
60
40
20
0
% ARRHYTHMIC
TOTAL BEATING CELLS
0
2
4
6
8
10
12
[CaCl2 mM]
4·0
3·0
2·0
1·0
0
MEAN SCORE for ARRHYTHMIAS

ouabain were not appropriate for K^+ owing to a lack of a distinct pattern of arrhythmic changes while the number of cells beating decreased with increasing K^+ concentrations. A concentration of 8 mM was chosen from these data.

Dinitrophenol

Cells responded rapidly to dinitrophenol (DNP) 2×10^{-4}M with rate falling to 50 per cent of control after 3 minutes exposure and rising to a stable 80 per cent of control after 8 minutes; rate range/rate rose to 550 per cent of control and then fell to a stable 230 per cent of control (Figure 31). The effect of different concentrations of DNP at 10 minutes is shown in Figure 30. Maintenance of beating rate became more erratic in the presence of DNP. Subjective arrhythmias resembled those with K^+ in that they were not pronounced and did not mimic those seen with ouabain and Ca^{++}.

The Effect of Anoxia on Cell Beating Behavior

Anoxia (100 per cent N_2 atmosphere) for periods of up to 60 minutes had little effect on the per cent of cells beating rhythmically (solid line) or on the per cent of cells beating arrhythmically out of the total number of cells beating (dotted line) (Figure 32). Prewarmed and moistened 100 per cent nitrogen was passed through a tissue culture flask for periods of up to 60 minutes. The pO_2 of the media fell to 0 mm Hg as measured by an oxygen electrode after 15 minutes.

Establishment of Suitable Concentrations of Lidocaine and Quinidine

For want of suitable comparison drugs for the study of prostaglandin actions on beating behavior abnormalities induc-

Figure 29: The effect of added potassium concentration on rate and rate range/rate in cultured heart cells. The initial control measurement was taken as 100%. Mean values for 20 cells ± S.E.M. are shown. The number of cells beating out of 100 sampled is shown by the small figures beside the data points.

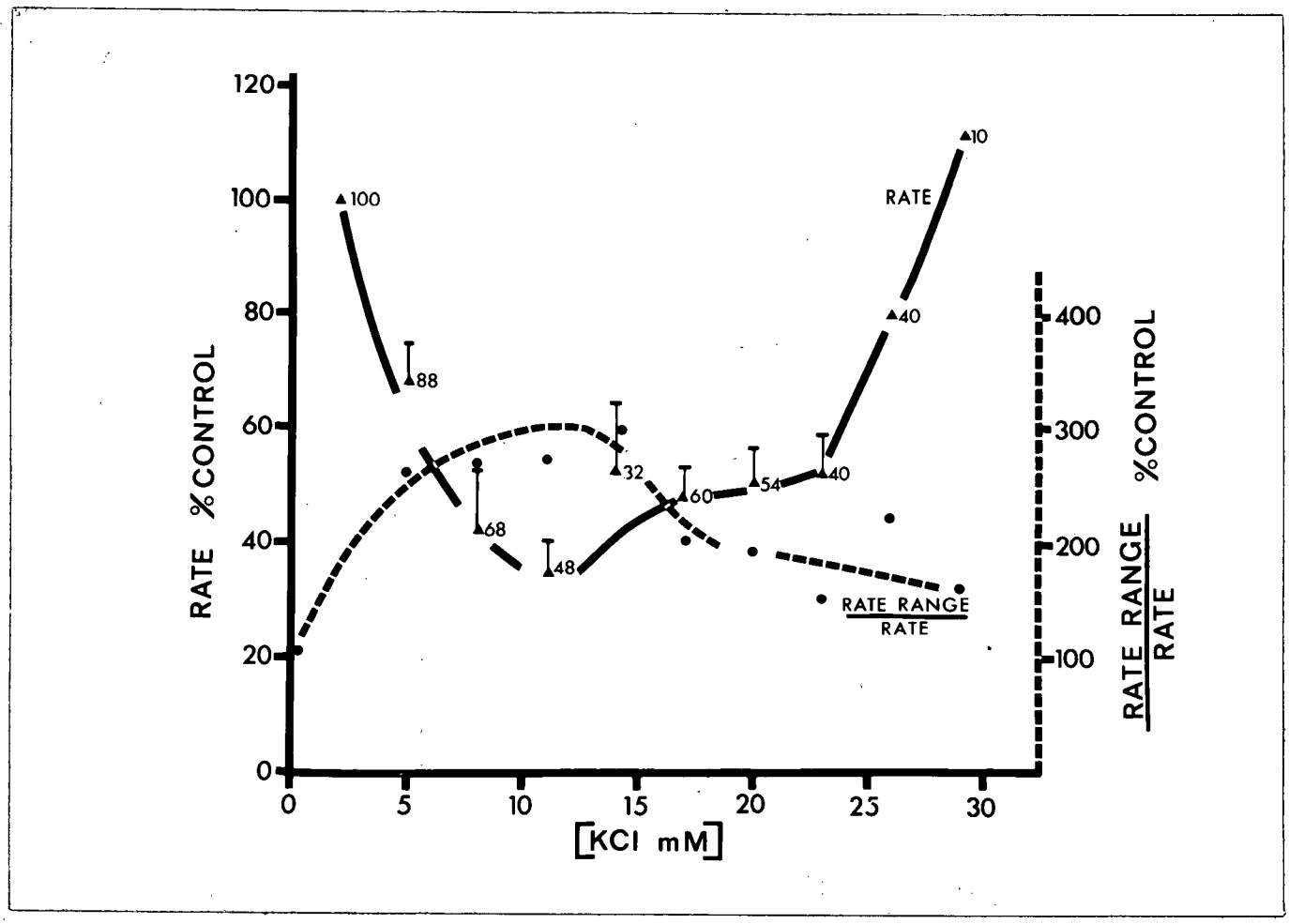
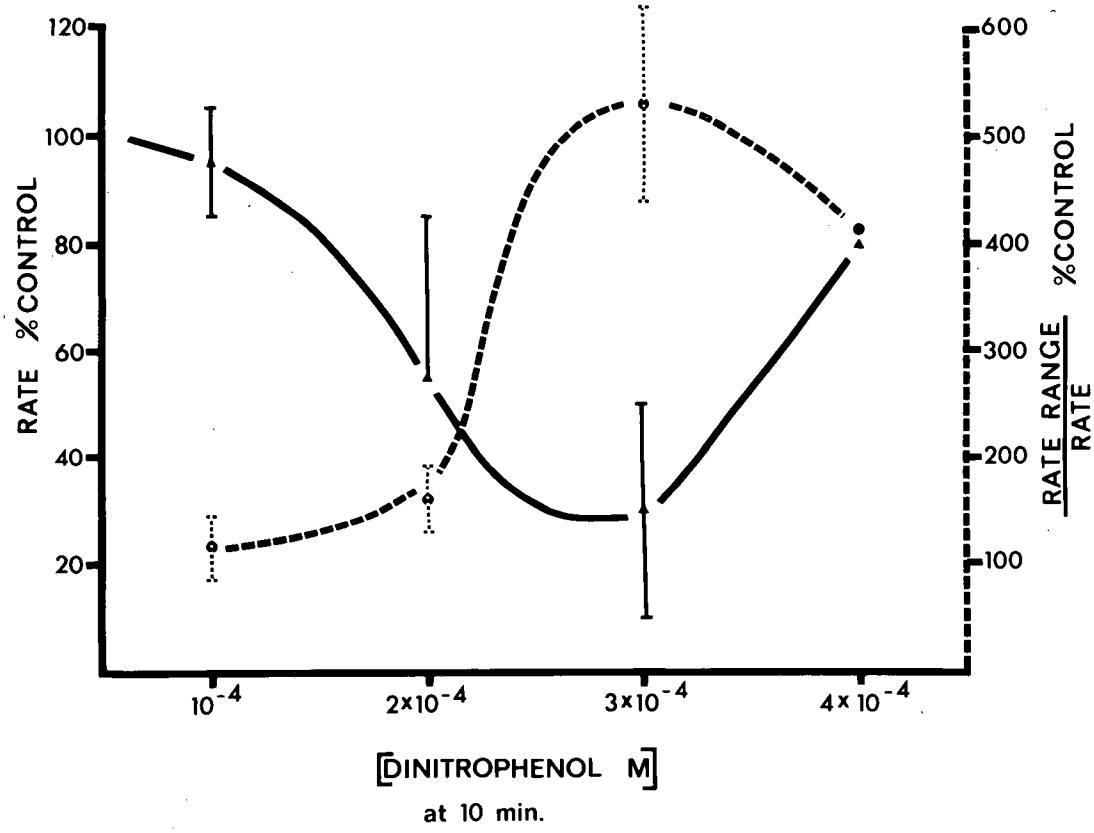

120
100
80
60
40
20
0
RATE %CONTROL
▲100
▲88
▲68
▲48
▲32
▲60
▲54
▲40
▲40
▲10
RATE
RATE RANGE
RATE
400
300
200
100
RATE RANGE / RATE %CONTROL
0
5
10
15
20
25
30
[KCl mM]

Figure 30: The effect of dinitrophenol concentration on rate (beats per minute) and rate range/rate in single cultured rat heart myoblasts. Mean values for 4 cells ± S.E.M. are shown.

Figure 31: The effect of dinitrophenol 2 x 10^{-4} M on rate and rate range/rate versus time in single cultured rat heart myoblasts. Mean values for 4 cells ± S.E.M. are shown.


RATE %CONTROL
120
100
80
60
40
20
600
500
400
300
200
100
RATE RANGE / RATE %CONTROL
10⁻⁴
2x10⁻⁴
3x10⁻⁴
4x10⁻⁴
[DINITROPHENOL M]
at 10 min.

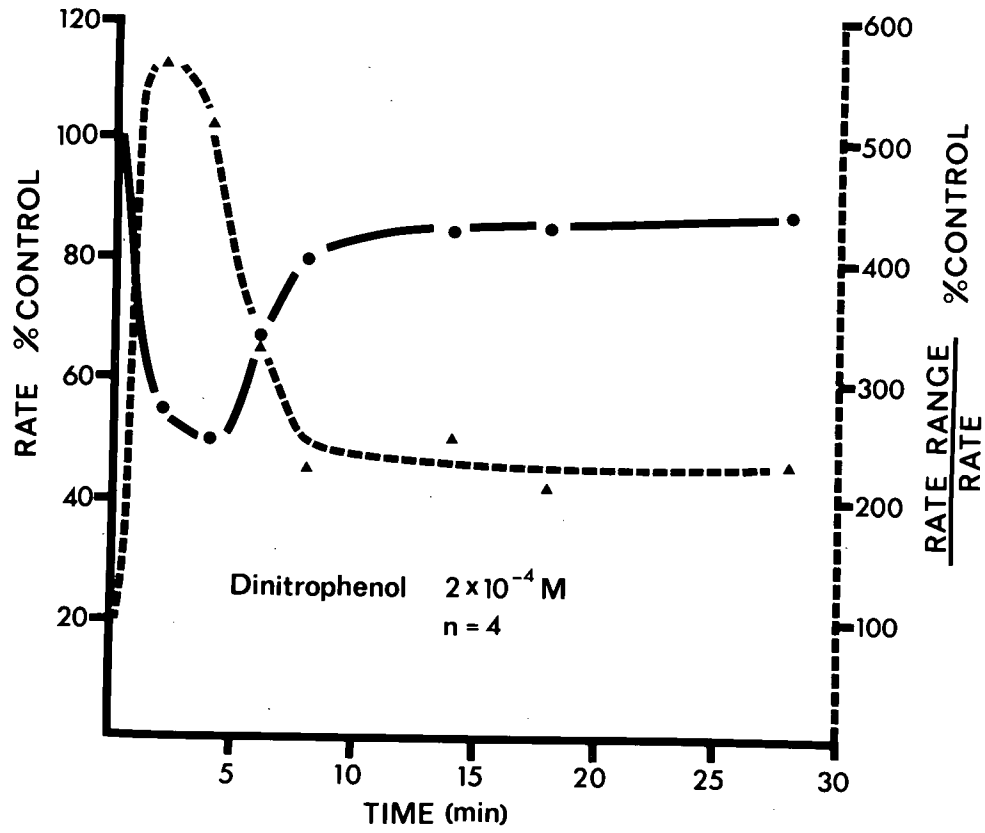
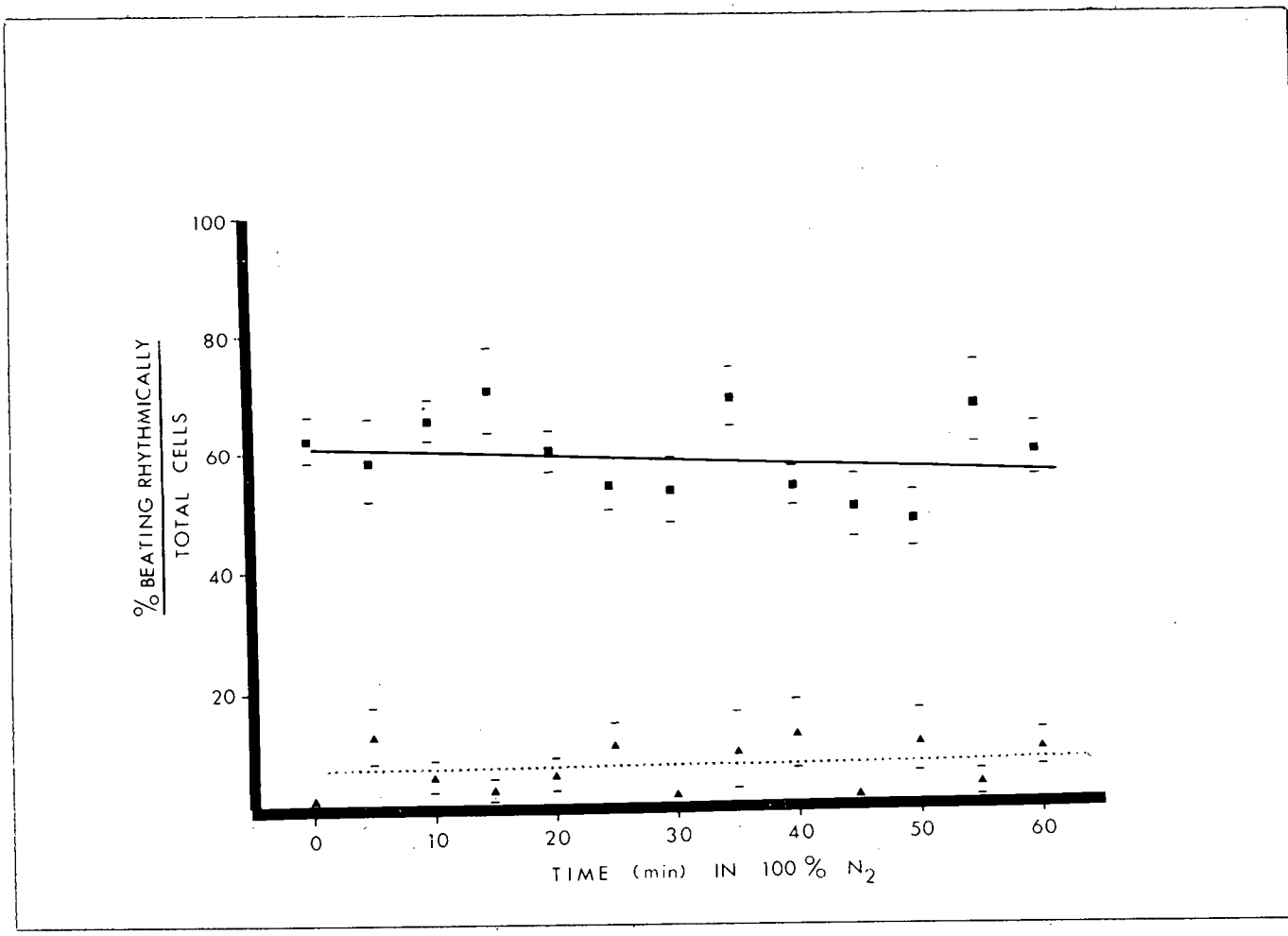

RATE %CONTROL
120
100
80
60
40
20
600
500
400
300
200
100
RATE RANGE / RATE %CONTROL
Dinitrophenol 2x10⁻⁴ M
n = 4
5
10
15
20
25
30
TIME (min)

Figure 32: The effect of anoxia on the percent of cells beating rhythmically/total cells (■————), and the percent of cells beating arrhythmically/total cells beating (▲- - - - - - - - -). Mean values for 10 cells are shown ± S.E.M.


% BEATING RHYTHMICALLY / TOTAL CELLS
100
80
60
40
20
0
10
20
30
40
50
60
TIME (min) IN 100 % N_2

ed by the previously discussed arrhythmogenic agents, it was decided to use two standard antiarrhythmics, namely lidocaine and quinidine. As both agents at high enough concentrations totally abolished cell beating, initial dose-response studies were performed in order to choose a suitable concentration.

Lidocaine and quinidine both stopped cells beating (Figure 33). The ED 50's were 10^{-6} and 10^{-4} M for quinidine and lidocaine respectively. Threshold values were 2×10^{-7} and 2×10^{-5} M. Parallel log-dose-response curves were observed. Exposure time for lidocaine and quinidine was 10 minutes.

Cyanea Toxin

In addition to examining the effects of ouabain, Ca^{++}, K^{+}, DNP, and anoxia on the production of beating abnormalities in cultured heart cells the effects of a cardiotoxic substance from the jellyfish Cyanea capillata were also studied. As previously discussed, the mechanism of action of this toxin is partially understood. It was felt that with little further study this toxin could be used as a tool to produce discrete, understood damage to cultured heart cells. The effect of prostaglandins against such damage could then be investigated.

The Effect of Cyanea Toxin on Cultured Heart Cells

Cultured Heart Cell Contractile Activity

Figure 34 shows the effect of 28 ng (Lowry protein) of Cyanea toxin per ml of culture media on a heart cell. An initial period during which the toxin produced no effect (0 to 10 min) was followed by a period of rapid increase in beating

Figure 33: The effect of quinidine and lidocaine concentration on the per cent of cells not beating. Mean values for 10 cells are shown.

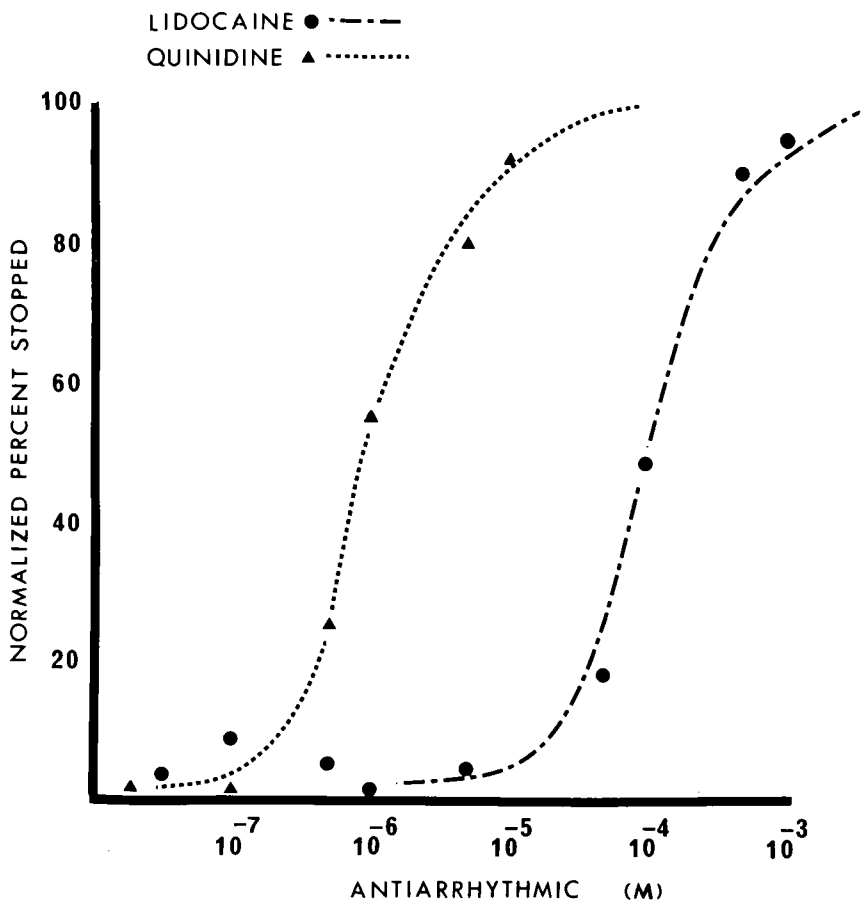

LIDOCAINE ●
QUINIDINE ▲
NORMALIZED PERCENT STOPPED
100
80
60
40
20
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
ANTIARRHYTHMIC (M)

Figure 34: The response of a single cultured heart cell to Cyanea toxin-containing material. A single heart cell was monitored before and after addition of 28 ng Cyanea toxin per ml at time zero. Optical density changes (with contractions), in a television image of the cell were measured by photo-resistors and recorded together with the electronically-derived beat-to-beat rate (beats per minute) and the first derivative (dOD/dt) of the density changes. Failure to exactly reposition the photoresistors at each time sample accounts for some of the optical density changes. Records were sampled at the times shown at a chart speed indicated by the 5 and 1 sec markers. Chart speed was increased at the 50 min sampling period.

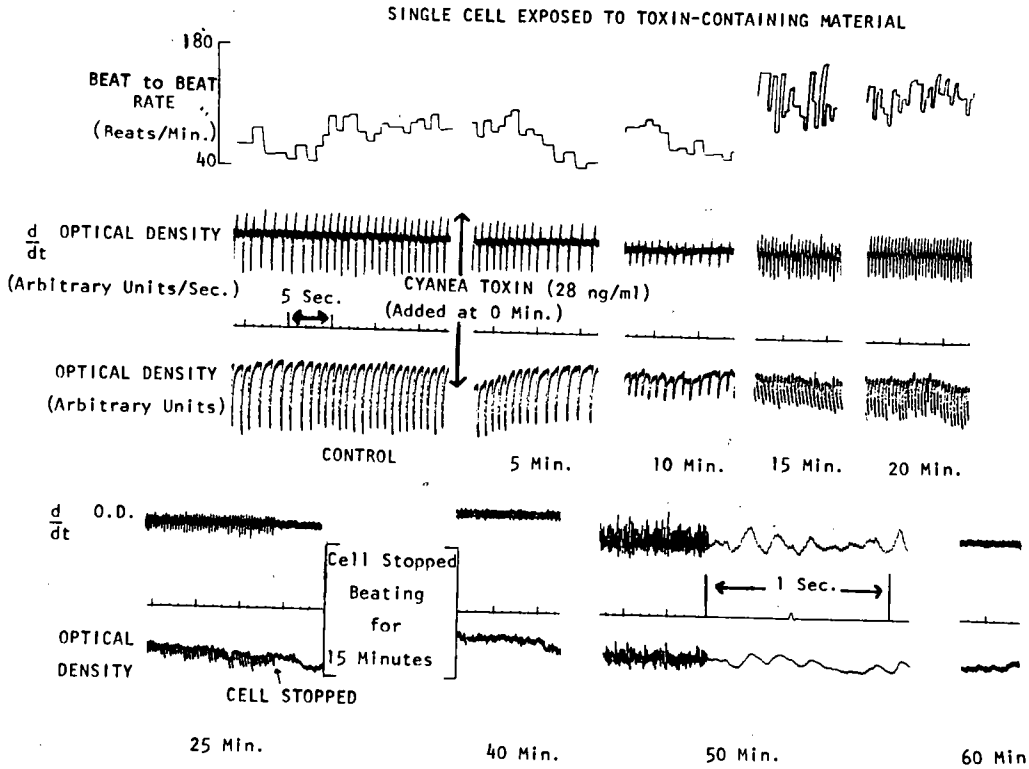

SINGLE CELL EXPOSED TO TOXIN-CONTAINING MATERIAL
180
BEAT to BEAT RATE
(Beats/Min.)
40
d/dt OPTICAL DENSITY
(Arbitrary Units/Sec.)
5 Sec.
CYANEA TOXIN (28 ng/ml)
(Added at 0 Min.)
OPTICAL DENSITY
(Arbitrary Units)
CONTROL
5 Min.
10 Min.
15 Min.
20 Min.
d/dt O.D.
Cell Stopped Beating for 15 Minutes
1 Sec.
OPTICAL DENSITY
CELL STOPPED
25 Min.
40 Min.
50 Min.
60 Min.

TABLE IX

Effect of Cyanea toxin-containing material on the time to various arrhythmic events in cultured single heart cells.

Conc of Cyanea toxin-containing material (ng/ml)	Time to dormant period (min)	Time to onset of fibrillation (min)	Time to final cessation (min)
28	25 ± 3	44 ± 2	74 ± 7
110	8 ± 1	21 ± 4	45 ± 10
440	6 ± 1	12 ± 2	25 ± 8

All figures are the mean and S.E.M. of 6 to 8 determinations on single cells from different cultures of myoblasts. The time to a particular event for a single cell was found from inspection of records of beating activity.

rate (15 to 25 min) before cellular fibrillation (40 to 45 min) and the final cessation of all beating activity at 60 min. Fibrillatory activity is shown at a faster chart speed in the 50 minute sample period. This sequence of events was found to be the same for all single cells and the time to each phase was dose-dependent (Table IX).

A dose-dependent increase in beating rate (expressed as a percentage of control) can be seen in Figure 35. From this figure it can also be seen that there was a dose-related decrease in the number of cells beating (due to individual cells reaching the dormant period) with time. At no time during these experiments was gross cellular disruption or vacuolization observed in any of the toxin-treated cells.

The induction of arrhythmias by various doses of Cyanea toxin is illustrated in Figures 36 - 38. In Figure 36 the proportion of cells beating arrhythmically with time can be seen to depend on Cyanea toxin concentration. A toxin concentration of 440 ng per ml induced all beating cells to beat arrhythmically within 10 minutes, whereas 24 and 56 minutes exposure was required for 110 and 28 ng per ml, respectively.

The percentage of total cells beating rhythmically initially increased on exposure to toxin before rapidly decreasing to zero at doses of 440 and 110 ng per ml (Figure 37). No initial increase in those beating rhythmically was seen at the lowest toxin concentration (28 ng per ml). The time required to reach 0 per cent beating rhythmically for 440, 110, and 28 ng per ml was dose-dependent (12, 24, and 44 minutes, respectively).

Figure 35: The effect of Cyanea toxin-containing material on the beating rate of single cultured heart cells. Cyanea toxin (at the concentrations of 28, 110, and 440 ng/ml) was added to cultures at time zero. Six cells were examined at 2 minute intervals for each concentration of toxin. Rate increases are expressed as percentage increases over control rates. Each point is the mean of the number of cells still beating at that time. The number of cells still beating is indicated by the figure next to a point.

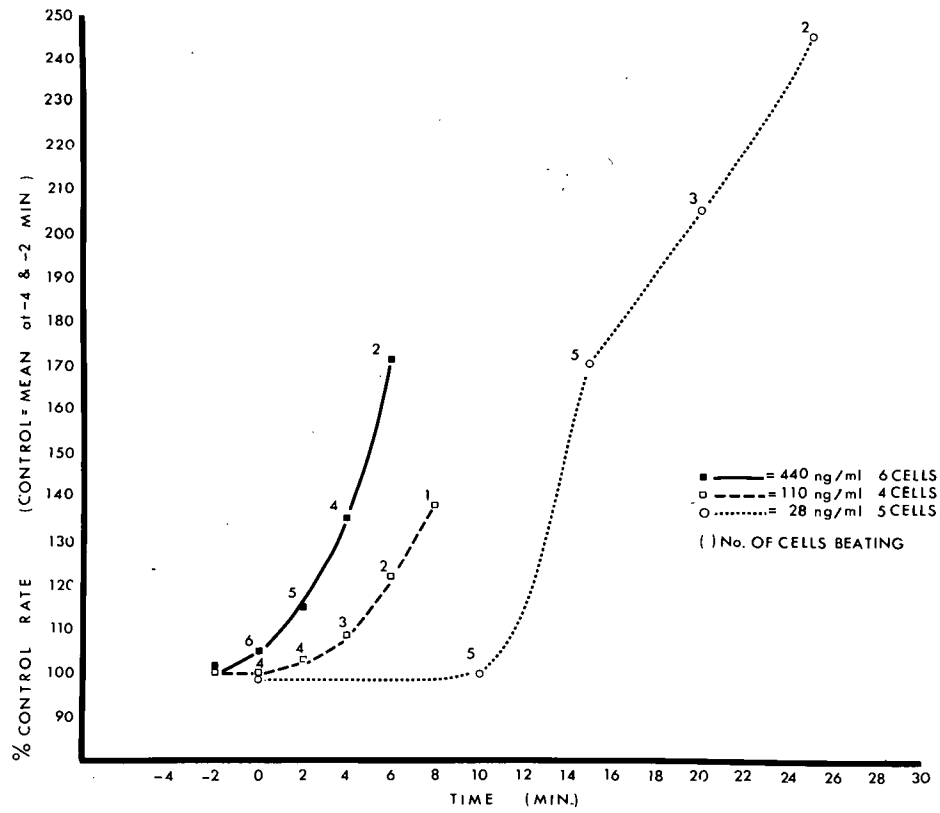

% CONTROL RATE (CONTROL = MEAN of -4 & -2 MIN)
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
-4 -2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
TIME (MIN.)
= 440 ng/ml 6 CELLS
= 110 ng/ml 4 CELLS
= 28 ng/ml 5 CELLS
( ) No. OF CELLS BEATING

Figure 36: The effect of Cyanea toxin-containing material on the percentage of beating cultured heart cells beating arrhythmically. Each point is the mean of determinations from three cultures with samples of ten cells taken from each culture. Ten different cells were randomly selected for each time period. Cyanea toxin (28, 110, and 440 ng/ml) was added at time zero. An arrhythmically beating cell was defined subjectively as indicated in Methods and by this method approximately 10 percent of a control culture of beating cells was beating arrhythmically before toxin addition.

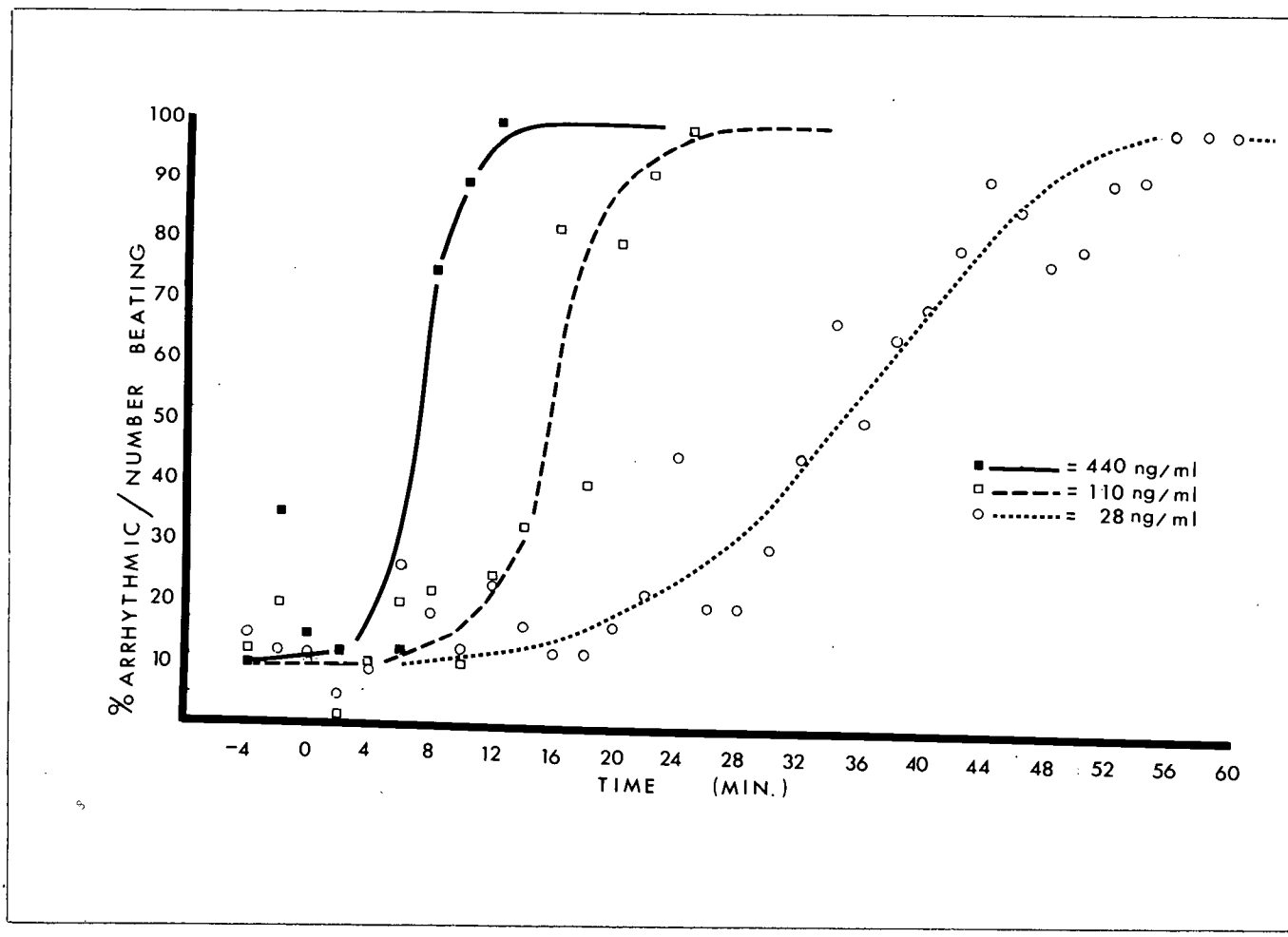

% ARRHYTHMIC / NUMBER BEATING
100
90
80
70
60
50
40
30
20
10
-4
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
TIME (MIN.)
= 440 ng/ml
= 110 ng/ml
= 28 ng/ml

Figure 37: The effect of Cyanea toxin-containing material on the percentage of total cells beating rhythmically. Each point is the mean of a sample of ten cells from three different cultures. Before the addition of Cyanea toxin at concentrations of 28, 110, and 440 ng/ml at zero time, approximately 50 percent of all (beating and non-beating) cells were beating rhythmically.

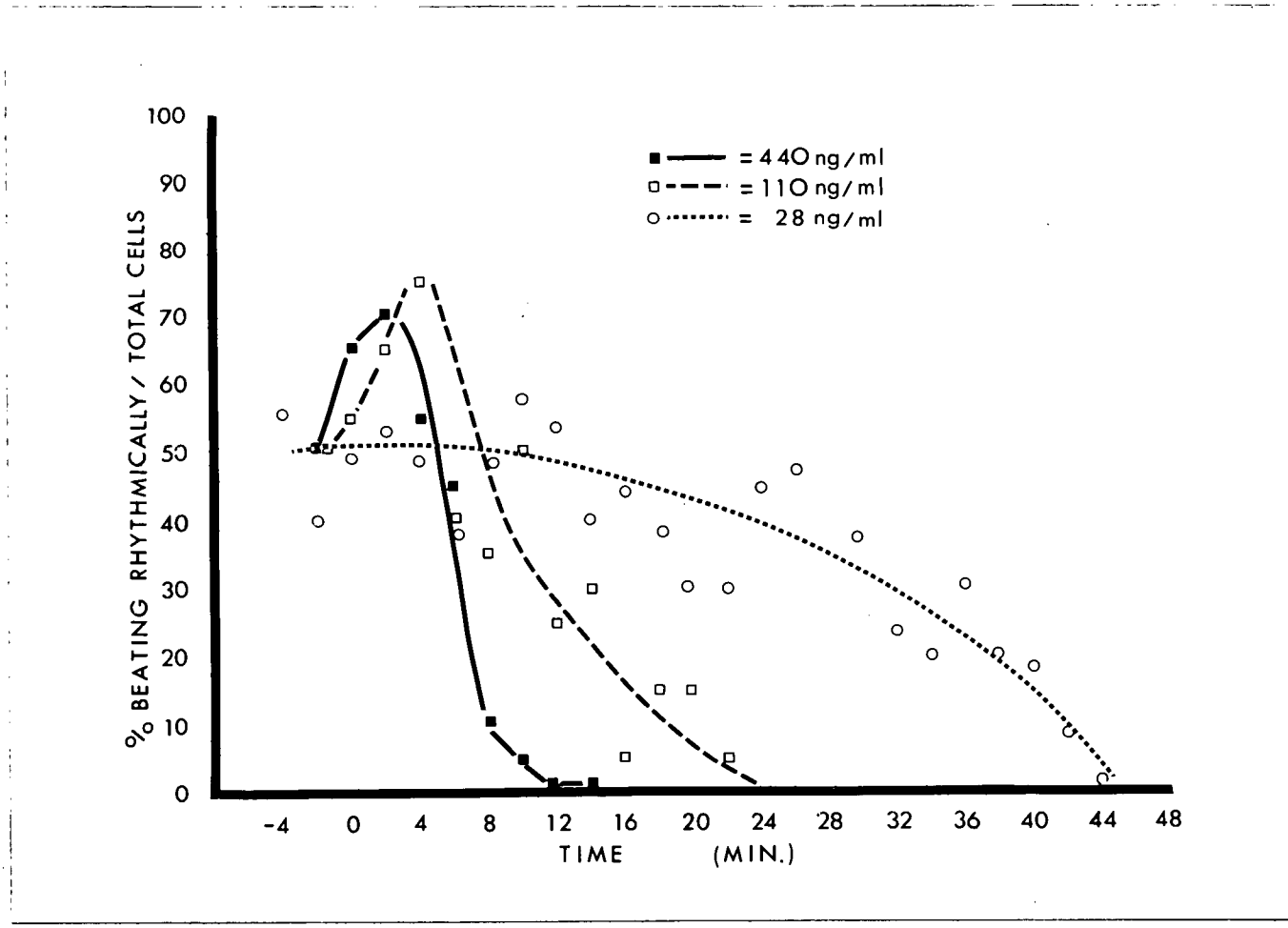
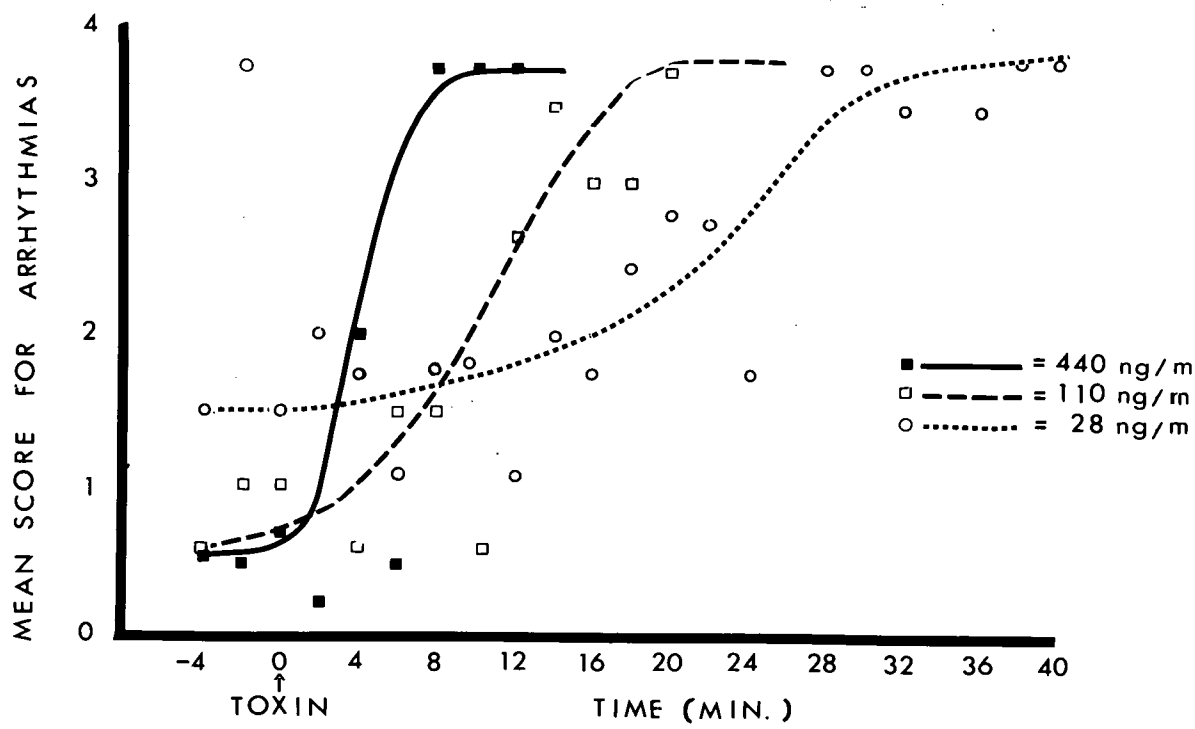

100
90
80
70
60
50
40
30
20
10
0
% BEATING RHYTHMICALLY / TOTAL CELLS
■ = 440 ng/ml
□ = 110 ng/ml
○ = 28 ng/ml
-4
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME (MIN.)

Figure 38: The effect of Cyanea toxin-containing material on the degree of arrhythmia in arrhythmically beating heart cells. Cyanea toxin at 440 ng/ml (■), 110 ng/ml (□), and 28 ng/ml (○) was added at zero time. The degree of arrhythmia in cells beating arrhythmically was scored subjectively on a scale of 0 to 4.0 (see Methods). Prior to addition of Cyanea toxin some 10 percent of cells were beating arrhythmically with a mean score of 0.5 - 1.5. This score was derived without consideration of the number of "arrhythmic cells" and therefore is subject to the greatest error where few cells were beating arrhythmically.


MEAN SCORE FOR ARRHYTHMIAS
0
1
2
3
4
-4
0
4
8
12
16
20
24
28
32
36
40
TOXIN
TIME (MIN.)
= 440 ng/ml
= 110 ng/ml
= 28 ng/ml

The mean score for arrhythmias (quantitative determination) is shown in Figure 38. Again, a dose-dependent relationship was seen with the severity of arrhythmias progressing rapidly from slight irregularities of rhythm, soon after addition of toxin, to cellular fibrillation for all cells at 8, 18, and 32 minutes for 440, 110, and 28 ng per ml toxin. Thus Cyanea toxin produced a dose-dependent progression of arrhythmic changes beginning with an initial increase in rhythmic rate and increase in the number of cells beating. These initial changes were followed by other abnormalities in rhythm until eventually all cells "fibrillated". In many respects the pattern of changes with toxin incubation time mimicked the changes seen with increasing Ca^{++} concentration.

The Effects of Prostaglandins, Lidocaine, and Quinidine on Abnormal Beating Induced in Cultured Heart Cells by Various Arrhythmogenic Agents

Five arrhythmogenic agents: ouabain (O) 30 μM, Ca^{++} 6 mM, K^{+} 8 mM, dinitrophenol (DNP) 0.2 mM, and adrenaline (Ad) 0.5 μM, induced abnormal beating in cultured rat heart myoblasts. Table X illustrates the general changes in beating behavior induced by the different agents. As indicated previously the dosages of the arrhythmogenic agents were chosen on the basis of the preceding experiments which quantitated their effect in cultured heart cells. The effects of prostaglandins, lidocaine, and quinidine on abnormal beating induced in cultured heart cells are shown in Tables XI, XII, and XIII. In a random double-blind fashion, arrhythmogenic agent and drug under test were added simultaneously and the flask 'read' 10 minutes later.

TABLES X, XI, XII, and XIII:

Five arrhythmogenic agents (ouabain "O" 30 μM, Ca^{++} 6 mM, K^{+} 8 mM, dinitrophenol "DNP" 0.2 mM, and adrenaline "Ad" 0.5 μm) induced abnormal beating in cultured rat heart myoblasts. Abnormalities were measured as:

1.) % beating normally (N)
2.) % stopped (S)
3.) % beating arrhythmically (A)
4.) mean subjective score of arrhythmias ($\bar{x}$A)
5.) rate (R)
6.) rate range (RR)

Prostaglandins A_2, E_2, $F_{1\alpha}$, $F_{2\beta}$ (10^{-5} M), lidocaine (L) 5 x 10^{-5} M and quinidine (Q) 5 x 10-7 M were tested for their ability to modify responses to arrhythmogenic agents. Significant potentiation ($p<0.05$) was judged arrhythmogenic and amelioriation was considered antiarrhythmic.

TABLE X

The general changes in beating behavior of cultured heart cells induced by different arrhythmogenic agents. See Methods p 47 for definition of terms.

AGENT	%N	%S	%A	$\bar{x}$A	R	RR
O	-	+	+	+	+	+
Ca^{++}	-	+	+	+	+	+
K^{+}	-	+	+	=	-	+
DNP	-	+	+	=	-	+
Ad	-	-	+	+	+	+

+ indicates an increase - indicates a decrease = indicates no change

TABLE XI

The effect of prostaglandins, lidocaine, and quinidine versus control (C) and arrhythmogenic agents-ouabain (O), calcium (Ca^{++}), potassium (K^{+}), dinitrophenol (DNP), and adrenaline (Ad)-on beating activity of cultured rat heart myoblasts. Results are expressed as the additional change after the effect of the arrhythmogenic agent alone has been subtracted.

% NORMAL (N)

	C	O	Ca	K	DNP	Ad
A_2	+11	-7	+10	-25	+4	-4
E_2	-8	+11	-5	-10	+21	-3
$F_{1\alpha}$	-5	+12	-3	-28	+10	-3
$F_{2\beta}$	+7	+17	-13	-2	+7	-3
L	-9	-16	+20	-27	+13	-4
Q	-17	-17	+21	-43	-4	-3

% STOPPED (S)

	C	O	Ca	K	DNP	Ad
A_2	-1	-5	+5	+19	-3	-9
E_2	+25	-6	-14	+8	-24	-5
$F_{1\alpha}$	+12	-9	-4	+24	-10	-3
$F_{2\beta}$	0	-24	-4	+2	-15	+1
L	+20	+9	+2	+29	-12	0
Q	+33	-6	-4	+45	-10	-2

% ARRHYTHMIC (A)

	C	O	Ca	K	DNP	Ad
A_2	-8	+12	-5	+9	+7	+16
E_2	-10	-5	-6	+2	+3	+8
$F_{1\alpha}$	-8	-4	-1	+2	0	+6
$F_{2\beta}$	-5	+7	0	0	+8	+8
L	-9	+7	-18	-2	-1	+20
Q	-12	+22	-22	-2	+15	+20

MEAN ARRHYTHMIC SCORE (MA)

	C	O	Ca	K	DNP	Ad
A_2		+.87	-.04			+0.7
E_2		+.65	0			+0.6
$F_{1\alpha}$		+.88	0			+0.5
$F_{2\beta}$		+.75	-0.3			+0.6
L		+.75	-0.6			0
Q		+1.15	-0.45			+0.5

RATE (Beats/min) (R)

	C	O	Ca	K	DNP	Ad
A_2	+7	+160	-68	+12	+19	+16
E_2	+8	+76	-6	+34	+45	+13
$F_{1\alpha}$	+40	+111	-101	+33	+52	+49
$F_{2\beta}$	-7	+165	-27	+5	+31	+13
L	-25	+68	-65	-29	+8	+15
Q	+6	+167	-89	-14	+6	+23

RATE RANGE (Beats/min) (RR)

	C	O	Ca	K	DNP	Ad
A_2	+114	+73	+113	-6	-41	+46
E_2	-11	-66	-47	-172	-209	+48
$F_{1\alpha}$	+5	-70	-120	-132	-251	+36
$F_{2\beta}$	-31	-45	-54	-70	+112	+108
L	+30	+91	-97	+226	-187	+33
Q	+16	+52	-19	-42	+472	+29

Where no values are shown for Mean Arrhythmic Score, too few cells were arrhythmic to provide meaningful conclusions.

TABLE XII

The ability of prostaglandins, lidocaine, and quinidine to modify the response of cultured rat heart myoblasts to five arrhythmogenic agents - ouabain, calcium, potassium, dinitrophenol, and adrenaline. Significant potentiation (arrhythmogenic effect) is indicated by a Y; amelioration (antiarrhythmic effect) is indicated by an X. Duncan's multiple range and multiple F test (Duncan, 1955) were used. See p. 50 for definition of terms.

OUABAIN

	N	S	A	xA	R	RR
A_2			Y		Y	
E_2						
$F_{1\alpha}$					Y	
$F_{2\beta}$	X	Y		Y	Y	
L	Y					
Q	Y		Y	Y	Y	

CALCIUM

	N	S	A	xA	R	RR
A_2				X		
E_2		X				
$F_{1\alpha}$						
$F_{2\beta}$	Y					
L	X		X	X		X
Q	X		X	X	X	

POTASSIUM

	N	S	A	xA	R	RR
A_2	Y					
E_2					X	X
$F_{1\alpha}$	Y	Y			X	X
$F_{2\beta}$						
L	Y	Y			Y	Y
Q	Y	Y			Y	

DINITROPHENOL

	N	S	A	xA	R	RR
A_2						X
E_2	X	X			X	X
$F_{1\alpha}$	X				X	X
$F_{2\beta}$		X				Y
L	X	X			X	X
Q			Y		Y	Y

ADRENALINE

	N	S	A	xA	R	RR
A_2			Y	Y	Y	Y
E_2				Y	Y	Y
$F_{1\alpha}$				Y	Y	Y
$F_{2\beta}$				Y	Y	Y
L			Y		Y	Y
Q			Y	Y	Y	Y

X = amelioration
Y = potentiation

TABLE XIII

The effects of prostaglandins, lidocaine, and quinidine on abnormal beating induced in cultured heart cells.

The first figure of each pair in the table gives the number of variables ameliorated; the second figure is the number of variables potentiated. Table XIII is a shortened form of table XII.

PROSTAGLANDIN	O	Ca^{++}	K^{+}	DNP	Ad
A_2	0/2	1/0	0/1	1/0	0/4
E_2	0/0	1/0	2/0	4/0	0/3
$F_{1\alpha}$	0/1	0/0	2/2	3/0	0/3
$F_{2\beta}$	1/3	0/1	0/0	1/0	0/3
L	0/1	4/0	0/4	4/0	0/3
Q	0/4	4/0	0/3	0/3	0/4

Prior to additions, overall control readings were obtained on each flask. Controls consisted of water for arrhythmogenic agents and appropriate ethanol for prostaglandins.*

The effects of prostaglandins (10^{-5} M), lidocaine (5×10^{-5} M), and quinidine (5×10^{-7} M) on 1) per cent of cells beating normally (N), 2) per cent stopped (S), 3) per cent of beating cells beating arrhythmically (A), 4) mean subjective score for arrhythmias of arrhythmically beating cells ($\bar{x}$A), 5) rate (R), and 6) rate range (RR) of beating cells can be seen in Table XI. Results are expressed as additional changes in the presence of drug under test (PGs L or Q) after the effect of the arrhythmogenic agent alone in the presence of appropriate controls was subtracted. Thus with ouabain and $PGF_{2\beta}$ the following results were obtained. In the presence of ouabain alone the percentage of cells beating normally fell from 56% ± 6.4 (S.E.M.) in the overall control situation to 37% ± 8.5 (S.E.M.) (difference 19%) in the presence of ouabain plus drug control (i.e. ethanol). With $F_{2\beta}$ in the presence of ouabain the number of cells beating normally only fell from 50% to 48% (difference 2%). Thus $F_{2\beta}$ treatment increased the per cent of cells beating normally in the presence of ouabain by 17%. Duncan's multiple range and multiple F test (Duncan, 1955) showed this change to be significant at the 5% level. Similar testing for the values in Table XI allowed the summaries in Tables XII and XIII to be constructed. Significance at the 5% level was chosen to differentiate between amelioration, potentiation and no change.

* Typical data sheets for this type of experiment are given in the appendix.

All drugs potentiated the effects of adrenaline. Tables XI, XII, and XIII) Prostaglandins and lidocaine ameliorated the effects of dinitrophenol; however, quinidine caused potentiation, decreasing the number stopped and increasing the number arrhythmic and the rate range. Prostaglandins had mixed effects against the other agents. With high potassium, prostaglandins decreased the number beating normally. The rate was increased and the rate range was decreased by prostaglandins in high potassium. With high calcium prostaglandins produced few significant changes. The mean score for arrhythmias was decreased with PGA_2, and the number stopped was decreased with PGE_2. Prostaglandin $F_{2\beta}$ reduced the number of beating normally in the presence of high Ca^{++}. In the presence of ouabain prostaglandins generally made the arrhythmogenic changes worse. The rate was increased by prostaglandins and PGA_2 increased the number of cells beating arrhythmically. The number of cells stopped was increased by $PGF_{2\beta}$ in the presence of ouabain, however, the number of cells beating normally was also increased (the only improvement seen with prostaglandins against ouabain arrhythmias). Lidocaine, and quinidine markedly opposed calcium arrhythmias but potentiated quabain and potassium arrhythmias. Some of these effects may not be due to a direct interaction between drug and arrhythmogenic agent but were probably due to physiological antagonisms. Thus among the prostaglandins both A_2 and $F_{2\beta}$ reduce rate and rate range in control cultures. Thus by physiological antagonism A_2 and $F_{2\beta}$ would be expected to reduce the effects of Ca^{++}, ouabain and adrenaline on rate but potentiate the

effects of K^+ and DNP. On rate range such a physiological antagonism would antagonise all agents. Lidocaine and quinidine both reduced the percentage of cells beating and slowed rates, actions which should directly antagonize ouabain, calcium, and adrenaline effects but potentiate K^+ and DNP. Such expected results for lidocaine and quinidine were seen with calcium and K^+ but not with adrenaline, ouabain and DNP.

The Effect of Prostaglandins, Lidocaine, and Quinidine on Arrhythmias Produced by Cyanea Toxin

Figure 39 shows the effect of prostaglandins, lidocaine, and quinidine on the per cent of cells arrhythmic/total beating versus time in the presence of Cyanea toxin 110 ng/ml. Each curve is the line of best fit obtained similarly to the results in Figure 36.* Prostaglandins, quinidine, and lidocaine all decreased the time for appearance of arrhythmias. The effect of prostaglandins, lidocaine, and quinidine on the per cent of cells beating normally versus time in the presence of Cyanea toxin is shown in Figure 40. The curves were distributed around the control. Similar results were obtained for the mean subjective arrhythmia score (Figure 41). Table XIV gives the tabulation of the above results in terms of the effect of the drugs on the time required for Cyanea toxin to produce a 50 per cent effect. It would therefore appear that neither prostaglandins, lidocaine, or quinidine significantly affect the course of Cyanea toxin cardiotoxicity except for a

*Note: the scatter of points for which lines are eye-lines of best fit in Figure 39-41 were the same as those seen in Figure 36-38.

Figure 39: The effect of prostaglandins, lidocaine, and quinidine on the percent of cells arrhythmic/total beating versus time, in the presence of Cyanea toxin 100 ng/ml.

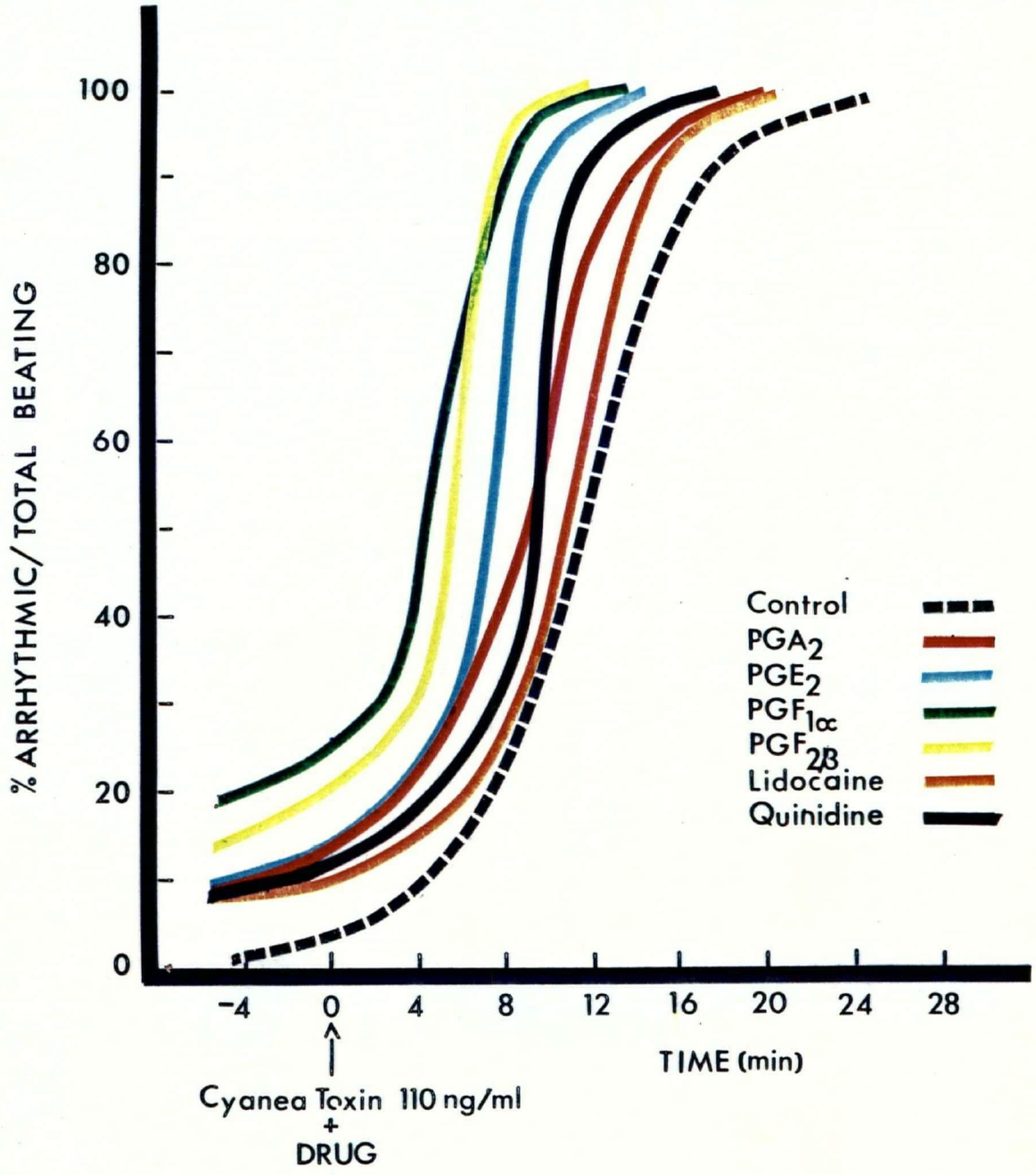

% ARRHYTHMIC/TOTAL BEATING
100
80
60
40
20
0
-4
0
4
8
12
16
20
24
28
TIME (min)
Cyanea Toxin 110 ng/ml
+
DRUG
Control
PGA2
PGE2
PGF1α
PGF2β
Lidocaine
Quinidine

Figure 40: The effect of prostaglandins, lidocaine, and quinidine on the percent of cells beating normally, versus time, in the presence of Cyanea toxin 110 ng/ml.

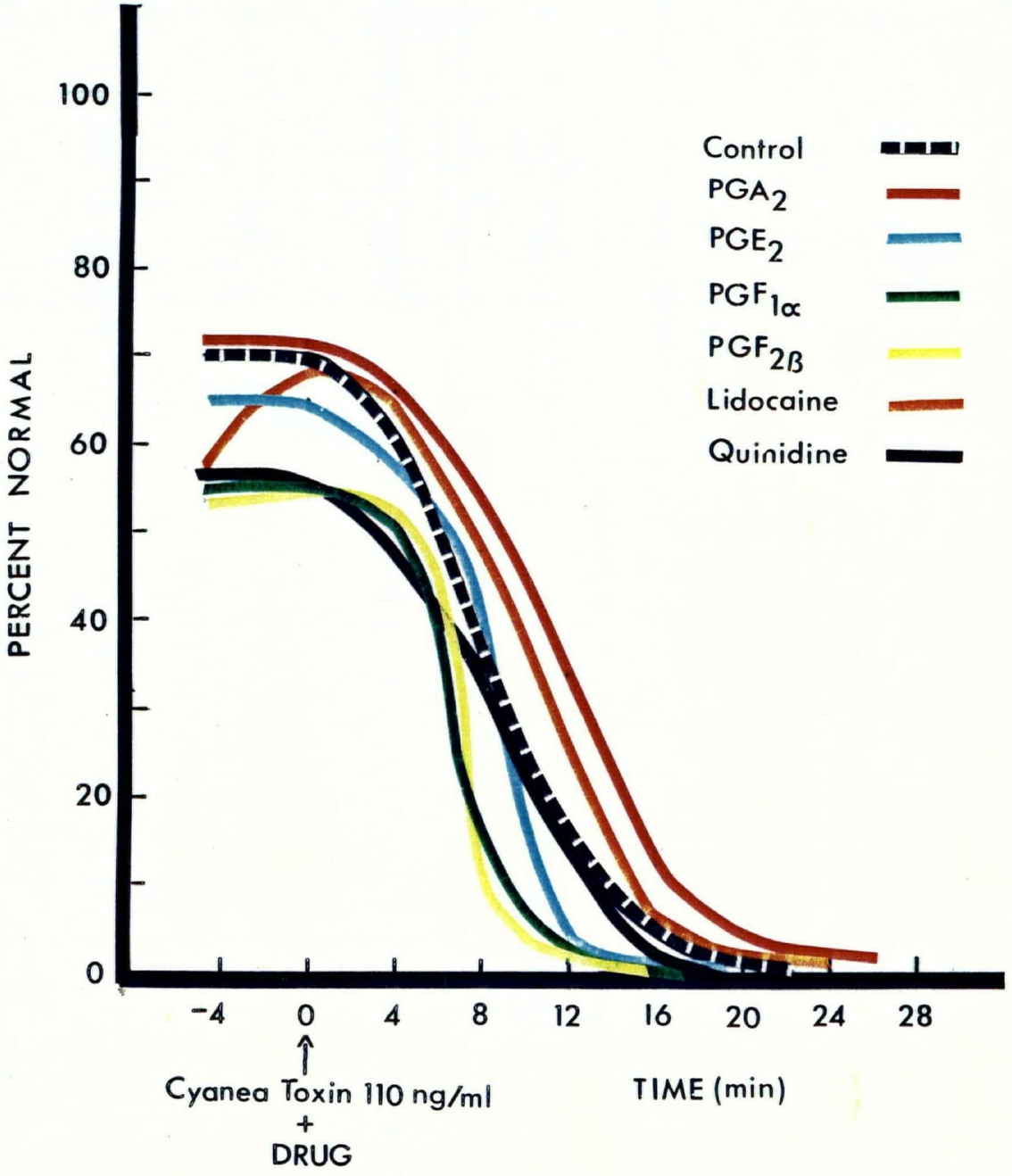

100
80
60
40
20
0
PERCENT NORMAL
Control
PGA2
PGE2
PGF1α
PGF2β
Lidocaine
Quinidine
-4
0
4
8
12
16
20
24
28
Cyanea Toxin 110 ng/ml
+
DRUG
TIME (min)

Figure 41: The effect of prostaglandins, lidocaine and quinidine on the mean subjective arrhythmic score versus time, in the presence of Cyanea toxin 110 ng/ml.

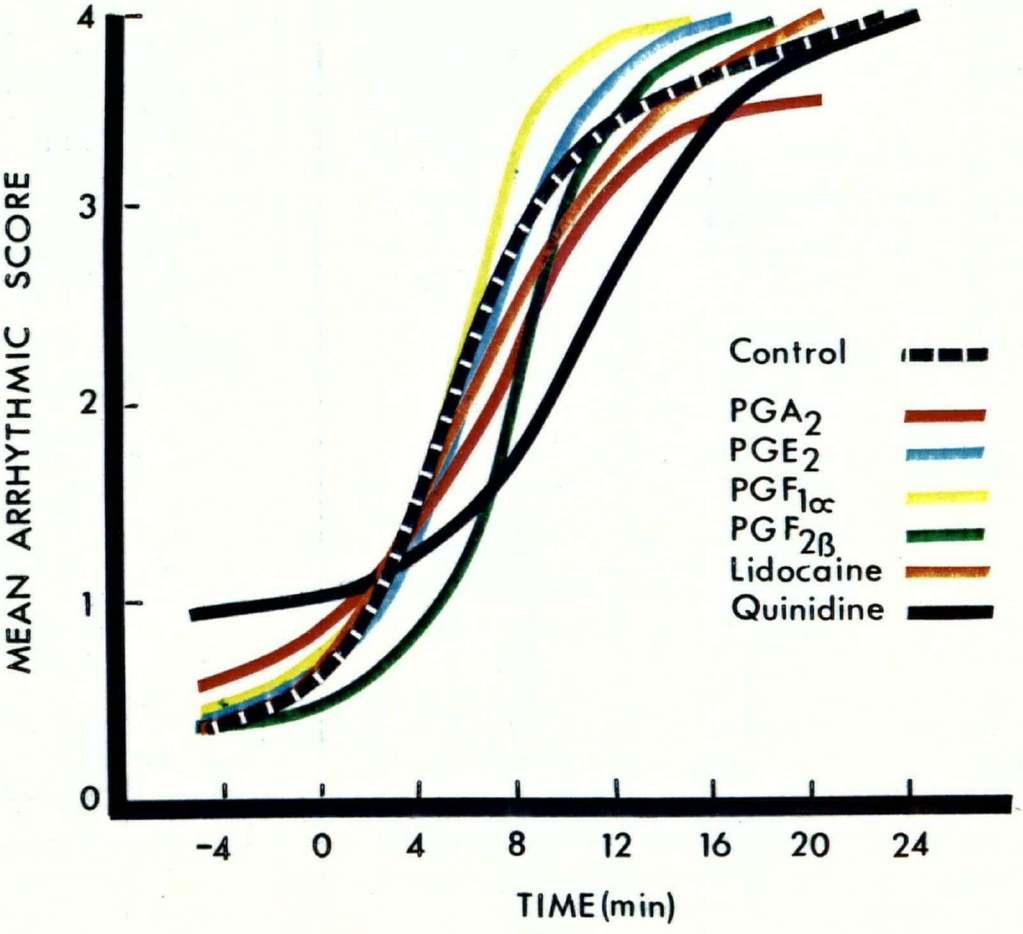

MEAN ARRHYTHMIC SCORE
4
3
2
1
0
-4
0
4
8
12
16
20
24
TIME (min)
Control
PGA2
PGE2
PGF1α
PGF2β
Lidocaine
Quinidine

TABLE XIV

The effect of quinidine, lidocaine, and prostaglandins on the time required for Cyanea toxin, 110 ng/ml, to produce a 50 per cent effect in the increase in per cent of single rat heart cells beating arrhythmically; the reduction in per cent of cells beating normally; and the increase in mean arrhythmic score.

	Shortest time (most toxic)	Longest time (least toxic)
Increase % Arrhythmic	$F_{1\alpha}$, $F_{2\beta}$, E_2, A_2, Q, L, C	
Reduction % Normal	$F_{1\alpha}$, $F_{2\beta}$, Q, C, E_2, L, A_2	
Increase in Arrhythmic Score	$F_{1\alpha}$, C, E_2, L, A_2, $F_{2\beta}$, Q	

C = control
Q = quinidine
L = lidocaine
all others are prostaglandins

possible slight effect with $PGF_{1\alpha}$, by increasing toxicity.

CHAPTER IV

DISCUSSION

The effect of Prostaglandins During Coronary Occlusion in Dogs

The first series of experiments was designed to determine the actions of prostaglandins E_2 and $F_{1\alpha}$ on arrhythmias occurring within the first 25 minutes following coronary artery occlusion. The results indicate that infusions of prostaglandin E_2 and $F_{1\alpha}$ are antiarrhythmic against ventricular arrhythmias which occur early following occlusion.

The best predictive tests for antiarrhythmics are probably those which closely resemble clinical situations, e.g. those following myocardial infarction. Such tests involve ligation of coronary arteries in experimental animals (TenHorr and Vergroesen, 1975). The first report of prostaglandin antiarrhythmic activity by Zijlstra et al. (1972) included the observation that ventricular tachycardia due to acute myocardial ischemia in dogs was changed to sinus rhythm by a single i.v. injection of prostaglandin E_1. The authors suggested that PGE_1 might act by moderating the sympathetic-parasympathetic balance. Other investigators (Mest et al., 1972, 1973, and Forster et al., 1973) reported on the antiarrhythmic effects of PGE_1, PGE_2, and $PGF_{2\alpha}$ against early arrhythmias produced by coronary artery ligation in mini-pigs,

dogs, and cats. They speculated that such protection might be the result of maintenance of lysosomal integrity in the ischemic tissue and prevention of the formantion of a cardiotoxic peptide. This suggestion was supported by a further study in dogs which showed that $PGF_{2\alpha}$ helped to preserve cardiac function after coronary occlusion while reducing lysosomal enzyme release (Glenn et al., 1975). Prostaglandin B_x, a stable free radical form of PGB_1, has also been reported to protect Rhesus monkeys from the effects of coronary artery ligation, while preserving oxidative phosphorylation in degenerated mitochondria (Angelakos et al., 1975). Thus it has been established that at least several different prostaglandins have antiarrhythmic activity against arrhythmias produced by coronary artery ligation in experimental animals. Several different mechanisms of action have been postulated although none has yet been established.

After a coronary occlusion, the reduction of blood supply to the deep ventricular muscle deprives the tissue of oxygen and metabolic substrates. As a result, the production of high energy phosphate bonds is drastically reduced (Braasch et al., 1968). Lack of sufficient supplies of high energy phosphate bonds may be responsible for the change in permeability of the myocardial cell membrane. There is a rapid loss of potassium and a gain of sodium in the ischemic musculature (Jennings et al., 1963; Harris et al., 1954). Since adenosine triphosphate is no longer available for proper functioning of the active ion pump, this imbalance cannot be corrected. As membrane potential declines the fast inward sodium current-carrying

mechanism is inactivated, but the slow inward current is not (Cranefield et al., 1973; Trautwein, 1973). Due to this depolarization the ischemic area becomes electrically negative with respect to the normally perfused muscle within one and one-half minutes of occlusion (Baley et al., 1944). This is the basis for the "injury current" - the flow of current from the pathologically depolarized to the normally polarized areas- which is seen in the electrocardiograms of patients with acute myocardial infarction (Guyton, 1976). These boundary currents may contribute to the ectopic activity. The increased potassium concentration in the extracellular fluid also has an effect on the heart (Guyton, 1976). Depolarized nerve terminals in the ischemic region release norepinephrine and ischemic ventricular muscle fibers release other substances such as enzymes and lactic acid which may also contribute to the pathologic changes (Carmiliet and Vereecke, 1969; Cranefield et al., 1972).

Powerful sympathetic reflexes develop following massive infarction due to the failure of the heart to pump an adequate volume of blood (Guyton, 1976). This sympathetic stimulation further increases the irritability of the heart muscle; however, it should be noted that the increase in arrhythmias during these first few minutes also occurs while the animal is under morphine-barbital or other barbiturate anesthesia of surgical depth (Harris, 1950; Martinez, Harvie and Walker, unpublished observations). It is therefore unlikely that reflex activity is a primary cause, but a local sympathetic effect cannot be entirely ruled out.

Prostaglandins E_2 and $F_{1\alpha}$ both markedly reduced the mean number of premature ventricular contractions in the 25 minute period following occlusion (Figure 10). These results are in agreement with the reports of previous workers (Zijlstra et al., 1972; Mest et al., 1972, 1973; Forster et al., 1973; Glenn et al., 1975; and Angelakos et al., 1975). In agreement with Harris (1950) we found that massive arrhythmias were always associated with the rapid release of the occlusion after 25 minutes and that this quickly produced ventricular fibrillation in many cases (Figure 10). Prostaglandins did not seem to be effective in preventing arrhythmias associated with occlusion release. One may presume that these arrhythmias are related to the rapid release of potassium, norepinephrine, enzymes, lactic acid and other metabolic products from the ischemic area.

The mechanism of prostaglandin antiarrhythmic activity may involve several possibilities. First, prostaglandins might act to depress automaticity in a manner similar to lidocaine or quinidine. Secondly, prostaglandins may act in some unknown manner to suppress the effects of ionic imbalance, catecholamine release, or the build-up of toxic metabolic products. Finally, prostaglandins may act before the onset of necrosis to prevent degenerative changes, thus enabling the tissue to continue functioning at a more nearly normal level of activity. Such a mechanism has already been suggested (Mest et al., 1972, 1973; Forster et al., 1973: Glenn et al., 1975; and Angelakos et al., 1975). Further studies are needed to distinguish between these possibilities.

The Effect of Prostaglandins, Lidocaine, and Quinidine on Arrhythmias Produced by Left Coronary Artery Ligation in Rats

The second series of experiments was designed to answer at least part of the question; Which prostaglandins are antiarrhythmic? The antiarrhythmic potency of several prostaglandins possessing different cardiovascular actions, together with lidocaine and quinidine, were compared to each other against early arrhythmias following left coronary artery occlusion in rats. The data obtained in this study indicate that antiarrhythmic activity is a characteristic possessed by several types of prostaglandins, although with differences in potency, and is apparently unrelated to their other cardiovascular effects.

As mentioned earlier, the best predictive tests for antiarrhythmic drugs are probably those which closely resemble the clinical situation. Recently, increasing attention has been paid to the problem of ventricular fibrillation developing in the "pre-hospital phase" of acute myocardial ischemia, with or without overt infarction. It is these early phase arrhythmias which account for nearly two-thirds of the mortality rate following acute coronary attack. (The mortality rate is approximately 40 per cent.) (Pantridge and Geddes, 1976) Prostaglandins have been shown to be effective against ventricular dysrhythmias produced in dogs and monkeys by myocardial ischemia resulting from acute coronary artery ligation (Zijlstra et al., 1972; Glenn et al., 1975; Angelakos et al., 1975). In the first set of experiments we confirmed this action with dogs, showing prostaglandins to be effective against early phase arrhythmias following occlusion. In the present set of

experiments we adapted coronary ligation techniques in rats (Johns and Olsen, 1954; Selye et al., 1960) to the study of antiarrhythmics. This technique allowed inexpensive experimental animals and relatively simple surgical techniques to be used for comparative testing with the ischemic model. In contrast to reports of other investigatiors (Johns and Olsen, 1954; Selye et al., 1960) we did not find rats to be able to survive occlusion of the left main coronary artery particularly well. Four out of five of the untreated control animals in our experiments died in ventricular fibrillation. It should be noted, however, that the previous authors used ether anesthesia, while pentobarbital was used in the present experiments.

We found the early sequence of events following coronary occlusion in rats to be in good agreement with reports using dogs (Harris, 1950; Sommers and Jennings, 1972; Wit and Friedman, 1975). Following occlusion in rats there was an initial latent period, of approximately 4 to 5 minutes, followed by PVCs, PVC salvos, and fibrillation. We did, however, note the ability of some hearts to spontaneously recover from prolonged flutter, an occurrence which is seldom seen in dogs. If the rat survived more than 12 minutes, sinus rhythm was restored. In comparison, Harris (1950) reported that the period of susceptibility to early fibrillation in dogs was brief and fibrillation did not occur later than the tenth minute following occlusion. These early arrhythmias may be attributed to electrophysiological events related to ischemia of ventricular cells in the region deprived of adequate coronary flow (Wit and

Friedman, 1975)- a process which would be similar in all species.

Other investigators have carried out studies with prostaglandins using experimental dysrhythmias such as those produced by aconitine, calcium chloride, barium chloride and ouabain (Chiba et al., 1972; Kelliher and Glenn, 1973; Mest et al., 1972, 1973, 1976; Forster et al., 1974b; Bayer et al., 1976; Mann et al., 1973). The experiments appear to be in agreement that the optimum in vivo antiarrhythmic dose range for prostaglandins in between 1 and 8 μg/Kg/min. On this basis our test dose of 2 μg/Kg/min was whose.

We found prostaglandin E_2, followed by $PGF_{2\beta}$ and quinidine, to be the most effective agents against these early phase, ischemic arrhythmias. Prostaglandins A_2, $F_{1\alpha}$, and lidocaine were less effective, although still considerably better than control. In comparison, Forster et al. (1973) reported the strongest effect against calcium chloride-induced arrhythmias in rats was shown by $PGF_{2\alpha}$, which protected a maximum of 84% of the animals for 10 minutes, followed in decreasing order of effectiveness by PGE_1, PGE_2, PGA_1, and $PGF_{2\beta}$. Against aconitine-induced arrhythmias, $PGF_{2\alpha}$ was again the most effective, followed by PGE_2, PGA_2, PGE_1, PGA_1, and $PGF_{2\beta}$. Against barium chloride-induced arrhythmias in unanesthetized rabbits (Mentz and Forster, 1974; Mentz et al., 1974) PGE_2 and PGA_1 showed the strongest effect, followed by PGA_2, $PGF_{2\alpha}$ and PGE_1. Finally, against ouabain arrhythmias in cats (Mest et al., 1976; Kelliher and Glenn, 1973) PGE_2 and PGA_1 were most effective, with lesser effects seen with $PGF_{2\alpha}$, PGA_2, and PGE_1. It appears that the

effectiveness of a prostaglandin may vary depending on the particular arrhythmia being tested against, although PGE_2 consistently scored high. The fact that we found $PGF_{2\beta}$ to be highly effective against ischemic arrhythmias is also of interest, as this prostaglandin possesses very low smooth muscle stimulating activity. This finding gives hope that prostaglandin analogs may be found which are devoid of any other pharmacological activity. In any case our experiment demonstrated that the prostaglandin antiarrhythmic effect is not a secondary effect, resulting from effects on blood pressure and heart rate for the prostaglandins caused only very minor changes in these variables.

It should be noted that there was no significant difference in the size of the infarct for any of the antiarrhythmic treatments. This would tend to argue against the possibility that the antiarrhythmic action of prostaglandins in the ischemic model results from an improvement in myocardial blood supply as had been suggested by Mest et al., (1975).

The standard antiarrhythmic drugs, lidocaine and quinidine, also proved effective against these early phase arrhythmias although prostaglandin E_2 appeared to be clearly superior to lidocaine and in some respects superior to quinidine as well. The dose of lidocaine (6 µg/Kg/min) was determined experimentally to be the largest dose which could be given for the time of infusion without producing a depression of blood pressure. The dose of quinidine (3 µg/Kg/min) was determined from the literature (Moe and Abildskov, 1975).

The fact that lidocaine was only moderately effective

against the early phase arrhythmias is not surprising, as controversy exists concerning the ability of lidocaine to prevent spontaneous ventricular fibrillation following coronary occlusion (Stephenson et al., 1960; Weisse et al., 1971). Our work is in good agreement with a recent study done in dogs which found lidocaine to be moderately effective in preventing arrhythmias in the early phase after coronary occlusion; in addition, lidocaine has been reported to be less effective during the acute phase following myocardial infarction than later in the attack (Pantridge and Geddes, 1976). The effectiveness of quinidine against these early arrhythmias in somewhat surprising since this drug is not commonly used in this situation. One may presume that this effect is related to the ability of this drug to suppress ectopic foci by its well-known depressant action on the myocardium. Prostaglandins have not been found to have a strong "quinidine-like" depressant effect (Forster, 1976; Mann, 1976), a consideration which would give them a clear therapeutic advantage.

In summary, a technique for comparative testing of antiarrhythmic drugs in the acute ischemic model using rats has been described. The time course for arrhythmia development and for types of arrhythmias produced are in good agreement with previous experiments in dogs.. Prostaglandins E_2, $F_{2\beta}$, and quinidine were found to be most effective against these early phase ischemic arrhythmias. The prostaglandin antiarrhythmic activity was not related to effects on blood pressure or heart rate. Since no significant differences were found in the size of the infarcts with different drug treatments, it seems unlike-

ly that the antiarrhythmic actions of prostaglandins result from any improvement in coronary circulation. The fact that prostaglandin $F_{2\beta}$, a prostaglandin with very weak activity on smooth muscle, had good antiarrhythmic activity gives hope that analogs with high specificity of action may be developed. The data in this study indicate that several classes of prostaglandins possess antiarrhythmic activity; they compare favorably with, and in some cases are superior to, the standard antiarrhythmic drugs lidocaine and quinidine.

The Effects of Prostaglandins, Lidocaine, and quinidine on Flutter Threshold and Maximum Following Frequency in In Situ Rat Heart:

Prostaglandins appear to be effective only against arrhythmias induced by damage or ionic disturbance and not against nondamaging disturbances such as electrical stimulation (Forster, 1976; Metz, 1976). This lack of activity, together with reports of only minor or controversial electrophysiological effects (Keckskemeti et al., 1976; Mentz et al., 1974; January and Schotelius, 1974), led us to investigate the actions of prostaglandins in comparison with lidocaine and quinidine on the flutter threshold and on maximum following frequency in in vivo rat hearts.

The results of the present study indicate that in the antiarrhythmic dose range (1-8 µg/Kg/min) Mentz and Forster, 1974; Mest et al., 1974) prostaglandins produce alterations in maximum following frequency which are dose- and prostaglandin-dependent. These changes are small (in the order of 10 per cent or less) in relation to the control and are much less than the

changes seen with the standard antiarrhythmic drugs lidocaine and quinidine. This finding, together with the fact that there was no change in flutter threshold with prostaglandins in contrast to the decrease seen with lidocaine and the increase seen with quinidine, would indicate that prostaglandins act, at least in part, in a manner different from most other antiarrhythmic drugs. These results are in accordance with the observation that in patients $PGF_{2\alpha}$ does not increase the diastolic stimulation threshold when used in doses required to suppress dysrhythmias encountered clinically (Mann, 1976).

In isolated atria and papillary muscles of guinea pigs a biphasic effect has been reported, with low concentrations of PGE_2 and $PGF_{2\alpha}$ producing an increase and higher concentrations a dimunution of the maximum rate of depolarization (Forster et al., 1974). Very high concentrations of $PGF_{2\alpha}$ (1 to 10 mg/ml) were required to reduce the conduction time in those isolated atria and such doses would not be tolerated in vivo due to toxic effects (Karim et al., 1971). In rat papillary muscle a lower dose of 10 or 100 ng/ml of $PGF_{2\alpha}$ was found to cause an increase in the action potential duration and plateau amplitude (January and Schotelius, 1974). In the present experiment prostaglandins $F_{2\alpha}$, E_2, and A_2 all had negative linear dose relationships for maximum following frequency in the antiarrhythmic dose range (1-8 µg/Kg/ml); in situ, however, the maximum change produced was small. Since measurements of maximum following frequency are receprocally related to the functional refractory period and the action potential duration (Szerkes, 1970), it would seem that our findings of a slight depressive action of

these prostaglandins on the myocardium is in good agreement with the *in vitro* findings. The fact that $PGF_{2\alpha}$ initially increased the maximum following frequency at 1 μg/Kg/min before causing a decrease may be related to its reported biphasic action (Forster *et al.*, 1974).

In situ electrophysiological effects of prostaglandins have been previously reported by Bayer *et al.* (1976) and Foster *et al.* (1974) in cats. Infusion of prostaglandins at 5 μg/Kg/min resulted in dose-dependent decreases in the velocity of conduction in the atria and in the AV node and extension of the functional refractory period for prostaglandins $F_{2\alpha}$, E_2, and E_1, with maximum effects of approximately 10 per cent. Prostaglandins E_2 and $F_{2\alpha}$ had little effect on the ventricular fibrillation threshold. Thus our results with *in vivo* rat heart are in close agreement with previous reports in cats. At least some prostaglandins have been found to have slight depressive actions on the *in vivo* heart, however, these effects are much weaker than quinidine-like agents.

In contrast, prostaglandin $F_{2\beta}$ caused a slightly positive linear response and prostaglandin $F_{1\alpha}$ had a biphasic effect (causing first a decrease, then an increase in maximum following frequency with increasing doses.) The fact that $PGF_{2\beta}$ was found to be the second most effective antiarrhythmic prostaglandin, after PGE_2, in the rat coronary artery ligation experiments while having virtually no effect on the electrical excitability measurements would tend to confirm the dissociation.

In conclusion, only some prostaglandins produce a slight depressive effect on the heart which is much less than that

which is seen with quinidine-like drugs. This depressive action does not correlate with the antidysrhythmic actions of prostaglandins.

The Effect of Prostaglandins on Isolated Rat Heart

Any drug which produces marked pharmacologic effects on the myocardium might have, in addition, antiarrhythmic or arrhythmogenic actions. It was therefore of interest to determine the pharmacology of prostaglandins used in our in vivo studies; conventional experiments measuring rate, force, and coronary flow were performed on unpaced Langendorf perfused hearts. It was also of interest to see whether prostaglandins would have a "protective" effect on the force of contraction at 10^{-7} M, however, this effect was not seen at other concentrations.

An examination of the literature shows that prostaglandins produce cardiac inotropic and chronotropic effects which are species dependent. For example, prostaglandin E_1 failed to alter the rate or force of contraction in the isolated heart of cat, dog, rabbit, or chicken, but produced positive chronotropic and inotropic effects in isolated guinea pig hearts (Berti et al., 1975; Mantegazza, 1965; Sunahara and Talesnik, 1974; Horton and Main, 1967). Quantitatively and qualitatively different results have been reported by other investigators. On the isolated cat papillary muscle, PGE_1 was found to markedly increase tension (Tucker et al., 1971), while on dog papillary muscle only a slight increase in tension was observed (Antonaccio and Lucchesi, 1970). Wennmalm and Hedqvist (1970) also observed a slight increase in both rate and contractile force of the

rabbit heart in response to PGE_1 and PGE_2. For the F series of prostaglandins the effects are just as varied. Prostaglandin $F_{1\alpha}$ did not affect the isolated guinea pig heart (Sobel and Robison, 1969) while $PGF_{2\alpha}$ produced a slight positive inotropic effect on isolated guinea pig atria (Nutter and Crumly, 1972) and had no effect on isolated chicken heart (Horton and Main, 1967) or dog and cat atria (Su et al., 1973). In rat myocardium, prostaglandin E_1 and E_2 only modestly increased the amplitude of contraction of the isolated heart and had no significant effect on rate (Berti et al., 1965; Vergroesen et al., 1967; Vergroesen and de Boer, 1968). Levy (1973), however, found that PGE_2 modestly increased both the force of contraction and the rate of atria from normal and genetically hypertensive rats. Our findings that prostaglandins have either no effect or produce only small positive effects on rate and force are therefore in good agreement with the results of previous workers.

Concerning the force of contraction. Post-infusion, hearts which had been exposed to PGE_2 or $PGF_{2\beta}$ at 10^{-7} M did not have the same loss of contractile force with time as did control. This slight stimulatory or "protective" effect continued even though prostaglandins were no longer present. Robert (1976) has postulated a cytoprotective property for prostaglandins in the gastrointestinal system as a partial explanation for their anti-ulcer activity; a similar suggestion has been made concerning the antiarrhythmic effects of prostaglandins in arrhythmias induced by coronary ligation (Angelakos et al., 1975). It should be noted that we found PGE_2 and $PGF_{2\beta}$ to be the most

effective prostaglandins A_2 and $F_{1\alpha}$ demonstrated little "protective" action in the present experiment (see Table III).

There was good cross species agreement between our *in vitro* and previous *in vivo* results. With dogs we found no significant change in rate or blood pressure when prostaglandins were infused. With prostaglandins in *in vivo* rats no change in rate and only a slight decrease in blood pressure, with PGA_2 and PGE_2 were noted.

It has been suggested (Mest *et al.*, 1975) that the anti-arrhythmic action of prostaglandins may result from improvement of the myocardial blood supply. This view was based on the observation of Szekeres *et al.* (1972) who found the rate of arrhythmias due to coronary occlusion to be decreased after infusion of nitroglycerin. Only PGE_2 produced an increase and $PGF_{2\beta}$ caused a decrease in coronary flow rate at 10^{-7} M in the present experiment. At 10^{-5} M all prostaglandins produced a marked increase. Most authors report an increase in coronary flow in isolated perfused hearts with prostaglandins. Mantegazza (1965) reported that PGE_1 enhanced coronary blood flow in cat, rat, and rabbit heart without significant inotropic or chronotropic effects. Vergroesen *et al.* (1967) also reported that PGE_1, PGE_2 and PGA_1 increased coronary flow in isolated rat heart; however, Berti *et al.* (1965) failed to find any effect of PGE_1 on heart rate and coronary flow in isolated rat heart. With the F series prostaglandins, in rat, Vergroesen *et al.* (1970) reported that $PGF_{1\alpha}$ or $PGF_{1\beta}$ failed to alter coronary flow. In contrast, Willebrands and Tasseman (1968) found an increase with $PGF_{1\alpha}$. Our data indicate marked increas-

es in coronary flow with several different series of prostaglandins, but only at high concentrations. It is possible that this dilation of the coronary vasculature may play a role in the antiarrhythmic action of prostaglandins, however, the fact that the size of the infarct produced was not significantly decreased by any prostaglandin in our in vivo rat experiments would tend to indicate that this role is of minor significance.

In summary, we have examined the cardiac pharmacology of several prostaglandins of the E, A, and F series in isolated Langendorff hearts and found them to have either minimal or slight effects on rate and force. These results are in good agreement with our in vivo observations, and it is unlikely that such weak effects could account for the strong antiarrhythmic actions of prostaglandins. Both PGE_2 and $PGF_{2\alpha}$ (the most effective prostaglandins against coronary occlusion arrhythmias) delayed the loss of contractile force with time at 10^{-7} M, demonstrating a slight "protective " or stimulating effect analogous to the cytoprotective proposal of Robert (1976), in the gut, or of Angelakos et al. (1976) in the heart. The significance of this protective effect cannot be determined from the present data. Finally, we found all prostaglandins tested to markedly increase coronary flow at 10^{-5} M. The fact that there was no significant difference in the size of the infarct produced in previous coronary ligation experiments makes it unlikely that dilation of the coronary vasculature is the prime mechanism for the antiarrhythmic action.

The Effects of Prostaglandins Alone on the Beating Activity of Cultured Heart Cells

Prostaglandins may produce either direct or indirect effects on rate and force in cardiac tissue. In vivo increases in heart rate reported in response to a number of prostaglandins have been shown in dog to be the result of reflex sympathetic activation and stimulation of cardioaccelerator centers in the medulla (Malik and McGiff, 1976). In contrast, direct chronotropic effects of prostaglandins have been demonstrated in vitro with guinea pig and frog heart (Berti, Lentati, and Usardi, 1965; Mantegazza, 1965). No in vivo studies on the effects of prostaglandins in rats are available; however, in isolated rat heart the E-series prostaglandins have been reported to have either no effects or the produce modest increases in rate (Berti, Lentati, and Usardi, 1965; Vergroesen, de Boer, and Gottenbos, 1967; Vergroesen and de Boer, 1968). The lack of marked direct effects observed in the present experiment are, therefore, in agreement with the results of previous workers. Only one exception, $PGF_{2\alpha}$, produced chronotropic effects, but even this was limited when compared with adrenaline. Other possible increases such as that noted with PGD_2 and the decrease with $PGF_{2\beta}$ were very limited and were not statistically significant.

Recent experiments (Goshima, 1976) have shown that the arrhythmic movements of both single isolated cells and cell clusters challenged with arrhythmogenic agents were improved by the addition of antiarrhythmic drugs such as quinidine or procaine amide. Since prostaglandins have been reported to have

antidysrhythmic properties (Forster, Mest and Mentz, 1973; Kelliher, Reynolds and Roberts, 1975; Mann, Meyer and Forster, 1973; Mentz and Forster, 1974), we hoped it would be possible to demonstrate this effect using the rate range as an index of rhythm variability. We found no significant change in the rate range for any of the prostaglandins tested, although there was a marked significant increase in this variable with both doses of adrenaline, which can be contrasted with $PGF_{2\alpha}$ which increased rate without increasing rate range. In the report noted above, antiarrhythmic drugs reduced the percentage of cells beating spontaneously. Although prostaglandins are reported as being antiarrhythmic, only $PGF_{1\alpha}$ and $PGF_{2\alpha}$ reduced the number of beating cells (i.e. tended to abolish automaticity). Prostaglandin $F_{2\alpha}$ had this action despite its positive chronotropic action.

Bucher and Kolb (1962) and Okarma and Kalman (1971) have reported that it is possible to interpret changes in optical density as corresponding to changes in the force of contraction in cultured heart cells. In the present experiments, the first derivative of optical density was also used to provide a second index of density change. Generally prostaglandins either produced no change or reduced both indices of contractile force with prostaglandins (Berti, Lentati and Usardi, 1965; Vergroesen, de Boer and Gottenbros, 1967; Vergroesen and de Boer, 1968). It is, therefore, not clear whether changes in optical density and the associated derivative are measurements of force effects in cultured heart cells. With adrenaline, however, density changes correlate well with what we

would expect as a force effect if a negative treppe effect occurs at high beating frequencies or if excess catecholamine stimulation reduces cultured cell energy levels. Further work is required to fully evaluate optical density changes, but whatever their exact meaning, it is significant that the majority of prostaglandins reduced both changes in optical density and its derivative, with the marked exception of $PGF_{2\beta}$. It is also to be noted that high concentrations of prostaglandins were used and therefore the effects may be non-specific. Fatty acids can have a depressant action on cardiac tissue (Szekeres, Borbola and Papp, 1976).

In this investigation the effect of various prostaglandins on cultured heart cell beating activity was assessed in terms of effects on various parameters of rate and contraction. It is interesting to note that activity on one parameter was not necessarily associated with activity on another. Thus, $PGF_{2\alpha}$ increased beating rate and the number of stopped cells but did not affect the optical density or its derivative, while $PGF_{1\alpha}$ affected the percentage beating and optical density but not rate. If the prostaglandins were listed in rank order for their effect on the different parameters only $F_{1\alpha}$, $F_{2\alpha}$, E_2, and 15 MeE_2 tended to be highly ranked, while B_1, A_2 and $F_{2\beta}$ were low ranked which would appear to indicate that prostaglandins have more than one pharmacological action on cultured cells, and that these actions are not necessarily related. For example, the mechanism responsible for the chronotropic action of $PGF_{2\alpha}$ is probably independent of that responsible for reducing the number of beating cells. Electrophysiologically prostaglandins

can hyperpolarize or depolarize cells, depending on concentration (Kecskemeti, Keleman and Knoll, 1974). The possibility of prostaglandins acting on more than one mechanism is increased by considering the actions of $PGF_{2\beta}$, which does not change the percentage of cells beating spontaneously, despite reducing rate and increasing optical density.

In conclusion, with the exception of $PGF_{2\alpha}$, which produced a marked chronotropic response, prostaglandins have limited direct actions in cultured heart cells. Furthermore, the positive chronotropic effect of $PGF_{2\alpha}$ is not associated with the dysrhythmic tendencies seen with catecholamines. Prostaglandins either produced no change or reduced indices of contractile force, with the exception of $PGF_{2\beta}$ which produced a positive force effect.

The Effects of Prostaglandins on Abnormalities of Beating in Cultured Heart Cells Induced by a Variety of Arrhythmogenic Agents

Our studies on the antiarrhythmic actions of prostaglandins have been directed at answering at least part of the question: 1) which prostaglandins are antiarrhythmic 2) under what circumstances, and 3) by what mechanisms? The present set of experiments deals with the third part of the original question; by what mechanisms are preostaglandins antiarrhythmic. Several possibilities have been proposed; 1) They may modulate sympathetic-para-sympathetic balance 2) they may affect blood flow and distribution 3) their antiarrhythmic activity may be secondary to their general cardiovascular actions and 4) they may have some "cellular protective" action which enables damaged cells

to carry on a more normal level of activity. We used cultured heart cells (which are free of adrenergic innervation and blood flow considerations) together with a number of specific arrhythmogenic agents to test the hypothesis that prostaglandins may be antiarrhythmic by means of some cellular protective action.

The Actions of Ouabain, Ionic Manipulation, Dinitrophenol, and Anoxia on Cultured Heart Cells

Arrhythmic behavior has already been described in cultured heart cells (Goshima, 1976; Boder and Johnson, 1972; Sane and Sawanoberi, 1970), but such descriptions lack qualitative and quantitative depth. The evaluation of arrhythmias in previous reports utilized subjective measurements (Goshima, 1976) and were limited quantitatively. The present experiment describes the action of a number of arrhythmogenic agents on the beating activity of single isolated cultured heart cells in both qualitative and quantitative terms using objective and subjective methods.

The types of arrhythmic responses varied with the agent considered. Ouabain and calcium produced similar effects with marked increases in rate terminating in fibrillatory activity. The most widely quoted mechanism of action for cardiac glycosides is inhibition of sodium and potassium-dependent membrane ATPase (Goodman and Gilman, 1975). The rapid increase in rate to a plateau (see Figure 24) may therefore be due to a depolarization of the resting membrane potential toward threshold with an elevation of intracellular sodium and calcium levels. A continued fall in membrane potential would eventually be arrhyth-

mogenic as the membrane potential approached threshold. Further depolarization would result in quiescence as the cells failed to repolarize. Figure 25 and 26 show good agreement with the expected results.

Potassium and dinitrophenol produced very different types of arrhythmias from ouabain and calcium; they were characterized by decreases in rate accompanied by an increase in rate variability while none of the subjective arrhythmic patterns seen with the first two agents was observed. Elevation of potassium first decreased rate up to 10 mM (Figure 29). This decrease in rate is most likely related to a lengthening of the action potential due to delayed or slowed repolarization. A higher concentration of extracellular potassium would oppose the potassium efflux during phase 3 of the cardiac action potential. The increase in rate seen with concentrations between 10 mM and 28 mM potassium is probably related to steepening of the slow depolarization (phase 4) portion of the cardiac action potential. Very high (10-28 mM) extracellular potassium might then tend to oppose the declining outward flow of potassium during this phase which partially balances the inward sodium leak (Trautwein, 1973). Dinitrophenol (DNP) is most widely known as an inhibitor of oxidative phosphorylation although it also inhibits anerobic metabolism as well (Lehninger, 1976). The initial rapid decrease in rate seen with DNP may be related to a partial depolarization of the cells due to inhibited sodium- and potassium-ATPase secondary to decreased ATP. The fact that although the cells partially recovered their initial rate, they appeared to beat more weakly would support this view.

Anoxia was found not to be arrhythmogenic. Similar results have been reported in the isolated heart (Mommaerts, 1966). Although cardiac tissue normally utilizes oxidative metabolism, apparently it is quite capable of switching to anaerobic metabolism and deriving sufficient energy by this means to continue normal functioning (at least when not pumping under load). In both the isolated heart and in cell culture the availability of glucose for an energy source and the removal of products of metabolism are not limiting factors.

Quinidine and lidocaine both decreased automaticity in cultured heart cells. Similar findings had previously been reported by Mercer and Dower (1966) although no complete log dose response curves were shown. Since we had previously found the F_{α} prostaglandins to decrease automaticity up to 33% in cultured heart cells the curves allowed us to select doses of quinidine and lidocaine with similar effects for comparison.

In conclusion, we have investigated the dose and time responses of several arrhythmogenic responses of several arrhythmogenic agents in cultured heart cells. It is possible to evaluate very different types of cellular arrhythmias in both qualitative and quantitative terms. The information from this study made it possible to select appropriate doses of our arrhythmogenic agents for testing with antiarrhythmic drugs. It was hoped that such a study might give insight into the antiarrhythmic mechanisms involved.

The Effect of Cyanea Toxin on Cultured Heart Cells

Walker (1977b) has shown the cardiotoxicity of Cyanea toxin-containing material to result from profound ionic disturbances including a loss of intracellular potassium and a gain in sodium and calcium associated with the expected electrophysiological changes of depolarization and reduction of action potential height with ultimate loss of action potentials. Cultured neonatal rat heart cells are believed to closely resemble intact heart tissue with regard to morphology and physiology (Halle and Wollenberger, 1970). Ion distribution (Halle and Wollenberger, 1970) and ionic mechanism are also similar (Lieberman et al., 1975), apart from the possibility of a greater importance of calcium and slow sodium currents (Sperelakis, 1972). From the above, one could predict that exposure of such cultured cells to Cyanea toxin should produce a characteristic progression of dysrhythmic changes. A toxin-induced fall in resting membrane potentials as seen in adult atria (Walker, 1976b), would account for the initial increase in beating rate if the maximum diastolic potentials in these cells are equivalent to atrial resting membrane potentials. A continued fall in membrane potential would eventually be arrhythmogenic as is the case with cells exposed to ouabain (Goshima, 1976). The quiescent period between the loss of the first type of arrhythmic activity and the appearance of cellular fibrillation may indicate a membrane potential falling below threshold for activation of a sodium dependent action potential. A further fall would then bring the potential into the area of spontaneous activation of slow calcium currents

(Cranefeild et al., 1972). Indeed, fibrillatory activity in Purkinje fibers may involve slow currents resistant to tetrodotoxin (TTX) and revealed by bathing with high K^+ solutions (Aronson and Cranefield, 1973). The final proof that the arrhythmic activity pattern results from membrane potential changes must await detailed electrophysiological investigation.

Despite the profound changes in activity with Cyanea toxin-containing material, it is of interest to note that morphological signs indicative of gross membrane damage (vacuolization and lysing), were not apparent. However, cells treated with Cyanea toxin did not recover activity when placed for 48 hours in control media. This lack of evidence of direct lysis is analogous to the finding of an unaltered intracellular space (Walker, 1977b) in toxin-treated atria.

In conclusion, Cyanea toxin produced the most severe type of arrhythmia encountered in cultured heart cells. We decided to use the characteristic series of changes in beating behavior produced by Cyanea toxin cultured cardiac cells as a further arrhythmogenic test for anti-arrhythmic drugs and prostaglandins.

The Effects of Lidocaine, Quinidine, and Prostaglandins on Abnormal Beating Induced in Cultured Heart Cells

The primary purpose of the present series of experiments was to determine the ability of prostaglandins to protect heart cells from a variety of damaging agents. The underlying rationale was that the anti-dysrhythmic actions of prostaglandins may be related toan ability to protect cardiac cells against damage which results in ionic redistribution, electrophysiological disturbances, and subsequent dysrhythmias. A similar "cyto-

protective" mechanism has been proposed by Robert (1976) for the anti-ulcer effects of prostaglandins and by Angelakos (1975) for the antiarrhythmic action in coronary occlusion. The data from the present study indicate that prostaglandins do not have any general "cytoprotective" action, although they do appear to oppose the inhibition of cell beating activity seen with dinitrophenol.

Part of the rationale underlying the present experiments was that behavior in cultured heart cells reflects that seen in intact tissue, to the extent that disturbances of beating rhythm may involve similar mechanisms to those occurring in the intact tissue. This is not withstanding the fact that re-entry models have an absolute dependency on heterogeneity in a population of cells. The morphology and physiology of cultured heart cells has been found to closely resemble the intact heart (Halle and Wollenberg, 1970).

Single isolated myocardial cells obtained from mammalian or chicken heart are known to beat spontaneously and independently in culture (Goshima, 1976; Wollenberger, 1964; Sperelakis and Lehmkuhl, 1964). The great majority of single isolated cells show regular, rhythmical beating under normal conditions and have regular arrhythmic movements analogous to fibrillation upon the addition of aconitine (Boder and Johnson, 1972) or of digitalis (Marke and Strasser, 1966; Mercer and Dower, 1966; Goshima, 1976) or in a medium of low potassium (Goshima, 1975) or of high calcium concentration (Goshima, 1975). The ability of standard antiarrhythmic drugs to improve these arrhythmic movements is controversial. Mercer and Dower (1966) and Klein-

feld et al. (1969) found that single isolated cells from embryonic chick heart showed arrhythmic movements on addition of antiarrhythmic drugs such as quinidine or procainamide. In contrast, Boder and Johnson (1972) reported that pre-treatment with lidocaine partially prevented aconitine-induced arrhythmias in cell clusters from neonatal mouse heart. Goshima (1975, 1976, 1977) reported that both cell clusters and single isolated cells from fetal mouse heart showed arrhythmic movements in media of low potassium, high calcium concentrations or with ouabain; these arrhythmic movements were markedly improved or completely disappeared post-treatment with quinidine, procainamide, or lidocaine.

In the present experiments we found lidocaine and quinidine to be effective only against cellular arrhythmias caused by high calcium concentration. In agreement with Mercer and Dower (1966) and Kleinfeld et al., (1969) and in contrast to the results of Goshima (1976), we did not find lidocaine or quinidine to be effective against cardiac glycoside-induced arrhythmias. In fact, our standard antiarrhythmic drugs made both ouabain and high potassium concentration arrhythmias worse, although the results with potassium could have been at least partially due to the cell's response to the drug alone. With dinitrophenol, lidocaine ameliorated the arrhythmias while quinidine had mixed effects which would tend to indicate some action other than direct depression of automaticity. All agents made adrenaline arrhythmias worse, indicating no direct sympathetic blocking action for quinidine, lidocaine, or prostaglandins. The prostaglandins had mixed effects except

for an ability to improve cells inhibited with dinitrophenol and the previously mentioned adverse effect seen with adrenaline. Dinitrophenol is a well-known inhibitor of oxidative phosphorylation which is capable of inhibiting anaerobic metabolism as well (Lehninger, 1976). Polis et al. (1972) have previously reported that prostaglandins are capable of stimulating oxidative phosphorylation in depressed mitochondrial preparations. Our results indicate that a similar effect is present at the cellular level.

We therefore found no over-all "protective" effect with prostaglandins in cell cultures. Only the effects of prostaglandins against dinitrophenol-induced arrhythmias appeared to bear any relationship to the antiarrhythmic actions of prostaglandins in vivo. The correlation, however, is not exact. Prostaglandin E_2 was most effective against DNP-induced cellular dysrhythmias and in previous experiments against in vivo coronary occlusion arrhythmias in rats. Prostaglandin E_2 had also previously been found to be most effective in maintaining contractile force in Langendorff hearts. In in vivo and in Langendorff hearts, however, $PGF_{2\beta}$ was the second most effective prostaglandin, followed by $PGF_{1\alpha}$ and PGA_2. In comparison, $PGF_{1\alpha}$ was more effective than $PGF_{2\beta}$ in cell culture. Lidocaine was effective against DNP-induced cellular arrhythmias, while quinidine was not, which may indicate that there is a fundamental difference in their mechanisms of action. At least with the prostaglandins, however, there is substantial agreement between the effects on DNP-induced cellular arrhythmias, maintenance of contractility in Langendorff hearts, and in vivo

antiarrhythmic activity in the rat coronary occlusion model. It is therefore possible that at least part of the antiarrhythmic action of prostaglandins may be due to their ability to maintain metabolic processes in depressed tissue. Such a mechanism would be compatible with the suggestions of Mest et al. (1972, 1973), Forster et al. (1973), Glenn et al. (1975), and Angelakos (1975) that the "protective" effect of prostaglandins following coronary artery ligation may be the result of maintenance of cellular function.

The Effect of Prostaglandins, Lidocaine, and Quinidine on Arrhythmic Changes in Cultured Rat Heart Cells with Cyanea Toxin

We had determined that the cardiotoxic activity of Cyanea toxin was not related to an ouabain-like inhibition of the membrane ATPase cation transport systems, and that Cyanea toxin produces a characteristic series of changes in beating behavior in cultured cardiac cells. This toxin was found to produce the most severe types of arrhythmias encountered in cultured myoblasts, and we therefore decided to make use of it as a further test for a "protective" effect with our antiarrhythmic drugs. The results indicate that neither prostaglandins or the standard antiarrhythmic drugs, lidocaine and quinidine, protect against Cyanea toxin-induced arrhythmias.

None of the antiarrhythmic agents altered the maximum effect produced by the toxin on arrhythmic score, per cent beating, or the per cent arrhythmic. The time to produce 100 per cent arrhythmic beating was, however, decreased by the prostaglandins. This change would be interpreted as being arrhythmogenic, not antiarrhythmic. Such a change may be related to an

increase in membrane permeability. The arrhythmias produced by *Cyanea* toxin are similar to the arrhythmias seen with high Ca^{++} or with low K^{+} media (Goshima, 1976), and the membrane depolarization seen with *Cyanea* toxin (Walker, 1977b) would also be consistent with increased membrane permeability. This may account for the fact that both *Cyanea* toxin and adrenaline arrhythmias were made worse by prostaglandins.

This experiment provides further evidence that there is no over-all "cellular protective" action responsible for the antiarrhythmic actions of the prostaglandins. Only the previously described effects against dinitrophenol-induced arrhythmias appear to bear any correlation with the *in vivo* results.

CHAPTER V

SUMMARY

1. Prostaglandins E_2 and $F_{1\alpha}$ are effective against early arrhythmias associated with coronary occlusion in dogs.

2. Prostaglandins E_2 and $F_{1\alpha}$ were not effective against arrhythmias associated with release of occlusion.

3. A technique for comparative testing of antiarrhythmic drugs in the acute ischemic model using rats has been described.

4. The time course for arrhythmia development and for types of arrhythmias produced in rats are in agreement with previous experiments in dogs.

5. Prostaglandins E_2, $F_{2\beta}$, and quinidine were found to be most effective against early phase ischemic arrhythmias in rats.

6. The antiarrhythmic activity of prostaglandins is not related to effects on blood pressure or heart rate.

7. Since the size of infarct is not altered by prostaglandins, it is unlikely that the antiarrhythmic actions of prostaglandins result from any marked improvement in coronary circulation.

8. The antiarrhythmic activity of prostaglandins does not correlate with their effects on smooth muscle, as shown by the results obtained with $PGF_{2\beta}$.

9. Several classes of prostaglandins possess antiarrhythmic activity; they compare favorably with, and in some cases are superior to, the standard antiarrhythmic drugs lidocaine and quinidine.

10. Results with representive prostaglandins of the E, A, and F series show that some, but not all, prostaglandins produce a slight depressive effect on maximum following frequency in in vivo rat heart at antiarrhythmic doses. No effect on flutter threshold is detectable. This depressive action does not correlate with the antidysrhythmic actions of prostaglandins.

11. Prostaglandins of the E, A, and F series have only minimal effects on rate and force in Langendorff rat hearts. It is unlikely that such weak effects could account for the strong antiarrhythmic actions of prostaglandins.

12. Both PGE_2 and $PGF_{1\alpha}$ delayed the loss of contractile force with time at 10^{-7} M in isolated hearts.

13. Prostaglandins E_2, A_2, $F_{1\alpha}$, and $F_{2\beta}$ all markedly increase coronary flow at 10^{-5} M in isolated rat hearts.

14. With the exception of $PGF_{2\alpha}$, which produced a marked chronotropic response, prostaglandins have limited direct action in cultured heart cells.

15. In cultured heart cells the positive chronotropic effect of $PGF_{2\alpha}$ is not associated with the dysrhythmic tendencies seen with catecholamines.

16. The effect of ouabain, calcium, potassium, and dinitrophenol on cultured heart cells has been investigated.

17. It is possible to evaluate very different types of cellular arrhythmias in both qualitative and quantitative terms.

18. Quinidine and lidocaine produce similar decreases in automaticity in cultured heart cells; however, quinidine is approximately 100 times more potent.

19. Cyanea toxin produces a characteristic series of arrhythmogenic changes in cultured heart cells which may be useful as a test agent for antiarrhythmic drugs.

20. Lidocaine and quinidine are effective only against cellular arrhythmias caused by high calcium concentration.

21. Prostaglandins were effective only against dinitrophenol-induced cellular arrhythmias.

22. There is no over-all "protective" effect with prostaglandins in cell culture.

23. At least part of the antiarrhythmic action of prostaglandins may be due to their ability to maintain metabolic processes in depressed tissue.

24. Cyanea toxin-induced cellular arrhythmias were made worse by prostaglandins, providing further evidence that there is no over-all "cellular protective" action responsible for antiarrhythmic action of prostaglandins.

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APPENDIX

TABLE A

The effect of prostaglandins, lidocaine, and quinidine on blood pressure and heart rate during left coronary artery ligation in rat. Standard errors are given unless too few samples were available for calculation.

	Control	A_2	E_2	$F_{1\alpha}$	$F_{2\beta}$	Lidocaine	Quinidine
B.P. Control	150 125 ±7 ±4	130 102 ±6 ±8	162 131 ±13 ±10	130 104 ±24 ±22	145 112 ±23 ±21	147 117 ±6 ±5	162 128 ±8 ±12
Post Surg	114 95 ±17 ±14	115 89 ±13 ±13	127 105 ±18 ±20	87 63 ±21 ±22	120 92 ±14 ±18	125 93 ±13 ±18	96 58 ±11 ±12
Infusion	116 96 ±11 ±10	87 63 ±4 ±5	95 70 ±12 ±14	91 67 ±17 ±17	124 99 ±15 ±12	109 80 ±12 ±15	-----
5 min post occlusion	104 87 ±10 ±11	104 80 ±20 ±23	99 78 ±15 ±16	73 51 ±23 ±22	112 90 ±11 ±11	93 70 ±4 ±7	69 35 ±11 ±6
10 min post occlusion	129 112 ±5 ±8	98 75 ±21 ±26	99 75 ±15 ±17	-----	112 87 ±11 ±11	99 78 ±6 ±9	72 40 ±7 ±8
Rate: Control	425±17	443± 14	427±17	412±11	404±18	424±25	399±25
Post Surg	441±30	445	493±38	448±15	467±18	443±24	397±27
Infusion	421±13	486±22	463±24	425±18	503±27	432±34	----
5 min post occlusion	446±6	475±20	453±20	469±45	483±18	458±27	410±37
10 Min post occlusion	455	496±17	458±17	520	490±23	447	398

PG BOOK #_____PAGE_____ FLASK#_____

DATE & TIME_____ DRUG_____

OPERATOR_____ CONC._____

	CONTROLS		DRUG			
	Rate	Rate Range / Rate	Rate	% Cont.	Rate Range / Rate	% Cont.
Cell #1						
2						
3						
4						
5						
Mean						
S.E.M.						
n						

PG BOOK #_____, PAGE_____ FLASK #_____

DATE & TIME_____ DRUG_____

OPERATOR_____ CONC._____

		No.
1	1 2 3 4 5 6 7 8 9 10	___
	S S S S S S S S S S	___
	A A A A A A A A A A	___
	1 1 1 1 1 1 1 1 1 1	
	2 2 2 2 2 2 2 2 2 2	
	3 3 3 3 3 3 3 3 3 3	
	4 4 4 4 4 4 4 4 4 4	

		No.
2	1 2 3 4 5 6 7 8 9 10	___
	S S S S S S S S S S	___
	A A A A A A A A A A	___
	1 1 1 1 1 1 1 1 1 1	
	2 2 2 2 2 2 2 2 2 2	
	3 3 3 3 3 3 3 3 3 3	
	4 4 4 4 4 4 4 4 4 4	

		No.
3	1 2 3 4 5 6 7 8 9 10	___
	S S S S S S S S S S	___
	A A A A A A A A A A	___
	1 1 1 1 1 1 1 1 1 1	
	2 2 2 2 2 2 2 2 2 2	
	3 3 3 3 3 3 3 3 3 3	
	4 4 4 4 4 4 4 4 4 4	

		No.
4	1 2 3 4 5 6 7 8 9 10	___
	S S S S S S S S S S	___
	A A A A A A A A A A	___
	1 1 1 1 1 1 1 1 1 1	
	2 2 2 2 2 2 2 2 2 2	
	3 3 3 3 3 3 3 3 3 3	
	4 4 4 4 4 4 4 4 4 4	

		No.
5	1 2 3 4 5 6 7 8 9 10	___
	S S S S S S S S S S	___
	A A A A A A A A A A	___
	1 1 1 1 1 1 1 1 1 1	
	2 2 2 2 2 2 2 2 2 2	
	3 3 3 3 3 3 3 3 3 3	
	4 4 4 4 4 4 4 4 4 4	

	$\bar{x}$	S.E.M.	n
% Norm beat	___	_____	___
% Stop	___	_____	___
% Arrhy.	___	_____	___
$\bar{x}$ Arr.	___	_____	___

NOTES

Cell Appearance: Good_____ Bad_____ Other_____

Fibroblasts: Many_____ Few_____ None_____

Age (days): 1 2 3 4 5 6 7 8 9 10

Special drug details (diln., soln., etc.)_____

General Notes: