CARDIAC EFFECTS OF PROSTAGLANDINS E_1 AND $F_{1\alpha}$

by

RAO VENKATA SATYA VEERABHADRA VADLAMUDI

M. PHARM, ANDHRA UNIVERSITY, WALTAIR, INDIA, 1976

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
DIVISION OF PHARMACOLOGY AND TOXICOLOGY
OF THE FACULTY OF PHARMACEUTICAL SCIENCES

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA, 1979

© RAO V.S.V. VADLAMUDI, 1979

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmaceutical Sciences

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date Feb. 12th 1980

ABSTRACT

The mechanical and biochemical effects of prostaglandins E_1 and $F_{1\alpha}$ were studied on rat heart using isolated right and left atria and the Langendorff perfused whole heart preparation.

Preliminary experiments were performed to establish optimal perfusion conditions for the Langendorff preparation. Hearts were perfused at different perfusate temperatures and at different filling pressures. Heart rate and coronary flow rate were monitored at all combinations of perfusate temperature and filling pressure. A constant temperature water recirculating pump setting of 38°C and a filling pressure of 40 cm of H_2O were chosen as the optimal perfusion conditions. Hearts perfused under the above conditions responded normally to bolus injections of isoproterenol. Isoproterenol produced a dose dependent increase in the contractile force of the Langendorff preparation and the cyclic AMP increasing effect of isoproterenol preceded the positive inotropic effect in a time course study.

Prostaglandin E_1 (PGE_1) did not produce any effect on heart rate or tension development in the Langendorff preparation, when infused over a dose range of 0.03 μg to 5.0 $\mu\text{g}/\text{min}$. Infusion of prostaglandin $F_{1\alpha}$ ($\text{PGF}_{1\alpha}$)

(0.1 to 5.0 $\mu\text{g}/\text{min}$) produced an increase in tension development which was associated with a negative chronotropic effect. The positive inotropic effect of $\text{PGF}_{1\alpha}$ was secondary to the drop in rate as the positive inotropic effect was completely abolished when the hearts were paced at 6 Hz.

In the rat right atrium, PGE_1 produced a dose dependent increase in the rate which developed very slowly. PGE_1 had no effect on the tension development of the rat left atrium. $\text{PGF}_{1\alpha}$ produced a slow, dose dependent positive chronotropic effect on the right atrium and a slight but not significant effect on the force of contraction of the left atrium. Both prostaglandins were equipotent in exerting their positive chronotropic effect on the right atrium. The PD_2 value for PGE_1 was 5.54 ± 0.25 and for $\text{PGF}_{1\alpha}$ 5.59 ± 0.18 .

In the right atrium 10^{-4}M PGE_1 increased the rate and cyclic AMP content without changing phosphorylase a activity or cyclic GMP content. $\text{PGE}_1(10^{-4}\text{M})$ slightly but not significantly increased the left atrial cyclic AMP content and did not change the cyclic GMP content. 10^{-4}M $\text{PGF}_{1\alpha}$ did not affect either right or left atrial cyclic AMP or cyclic GMP content.

The effect of a 1 $\mu\text{g}/\text{min}$ infusion of either PGE_1 or $\text{PGF}_{1\alpha}$ on the changes of cyclic AMP and cyclic GMP contents and phosphorylase a activity with time were studied

in the Langendorff preparation. A 1 $\mu\text{g}/\text{min}$ infusion of PGE_1 increased the myocardial cyclic AMP levels by about 57 per cent above control at 30 secs after starting the infusion and the cyclic AMP levels were still elevated by 50 per cent over control at the end of a one minute period of infusion. PGE_1 did not change cyclic GMP content or phosphorylase a activity at any time point. A 1 $\mu\text{g}/\text{min}$ infusion of $\text{PGF}_{1\alpha}$ did not alter cyclic AMP and cyclic GMP levels or phosphorylase a activity in the rat heart within one minute.

These results supported the earlier reported observation that PGE_1 selectively increased rat myocardial cyclic AMP content without altering myocardial contractile force or phosphorylase a activity. PGE_1 might be selectively increasing a pool of cyclic AMP and activating a cyclic AMP-dependent protein kinase in the cardiac cells that is not associated with contractile force or phosphorylase activation. $\text{PGF}_{1\alpha}$ did not possess this selective effect of PGE_1 . Cyclic GMP is not involved in the mediation of the actions of either PGE_1 or $\text{PGF}_{1\alpha}$ on the rat heart.

LIST OF CONTENTS

	<u>Page</u>
ABSTRACT	i
LIST OF FIGURES AND TABLES	v
ACKNOWLEDGEMENT	viii
INTRODUCTION	1
History of Prostaglandins	1
Chemistry	2
Biosynthesis	3
Cardiovascular Effects of Prostaglandins	9
Blood Pressure	9
Inotropic and Chronotropic Effects	10
Effect on Coronary Circulation	13
Prostaglandins and Autonomic Neuro- transmission in the Heart	15
Prostaglandins and Cyclic Nucleotides	17
Role of Cyclic AMP in Cardiac Contraction	21
Purpose of the Investigation	25
MATERIALS AND METHODS	28
RESULTS	43
DISCUSSION	82
BIBILIOGRAPHY	93

LIST OF FIGURES

		<u>Page</u>
Fig. 1	Structural aspects prostaglandins	6
Fig. 2	Biosynthetic pathway of prostaglandins ...	8
Fig. 3	Typical standard curves for cyclic AMP and cyclic GMP	36
Fig. 4	Effect of changing filling pressures on rate of isolated rat hearts perfused at different temperatures	53
Fig. 5	Effect of changing filling pressures on coronary flow rate of isolated rat hearts perfused at different temperatures	55
Fig. 6	Effect of isoproterenol on the force development of rat Langendorff preparation	57
Fig. 7	Effect of isoproterenol on changes in cyclic AMP content and contractile force with time in isolated perfused rat hearts.	59
Fig. 8	Effect of prostaglandin $F_{1\alpha}$ on rat Langendorff preparation	61
Fig. 9	Effect of prostaglandin E_1 on spontane- ously beating rat right atrium	63
Fig. 10	Effect of prostaglandin E_1 on the tension development of the electrically paced rat left atrium	65

	<u>Page</u>
Fig. 11 Effect of prostaglandin $F_{1\alpha}$ on spontaneously beating rat right atrium	67
Fig. 12 Effect of prostaglandin $F_{1\alpha}$ on electrically paced rat left atrium	69
Fig. 13 Effect of 10^{-4} M PGE_1 on right atrial rate, cyclic AMP content and phosphorylase <u>a</u> activity	71
Fig. 14 Effect of 10^{-4} M $PGF_{1\alpha}$ on the right atrial rate and cyclic AMP content	73
Fig. 15 Scheme representing compartmentalization of cardiac cell	89

LIST OF TABLES

		<u>Page</u>
Table 1	Effect of 10^{-4} M PGE ₁ and 10^{-4} M PGF _{1α} on right atrial cyclic AMP and cyclic GMP content	75
Table 2	Effect of 10^{-4} M PGE ₁ and 10^{-4} M PGF _{1α} on left atrial cyclic AMP and cyclic GMP content	77
Table 3	Effect of PGE ₁ on cyclic AMP and cyclic GMP content and phosphorylase <u>a</u> activity of the rat Langendorff preparation	79
Table 4	Effect of PGF _{1α} on cyclic AMP and cyclic GMP content and phosphorylase <u>a</u> activity of the rat Langendorff preparation	81
Table 5	Summary of the effects of PGE ₁ and PGF _{1α} on rat isolated atria and Langendorff preparation	83

ACKNOWLEDGEMENT

I am deeply indebted to Dr. J.H. McNeill for his valuable guidance and encouragement throughout this study. I would like to thank the Upjohn Company for providing us with Prostaglandins E_1 and $F_{1\alpha}$, and I would also like to acknowledge the financial assistance of the British Columbia Heart Foundation.

INTRODUCTION

HISTORY:

The prostaglandins are naturally occurring, unsaturated fatty acid derivatives which possess a wide range of biological activity and are implicated in the regulation of many cell processes. The first report leading to the discovery of prostaglandins was made by Kurzrok and Leib (1930). They reported that fresh human semen could induce strong contraction or relaxation of human uterus. Five years later Goldblatt (1935) reported on the smooth muscle stimulatory effect of human semen. In 1936 Euler reported that lipid soluble extracts of human and certain mammalian seminal fluid had smooth muscle stimulating and blood pressure lowering effects. Euler gave the name 'prostaglandin' to the active factor present in the extract, indicating that the origin of the active factor was the prostate gland. Isolation of the first pure prostaglandin was achieved in 1960 by Bergstrom et al. Bergstrom et al. in 1962 reported that prostaglandin was not a single substance but a group of chemically related compounds. Initially prostaglandins were thought to be present only in male accessory sexual glands and their secretions, but later they were found to

occur in almost every animal species and every animal cell or tissue that has been studied.

CHEMISTRY:

Structurally prostaglandins are derivatives of the hypothetical compound prostanoic acid (Fig. 1). They contain a cyclopentane ring with two adjacent carbon side chains, one of the chains containing a carboxyl group at the terminal position. The prostaglandins were categorized into nine groups which were conveniently referred to by letters E, F, A, B, C, D, G, H and I. Members of each group differ from the others by having different functional groups at different positions on the cyclopentane ring (Fig. 1).

As a general rule all the prostaglandins belonging to the above groups contain a C_{13-14} trans double bond and a $C_{15-\alpha}$ -hydroxyl group. The prostaglandins were further divided into subgroups, which were referred to by numbers: 1, 2 and 3, indicating the number of double bonds present in the side chains.

In 1976, Moncada et al. discovered a potent unstable prostaglandin derivative which had vasodilator and platelet aggregation inhibitory properties, which initially was named

PGX. It was chemically characterized and was found to be a prostaglandin derivative with a different ring structure (Fig. 1). Johnson et al. in 1976 renamed PGX as prostacyclin and this compound is referred to as PGI_2 .

BIOSYNTHESIS:

The biological precursors of prostaglandins are C_{20} unsaturated fatty acids such as arachidonic acid, dihomono- γ -linolenic acid and eicosapentaenoic acid (Moncada and Vane, 1979). In mammalian tissue arachidonic acid is the main precursor for all bis-enoic prostaglandins and arachidonic acid can be obtained from the diet or by dehydrogenation and chain elongation of the essential fatty acid linoleic acid (Moncada and Vane, 1979). Arachidonic acid is usually incorporated into the phospholipids of the cell membrane in all tissues (Ramwell et al., 1977). Arachidonic acid can be released from the cell membrane phospholipids by the action of the enzyme phospholipase A_2 [Flower and Blackwell (1976)]. The released arachidonic acid is converted to various prostaglandins through the formation of various active intermediates, by an enzyme complex known as prostaglandin synthetase (Fig. 2). Prostaglandin biosynthesis essentially consists of three steps. In the first step arachidonic acid undergoes a two-fold oxygenation forming

a 15-hydroperoxy endoperoxide intermediate. This reaction is catalysed by an enzyme called arachidonic acid cyclooxygenase. The 15-hydroperoxy endoperoxide intermediate has been termed as PGG_2 by Hamberg and Samuelsson (1973). PGG_2 is very unstable and undergoes enzymatic reduction by glutathione, in the presence of glutathione peroxidase resulting in the formation of a second unstable, highly reactive intermediate, the 15-hydroxy endoperoxide, which was given the name PGH_2 (Hamberg and Samuelsson, 1973). This reaction is the second step in prostaglandin synthesis.

The third step involves isomerization of PGH_2 into various prostaglandins. These reactions are catalysed by various prostaglandin isomerases. In different tissues different prostaglandins are formed as major prostaglandins. For example prostacyclin is the major prostaglandin produced by the vascular wall in most of the animal species studied so far (Dusting et al., 1977). Thromboxane A_2 which is produced by the action of thromboxane synthetase on PGH_2 , has been shown to be the major prostaglandin compound produced in platelets (Hammarstrom and Falardeau, 1977).

5.

Figure 1

Structural Aspects of Prostaglandins

Structural formulae for the hypothetical compound prostanoic acid and various substituents on the cyclopentane ring of different prostaglandins are shown.

PROSTANOIC ACID

I

7.

Figure 2

Biosynthetic pathway of prostaglandins

BIOSYNTHESIS

CARDIO VASCULAR EFFECTS OF PROSTAGLANDINS

BLOOD PRESSURE:

The blood pressure lowering property of prostaglandins was observed initially by Goldblatt (1933, 1935) and Euler (1936). These investigators independently reported that extracts of human seminal plasma produced a fall in blood pressure. Prostaglandins of the E series lower arterial blood pressure in dog (Bergstrom et al., 1964), cat (Koss et al., 1973), rabbit (Bergstrom and Euler, 1963), rat (Weeks et al., 1969) and mouse (Weeks, 1969). The blood pressure lowering effects of E prostaglandins are dependent on the route of parenteral administration, due to rapid uptake and metabolism of these compounds by the liver and lungs (Ferreira and Vane, 1967). E prostaglandins produce a fall in blood pressure due to their direct vasodilator action on resistance vessels thus causing a decrease in total peripheral resistance. This effect of E-prostaglandins is independent of cholinergic mechanisms (Bergstrom, 1959) and adrenergic mechanisms (Nakano and McCurdy, 1967). Prostaglandins of the A series have qualitatively similar effects to PGE compounds and they exhibit potent blood pressure lowering effects.

The effect of F-prostaglandins on blood pressure varies with the species. PGF compounds lower blood pressure in the rabbit (Bergstrom and Euler, 1963) and cat (Koss et al., 1973), and produce a rise in the blood pressure in rat and dog (Nakano and McCurdy, 1968). The recently discovered prostacyclin (PGI_2) lowers blood pressure in the dog and rat (Weeks, 1978).

INOTROPIC AND CHRONOTROPIC EFFECTS:

The effects of prostaglandins on myocardial contractile force and heart rate vary with the species and preparation used and the experimental conditions employed.

Nakano and McCurdy (1968) showed that the intravenous administration of PGE compounds or PGA compounds to the anesthetized dog produced an increase in myocardial contractile force and heart rate. However, administration of PGE_1 , PGA, or $\text{PGF}_{2\alpha}$ to isolated atria of dog did not produce any change in the force and frequency of contraction (Su et al., 1973). PGE_1 when administered to the isolated blood perfused sinus node of the dog did not produce a significant change in the rate of contraction, but did produce a significant, dose-dependent increase in the tension development and dT/dt of the dog isolated papillary muscle (Endoh, 1976).

In the cat, Koss et al. (1973) showed that PGE_1 produced a delayed tachycardia and $\text{PGF}_{2\alpha}$ produced bradycardia. PGE_1 did not exert any effect on heart rate or force of contraction in the isolated cat heart (Berti et al., 1965). Su et al. (1973) demonstrated that PGE_1 , PGA_1 and $\text{PGF}_{2\alpha}$, all produced an increase in the rate of cat right atria without exerting any effect on the tension development of either the right atria or the right ventricle strip. PGE_1 was also shown to have a positive chronotropic effect on the cat right atrium by Kaumann et al. in 1974.

Berti et al. (1965) reported that PGE_1 did not change heart rate or force of contraction in isolated perfused rabbit hearts at low doses, but produced a negative inotropic effect when administered in high doses. PGE_1 , PGA_1 and $\text{PGF}_{1\alpha}$ when administered to isolated rabbit atria, did not exert any effect on the force or frequency of contraction (Nutter and Crumley, 1972; Nutter and Ratts, 1973).

In the guinea pig, PGE_1 increased heart rate and force of contraction in the isolated perfused hearts (Berti et al., 1965). The E series and F series prostaglandins increased both the rate and contractile force of isolated guinea pig atria, and slightly increased the tension development in papillary muscles (Förster et al., 1974).

PGF compounds did not show any effect on the guinea pig isolated perfused heart (Sobel and Robison, 1969). PGA compounds were shown to exert a positive inotropic effect on the guinea pig isolated left atria (Nutter and Ratts, 1973).

Reports of the effects of prostaglandins, particularly those of the E series, on the rat heart are controversial. A high dose of PGE_1 produced a positive inotropic effect in the isolated perfused rat heart, without producing any change in coronary flow or heart rate (Berti et al., 1965). Vergroesen et al. in 1968 studied the effects of PGE_1 , PGE_2 and PGF_1 on isolated rat hearts and they reported that PGE_1 produced a moderate increase in contractile force without having any effect on the heart rate while $\text{PGF}_{1\alpha}$ produced a sharp increase in contractile force without altering heart rate. PGE_1 produced a positive inotropic effect on the spontaneously beating rat atrium when administered in a concentration range of $1 \times 10^{-7}\text{M}$ to $1 \times 10^{-4}\text{M}$ (Kangasaho et al., 1978). The positive inotropic effect produced by PGE_1 developed slowly and the prostaglandin did not change atrial rate. However, recent studies by Keely (1977, 1979) and Reese et al. (1978) showed that PGE_1 administration to the rat Langendorff preparation was without any effect on the tension development or heart rate.

EFFECT ON CORONARY CIRCULATION:

Prostaglandins of E and A series increased coronary blood flow and decreased coronary vascular resistance in most of the animal species studied, both in intact animal and in isolated heart preparations (Berti et al., 1965; Bloor and Sobel, 1970; Higgins et al., 1971; Nakano and McCurdy, 1968; Nutter and Crumly, 1970 and Vergroesen et al., 1967). PGE_1 is the most potent agent among the E and A series prostaglandins, in producing coronary vasodilation. Though the F prostaglandins are pressor in some animal species, they do not influence the coronary circulation (DuCharme et al., 1968). DeDeckere et al. in 1979 showed that $\text{PGF}_{2\alpha}$, a pressor agent in the rat, produced a delayed increase in coronary flow in isolated rat heart. These authors demonstrated that $\text{PGF}_{2\alpha}$ stimulated the release of PGE_2 and PGI_2 , which are potent coronary vasodilators. Prostacyclin, the major prostaglandin synthesised and released in hearts, causes coronary vasodilation in isolated hearts from rabbit, guinea-pig and rat (Schrör et al., 1978).

Ten Hoor and Vergroesen in 1975 suggested that prostaglandins might be involved in the regulation of coronary blood flow. The experimental evidence supporting this hypothesis has been recently reviewed by Parratt and Marshall (1978). These authors categorized the

evidence into the following classes.

i) Effect of prostaglandins on coronary blood flow

Enough experimental data has been reported in recent years showing that prostaglandins of the E and A series and PGI_2 are very potent coronary vasodilators and that these compounds cause vasodilation by a direct action on vascular smooth muscle.

ii) Synthesis of Prostaglandins in the heart

Prostacyclin is the major prostaglandin produced in the heart from arachidonic acid (Needleman, 1978). It was shown that administration of arachidonic acid to isolated perfused hearts or intact animals leads to increased production of prostaglandin-like substances in the coronary vessels and a marked increase in coronary blood flow (Needleman, 1976; Hintz and Kaley, 1977).

iii) Effect of pathological conditions

Prostaglandins, particularly PGE_2 and PGA_2 were released in the heart during experimentally-induced ischemia by occluding the left anterior descending coronary artery for ten minutes (Kraemer et al., 1976). Prostaglandin-like substances were shown to be released into the coronary venous effluent in isolated perfused rabbit hearts during anoxia and hypoxia (Needleman et al., 1975).

iv) Effects of vasoactive drugs

Vasoactive drugs such as bradykinin (Needleman et al., 1975), noradrenaline, acetylcholine (Wennmalm, 1975) angiotensin I and II (Needleman et al., 1973) have all been shown to induce myocardial release of prostaglandins. Most of these compounds increase coronary blood flow and they might be doing so by releasing prostaglandin-like substances in the heart.

Experimental evidence presented above suggests a role for prostaglandins in the regulation of coronary blood flow. Adenosine might be playing a role in the regulation of coronary blood flow and observations made by Zehl et al. in 1976, showed that prostaglandins release adenosine and adenosine releases prostaglandins in the heart. These observations support the hypothesis that both prostaglandins and adenosine are involved in the regulation of coronary blood flow.

PROSTAGLANDINS AND AUTONOMIC NEUROTRANSMISSION IN THE HEART:

Apart from being involved in a possible regulatory role in the maintenance of coronary blood flow, prostaglandins might be playing a role in the regulation of autonomic neurotransmission in the heart.

Hedquist and Brundin (1969) showed that PGE_1 inhibited the mechanical responses induced by sympathetic nerve stimulation in perfused cat spleen. This observation suggested that PGE compounds might be acting as regulators of transmitter release at the sympathetic neuro-effector junction. Hedquist et al. (1970) showed in their experiments with isolated perfused rabbit hearts that exogenously administered PGE_1 inhibited the release of noradrenaline from sympathetic nerve endings. Sympathetic nerve stimulation, or noradrenaline infusion increased the rate of synthesis of prostaglandins in rabbit hearts, as detected by an increased outflow of prostaglandin-like substances in the venous effluent (Wennmalm, 1971). The prostaglandin like materials released by sympathetic nerve stimulation from isolated hearts labelled with ^{14}C -arachidonic acid were shown to be capable of inhibiting the sympathetic neurotransmitter release in guinea-pig vas deferens (Wennmalm, 1978). Wennmalm in 1976 showed that indomethacin (a prostaglandin synthetase inhibitor) pretreatment caused an increased outflow of noradrenaline after sympathetic nerve stimulation in the rabbit hearts. Wennmalm (1976) concluded that all the experimental results so far obtained support the hypothesis that endogenous PGE compounds function as a link in the

negative feed-back regulation of transmitter release from sympathetic nerve endings, as originally suggested by Hedquist in 1970.

Endogenous prostaglandins might not be playing a key role in the regulation of transmitter release from the parasympathetic nerve endings. Vagal nerve stimulation or infusion of acetylcholine into isolated rabbit hearts stimulated the release of prostaglandins and this release was blocked by atropine (Junstad and Wennmalm, 1974). Hadhazy et al. in 1973 showed that exogenously administered PGE_1 inhibited the negative chronotropic effects of either vagal nerve stimulation or acetylcholine administration. These authors also showed that exogenously administered PGE_1 did not inhibit acetylcholine release in the guinea pig ileum. They concluded that the anticholinergic effect of PGE_1 in guinea pig atria might be due to its effect on cyclic AMP system and endogenous prostaglandins might not be involved in the regulation of parasympathetic system in the heart.

PROSTAGLANDINS AND CYCLIC NUCLEOTIDES

The mechanism of action of prostaglandins on various biological systems has only been partly elucidated

and might proceed through influencing intracellular cyclic nucleotide levels. The interaction of prostaglandins with cyclic AMP system was first discovered by Steinberg et al. (1964). They showed that in fat cells, prostaglandins inhibit the actions of lipolytic agents by decreasing the levels of cyclic AMP. Based on the above observation Ramwell and Shaw in 1966 proposed that prostaglandins may be acting as negative feed back regulators of cyclic AMP action. This negative feed back concept was ruled out as an exception in adipose tissue, as prostaglandins were shown to stimulate cyclic AMP production in several other tissues (Butcher and Baird, 1968). Out of all the prostaglandins studied, the E-prostaglandins are very efficacious in elevating the cyclic AMP levels, particularly in lymphocytes (Bourne et al., 1972) and platelets (Wolf and Shulman, 1969).

A binding protein with high-affinity for E-type prostaglandins has been shown to exist in isolated fat cells (Kuehl and Humes, 1972). This binding protein is in fact a prostaglandin E receptor and was demonstrated to be present in many other tissues such as rat stomach, liver, thyroid and leuteal cell membranes (Kuehl, 1974). The PGE receptor was shown to be membraneous in nature and might have been linked with the enzyme adenylate cyclase (Oien et al., 1973).

The F-type prostaglandins mimic PGE compounds at at least some of the PGE receptors and stimulate cyclic AMP production (Kuehl, 1974). But the higher concentrations of PGF compounds required to mimic the PGE-compounds led to the suggestion that PGF compounds might act at a different receptor site and that their actions were mediated through a different transmitter (Kuehl et al., 1973). Evidence for the existence of a unique PGF receptor site in the homogenates of ovine corpora lutea has been reported and the mediator involved in the mechanism of action of PGF compounds in this tissue was found to be cyclic GMP (Powell et al., 1973). PGF compounds produce opposite effects to those produced by PGE compounds in certain tissues such as uterus, bronchioles and blood vessels. The fact that PGE compounds act through cyclic AMP and PGF compounds act through cyclic GMP correlates well with Goldberg's yin-yang hypothesis of cell regulation (Kuehl, 1974).

Prostaglandins have been found to increase cyclic AMP levels in the myocardium. Sobel and Robison (1969) reported that PGE_1 and $\text{PGF}_{1\alpha}$ stimulated adenylate cyclase activity in guinea pig heart homogenates. Klein and Levey in 1971 studied the effect of prostaglandins E_1 , E_2 , A_1 , $\text{F}_{1\alpha}$ and $\text{F}_{2\alpha}$ on the adenylate cyclase activity of the particulate fraction of guinea pig heart homogenates. They reported

that PGE_1 , E_2 and A_1 produced increases in cyclic AMP content whereas $\text{PGF}_{1\alpha}$ and $\text{PGF}_{2\alpha}$ did not alter cyclic AMP production. However Levey and Klein in 1973 showed that $\text{PGF}_{1\alpha}$ and $\text{PGF}_{2\alpha}$ stimulated adenylate cyclase activity of the solubilised myocardial adenylate cyclase preparation from guinea pigs and cats. It is well documented in the literature that increasing the levels of cyclic AMP will lead to the activation of the enzyme phosphorylase a (Namm and Mayer, 1968; Namm et al., 1968). Piccinini in 1965 and Curnow and Nuttal in 1971 and 1974 reported that prostaglandin E_1 caused activation of phosphorylase a in the rat heart. Recent observations made by Keely in 1977 and 1979 and Reese et al. in 1978 on the effect of PGE_1 on isolated perfused rat hearts showed that PGE_1 increased myocardial cyclic AMP levels and stimulated the cyclic AMP-dependent protein kinase activity without altering myocardial contractile force or phosphorylase a activity. Keely (1979) and Reese et al. (1978) also showed that the myocardial phosphorylase kinase activity was unaffected. However, Kangasaho et al. in 1978 reported that PGE_1 , when administered to spontaneously beating rat atria, produced a positive inotropic effect and elevated the cyclic AMP content of the atria. They also showed that PGE_1 had no effect on the atrial rate and concluded that the positive inotropic

effect of PGE_1 might be mediated through elevation of the cyclic AMP content.

ROLE OF CYCLIC AMP IN CARDIAC CONTRACTION:

Sutherland and Rall in 1958 discovered cyclic AMP in the liver as a heat stable factor which increased after the administration of adrenaline. Various experimental studies indicated that the actions of a variety of drugs and hormones are associated with the elevation of intracellular cyclic AMP levels. Sutherland and coworkers in 1968 put forward the famous second messenger hypothesis, stating that cyclic AMP is the second messenger involved in the mediation of effects of various drugs and hormones which are the first messengers. The possible involvement of cyclic AMP in the mediation of cardiac effects of catecholamines was first reported by Murad et al. (1962). Robison et al. (1965) and Cheung and Williamson (1965) presented experimental evidence for the association between cyclic AMP and the cardiac effects of epinephrine. These workers showed that injection of epinephrine into isolated perfused rat hearts elevated the cyclic AMP content and that this elevation preceded the positive inotropic and glycogenolytic effects of epinephrine. The positive inotropic effects of other cardiotonic drugs such as histamine (McNeill and Verma, 1974)

and glucagon (Brunt and McNeill, 1978) have also been suggested to be mediated through elevated myocardial cyclic AMP levels. The possible mechanism by which cyclic AMP mediates the inotropic and metabolic effects of the cardio active drugs is as follows: The cardio-active drugs stimulate the activity of the membrane-bound enzyme adenylate cyclase by binding to the corresponding membrane-bound receptors. The activated adenylate cyclase catalyses the hydrolysis of ATP to cyclic AMP. The cyclic AMP thus formed, before being inactivated by the enzyme phosphodiesterase, activates the cyclic AMP-dependent protein kinase in the cell. The activated protein kinase phosphorylates troponin of the contractile proteins and the sarcoplasmic reticulum (SR) membrane protein. Phosphorylation of troponin enhances binding of Ca^{++} to the contractile elements, thereby increasing the contractile activity. Phosphorylation of SR membrane protein results in increased rate of Ca^{++} uptake by SR, thereby increasing the rate of relaxation of cardiac muscle. The cyclic AMP-dependent protein kinase also phosphorylates phosphorylase kinase to an active state, which in turn catalyses the conversion of the inactive phosphosylase b enzyme to the active phosphorylase a enzyme. Phosphorylase in the a form causes the breakdown of glycogen to glucose-1-phosphate in cardiac muscle (Nayler, 1977)

Recently, a number of studies have been published which question the association between cyclic AMP and myocardial contractility or glycogen metabolism. For example, Henry et al. (1975) showed that glucagon produced a positive inotropic effect in both guinea pig and rat hearts, but the positive inotropic effect was associated with an increase in cyclic AMP content only in the rat heart. Venter and coworkers in 1975 reported that, in cat papillary muscle preparation, isoproterenol bound covalently to glass beads produced a positive inotropic effect without producing any detectable change in cyclic AMP levels. Keely (1977, 1979) and Reese et al. (1978) reported a dissociation between cyclic AMP and glycogen metabolism by showing that PGE_1 produced an increase in cyclic AMP levels and activated cyclic AMP dependent protein kinase without activating the phosphorylase a system in perfused rat hearts.

Cyclic AMP-dependent protein kinases play a major role in the functioning of cyclic AMP as a second messenger. Recently, experimental evidence was presented showing that the positive inotropic effects of epinephrine (Keely and Corbin, 1977) and histamine (Keely and Eiring, 1979) are mediated through or at least associated with, the activation of cyclic AMP-dependent protein kinases. Cyclic AMP-dependent protein kinases are widely distributed in most

of the tissues examined and they perform different functions. There exists a possibility that the cyclic AMP dependent protein kinases might be distributed in different compartments in a cell and may be performing different functions. Recently, experimental evidence has been presented for the existence of two different types of cyclic AMP-dependent protein kinases in cardiac tissue (Corbin et al., 1977; Corbin and Keely, 1977). These authors showed that cyclic AMP-dependent protein kinase from myocardial tissue exists in two forms, isozymes I and II, based on the different elution profiles of these protein kinase isozymes from DEAE-cellulose column. The protein kinase isozymes showed broad species variation in their distribution. Rat and mouse hearts contained predominantly isozyme I whereas beef and guinea pig hearts contained isozyme II. Rabbit and human hearts contained equal amounts of both isozymes (Corbin and Keely, 1977). Both the isozymes contained the same catalytic subunit but the regulatory subunits were different. Both of the isozymes exhibited different association and dissociation patterns between the catalytic and regulatory subunits.

In the in vivo situation isozyme II is essentially membrane bound and when the intracellular cyclic AMP content increases, the active catalytic subunit is

dissociated into the cytoplasm due to the formation of a cyclic AMP and regulatory subunit complex. Activation of type II isozyme might mediate the inotropic and metabolic effects of drugs and hormones. Recently experimental evidence was presented showing that isozyme II is involved in the mediation of the effects of epinephrine in the rabbit heart (Hayes et al., 1979). These authors reported that epinephrine produced a positive inotropic effect and activated phosphorylase a by activating the particulate bound cyclic AMP-dependent-protein kinase (isozyme II) whereas PGE₁ did not activate particulate protein kinase and did not affect myocardial contractile force or the phosphorylase a enzyme. This evidence clearly demonstrates a relationship between type II protein kinase and the inotropic and metabolic effects of epinephrine.

PURPOSE OF THE INVESTIGATION

From the above discussion, it is evident that the previous studies of biochemical and mechanical effects of PGE₁ and PGF_{1 α} on the rat heart agree in some areas and conflict in some others. Studies indicate that PGE₁ increases myocardial cyclic AMP levels and PGF_{1 α} increased contractile force. However, the effect of PGE₁ on myocardial

contractile force and phosphorylase a activity are not clearly established and studies published thus far are conflicting. Further, the effect of $\text{PGF}_{1\alpha}$ on myocardial cyclic AMP levels and the phosphorylase a system and the effects of either PGE_1 or $\text{PGF}_{1\alpha}$ on myocardial cyclic GMP levels remain unknown.

The mechanism of action of PGE_1 and $\text{PGF}_{1\alpha}$ on the heart has not been clearly established. Previous studies on isolated perfused rat hearts indicated that PGE_1 increased myocardial cyclic AMP levels without exerting any effect on either myocardial contractile force or the phosphorylase a system (Keely, 1977, 1979; Reese et al., 1978 and Hayes et al., 1979). However, studies on the effect of PGE_1 on isolated spontaneously beating rat atria showed that PGE_1 produced a positive inotropic effect and increased atrial cyclic AMP levels (Kangasaho et al., 1978). The former studies indicated either a dissociation between cyclic AMP and the inotropic effect, or a compartmentalization of cyclic AMP where PGE_1 specifically elevated the cyclic AMP content in a particular compartment which is not involved in the mediation of the inotropic effect. However, the latter study (Kangasaho et al., 1978) showed an association between cyclic AMP and inotropy.

The main purpose of this investigation was to verify whether PGE_1 specifically increased myocardial cyclic AMP

levels without altering contractile force or phosphorylase a activity and also to determine its effects on isolated atria. The effect of $\text{PGF}_{1\alpha}$ on the myocardial cyclic AMP, contractile force and phosphorylase a activity were also studied and were compared with that of PGE_1 to determine if $\text{PGF}_{1\alpha}$ also possessed the specific effect of PGE_1 on the rat heart. The effect of $\text{PGF}_{1\alpha}$ on isolated atria was also investigated.

We also wished to study the involvement of cyclic GMP in the mechanism of action of both PGE_1 and $\text{PGF}_{1\alpha}$ on rat heart. The effects of PGE_1 and $\text{PGF}_{1\alpha}$ on changes of myocardial contractile force and cyclic nucleotides with time were studied and compared.

MATERIALS AND METHODS

MATERIALS

Heparin sodium and DL-isoproterenol hydrochloride were obtained from Sigma Chemical Company, St. Louis, Missouri, U.S.A. Prostaglandin E_1 and Prostaglandin $F_{1\alpha}$ were generous gifts from the Upjohn Company, Kalamazoo, Michigan, U.S.A. Radioimmuno assay kits for cyclic AMP and cyclic GMP were obtained from Becton-Dickenson immuno diagnostics, New York, U.S.A. Reagents for phosphorylase assay such as, Tris, glycogen, D-glucose-1-phosphate, 5'-adenosine monophosphate and 1-amino-2-naphthol-4-sulfonic acid were all obtained from Sigma Chemical Company. The prostaglandins were dissolved in absolute alcohol (10 mg/ml stock solution) and were made up to the final concentration with Chenoweth-Koelle buffer. All of the radioimmuno assay kit reagents were reconstituted with either distilled water or sodium acetate buffer.

METHODS

(i) Removal of Heart:

Wistar rats of either sex (250-300 g) were used throughout the study. The rats were given food (Purina Lab

chow) and water ad libitum. Heparin sodium was injected intraperitoneally (1000 I.U./kg) ten minutes before sacrifice. The rats were stunned by a sharp blow on the base of the skull and were decapitated using a guillotine. Hearts were quickly excised and were placed in oxygenated Chenoweth-Koelle solution (Chenoweth and Koelle, 1946) at room temperature. Hearts were perfused through the aorta according to the constant pressure Langendorff technique (Langendorff, 1895).

(ii) Heart Perfusion

The constant pressure perfusion apparatus consisted of a movable jacketed reservoir connected to the perfusion cannula through a Pyrex[®] jacketed water condenser, and a one way stop-cock (B-D[®], 5035-MS type). The perfusion pressure was controlled by adjusting the height of the movable reservoir. The perfusate level in the reservoir was maintained by delivering the perfusate from a second reservoir at a constant rate. The perfusate was constantly aerated with a mixture of 95% oxygen and 5% carbon dioxide. The temperature of the perfusate was maintained constant by circulating water at the required temperature through the jacketed reservoir and condenser by means of a water recirculating pump (Haake[®] - constant temperature water

recirculating pump). The hearts were perfused with Chenoweth-Koelle solution containing the following in millimolar quantities: NaCl - 119.8; KCl - 5.63; CaCl_2 - 2.18; MgCl_2 - 2.12; D-glucose - 9.99 and NaHCO_3 - 25.0. The solution when aerated with a mixture of 95% oxygen and 5% carbon dioxide, at 37°C had a pH of approximately 7.4.

The hearts were secured to the inflow cannula through the aortic stump, by means of a silk thread. The cannula was tied in such a way that it did not occlude the coronary artery. A silk thread was attached to the apex by means of a Palmer clip. The other end of the thread was attached to a force displacement transducer (Grass® FT03 type), through a steel pulley. Diastolic tension was adjusted to 1 gm and the heart rate and tension developed were recorded on a Polygraph (Grass® model 79). The hearts were allowed to equilibrate for 20 minutes before any drug administrations were made. Drugs were administered by either bolus injection or by infusing at a rate of 0.5 ml/min. Bolus injections were given through 'Intramedic', PE-10 gauge, polyethylene tubing, which enabled the drugs to be delivered at a fixed point above the aorta. Infusions were made by means of a Harvard apparatus compact infusion pump, and the infusate was delivered at a fixed point into the latex tubing above the aorta. Hearts were frozen by the

application of modified Wollenberger tongs (Wollenberger et al., 1960) precooled in liquid nitrogen before and at various times after the administration of drugs. The frozen hearts were stored at -80°C until being assayed for cyclic nucleotides and phosphorylase a activity.

ISOLATED ATRIAL PREPARATION.

After the hearts were quickly removed from the rats, they were placed in oxygenated Chenoweth-Koelle solution. The atrial pair was separated by removing ventricular and other extraneous tissue with fine scissors. The right and left atria were separated from each other, such that the sino atrial (SA) node was attached to the right atrium. The separated atria were suspended in 20 ml baths containing oxygenated Chenoweth Koelle solution at 37°C . One end of the atrium was attached to a stimulating electrode and the other end to a force displacement transducer by means of a silk thread. Spontaneously beating right atria were used to measure changes in heart rate. The left atria were electrically paced at 0.7 Hz and were used to measure changes in contractile force. The heart rate and tension developed were recorded on a Grass model polygraph. The tissues were allowed to equilibrate for half an hour with frequent washings,

usually once every 10 minutes. Drug solutions were added to the 20 ml baths, to make the required final drug concentrations. The atria were frozen either before or after drug administration, by applying tongs precooled in liquid nitrogen and the frozen tissues were stored at -80°C until assayed for cyclic nucleotides and phosphorylase a activity.

CYCLIC NUCLEOTIDE ASSAYS

The cyclic AMP and cyclic GMP contents of the frozen myocardial tissue were estimated by radioimmuno assay according to Steiner et al. (1972) using commercially available Becton-Dickenson radioimmuno assay kits.

EXTRACTION OF CYCLIC NUCLEOTIDES

About 50-70 mg of the frozen tissue was weighed (weighing was completed while the tissue was still frozen) and was homogenized in 2 ml of 6% trichloroacetic acid (TCA) using a Polytron[®] homogeniser. The homogenised sample was centrifuged at 4°C , for 20 minutes at 2000 RPM in a swinging bucket centrifuge. The supernatant was recovered and extracted three times with 10 ml portions of water saturated ether, each time aspirating the ether layer out. The ether extraction was to remove traces of trichloroacetic acid,

which might interfere in the assay. After the extraction was completed, the aqueous phase was dried free of ether under a stream of nitrogen. The aqueous extract was used as such for the estimation of cyclic AMP content and a 0.5 ml aliquot was acetylated and used for the estimation of cyclic GMP content.

ACETYLATION

A portion of the extract (0.5 ml) was acetylated for cyclic GMP estimation. A 1:2 mixture of acetic anhydride and triethylamine was prepared and 15 μ l of this mixture was added to each 0.5 ml aliquot of the extract and vortexed immediately.

PREPARATION OF STANDARD CYCLIC AMP AND CYCLIC GMP SOLUTIONS

A standard stock solution of cyclic AMP containing 1 μ mole/ml was prepared. A series of standard solutions of cyclic AMP containing 0.25, 0.5, 1, 2.5, 5 and 10 p moles/100 μ l were prepared by appropriate dilution of the stock solution with a 6% TCA solution. The standard solutions were extracted with water saturated ether, and were dried over a stream of nitrogen.

A standard stock solution of cyclic GMP containing 5000 p moles/ml was prepared. A series of standard solutions containing 5, 10, 50, 100 and 200 f moles of cyclic GMP/100 μ l were prepared by appropriate dilutions of the stock solutions with a 6% TCA solution. The TCA was removed by extraction with water saturated ether. Portions of standard solutions (0.5 ml) were acetylated with a mixture of acetic anhydride and triethylamine and the acetylated standards were used in the cyclic GMP assay. Typical standard curves for cyclic AMP and cyclic GMP are shown in Figure 3.

CYCLIC AMP AND CYCLIC GMP ASSAY PROTOCOL

Incubation tubes were prepared according to the protocol given in the next page.

Figure 3

Typical standard curves for cyclic AMP and cyclic GMP. Standard concentrations of cyclic GMP are plotted against corresponding percent trace binding.

Concentrations of cyclic AMP and cyclic GMP (X-axis) are expressed in picomoles/tube and femtomoles/tube respectively.

Percent trace binding (Y-axis) represents binding of a fixed concentration of labelled cyclic nucleotide in the presence of varying concentrations of unlabelled cyclic nucleotide, as a percent of total binding of that concentration of labelled nucleotide, and is calculated according to:

$$\text{Percent trace bind} = \frac{\text{counts for labelled cyclic nucleotide} + \text{standard cyclic nucleotide}}{\text{counts for labelled cyclic nucleotide alone}} \times 100$$

Vertical bars represent standard error of the mean.

Numbers in parenthesis represent sample size.

PROTOCOL

Incubation Tubes	¹²⁵ I Derivative of cyclic Nucleotide* μl	Standard or Sample μl	Cyclic Nucleotide Antiserum** μl	Sodium Acetate Buffer μl
Total Counts	100	-	-	-
Blank	100	-	-	400
Trace Bind	100	-	100	300
Standards	100	100	100	200
Samples	100	100	100	200

* ¹²⁵I succinyl cyclic AMP tyrosine methyl ester for cyclic AMP assay and ¹²⁵I succinyl cyclic GMP tyrosine methyl ester for cyclic GMP assay.

** Cyclic AMP rabbit antiserum or cyclic GMP rabbit antiserum.

ASSAY

The assay involves competition between radioactive cyclic nucleotide and the non-radioactive cyclic nucleotide present in the standard or sample for the binding sites on the antibody, present in the antiserum. Equilibrium of the binding reaction is attained after 18 hrs of incubation at 4°C. All the assay tubes were prepared according to the

above given protocol and were incubated at 4°C for 18 to 20 hrs. After incubation was over, the cyclic nucleotide anti-body complex was precipitated by the addition of 2 ml of 60% saturated ammonium sulfate solution. The precipitated complex was separated by centrifuging at 2000 RPM at 4°C for about 30 minutes. The supernatant liquid was drained and the pellets were counted for radio-activity in a gamma counter (Nuclear[®], Chicago).

PHOSPHORYLASE a ASSAY

The phosphorylase a activity was measured in the direction of glycogen synthesis by a modification of the method of Cori and Cori (1940) as described by McNeill and Brody (1966).

EXTRACTION

Heart samples were weighed (50-100 mg), while in the frozen condition and homogenised in 5 ml of Tris buffer using a polytron[®] homogenizer. The homogenate was transferred to centrifuge tubes and the homogenising tubes were rinsed with appropriate volume of Tris buffer to bring the final dilution of the tissue to 1:200. The rinsings were also added to the centrifuge tubes. The

homogenate was centrifuged at 0°C for 10 minutes at 10,000 RPM.

REAGENTS

10% TCA solution: TCA solution (10%) was made by dissolving 200 gm of trichloroacetic acid in distilled water and making up the final volume to 200 ml with distilled water.

Tris Buffer: Trish [Tris (hydroxymethyl) amino methane] (12 gm), 6 gm of bovine albumin fraction V, 0.672 gm of disodium salt of EDTA and 0.84 gm of sodium fluoride were dissolved in distilled water and the volume was made up to 2 litres. The pH of this solution was about 9. The pH of the tris buffer was adjusted to 6.8 by the addition of conc. hydrochloric acid.

Molybdate Solution: Concentrated sulfuric acid (100 ml) was carefully diluted with 800 ml of distilled water. Ammonium molybdate (22.5 g) was dissolved in the diluted sulfuric acid and the final volume of the solution was made up to 1000 ml.

4% Glycogen: Glycogen (4 g) was dissolved in distilled water, the final volume was made up to 100 ml and the glycogen solution was passed through a Dowex 1-X4 column,

previously treated with 1N hydrochloric acid and washed free of chloride.

Fiske and Subba Row Solution: Sodium bisulfite (30 gm)

and 1 gm of sodium sulfite were dissolved in 120 ml of distilled water. 1-amino-2-naphthol-4-sulfonic acid (500 mg) was mixed with a small quantity of water and this mixture was added to the sodium bisulfite and sodium sulfite solution. The volume was made up to 200 ml and the mixture was stirred overnight in a foil covered dark bottle. After overnight stirring the solution was filtered into a foil covered amber coloured bottle and stored in a dark cupboard.

Glycogen, Glucose-1-Phosphate Buffer: Glucose-1-phosphate (222 mg) was dissolved in 11 ml of glycogen solution. The glucose-1-phosphate solution in glycogen (5 ml) was with 35 ml of tris buffer to make glycogen, glucose-1-phosphate buffer reagent.

Glycogen, Glucose-1-Phosphate, 5'-AMP Buffer: 5'-AMP (24 mg) was dissolved in 40 ml of tris buffer. The 5'-AMP solution in tris buffer (35 ml) was mixed with 5 ml of glucose-1-phosphate solution in glycogen to make glycogen, glucose-1-phosphate, 5'-AMP buffer reagent.

Incubation tubes for each heart sample were prepared according to the protocol given below.

PROTOCOL

Reagents	Tubes					
	1	2	3	4	5	6
Glycogen+glucose-1-phosphate buffer	0.8ml	-	0.8ml	0.8ml	-	-
Glycogen+glucose-1-phosphate+5'-AMP buffer	-	0.8ml	-	-	0.8ml	0.8ml
Enzyme preparation	-	-	0.2ml	0.2ml	0.2ml	0.2ml

All the tubes were incubated at 37°C for 20 minutes

10% TCA solution	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml
Enzyme preparation	0.2ml	0.2ml	-	-	-	-

After incubated for 20 minutes, the tubes were placed over ice and the reaction was stopped by precipitating the proteins with 2.0 ml of 10% TCA solution. To the number 1 and 2 tubes, 0.2 ml of enzyme preparation was added after precipitating the proteins with 10% TCA solution, as these tubes were blank determinations. The contents of the tubes were centrifuged at 2,500 x g for 10 minutes to spin down the protein precipitate. The supernatant solutions were separated and were photometrically assayed for inorganic phosphate according to Fiske and Subba Row (1928). The

phosphorylase a activity was represented as a percent of total phosphorylase activity. Total phosphorylase activity can be measured in the presence of 5'-AMP and phosphorylase a activity in the absence of 5'-AMP. The phosphorylase a activity was represented as

$$\frac{\text{Phosphorylase activity in the absence of 5'-AMP}}{\text{Phosphorylase activity in the presence of 5'-AMP}} \times 100 .$$

RESULTS

DETERMINATION OF OPTIMUM PERFUSION CONDITIONS

A series of preliminary experiments were conducted in order to establish optimal perfusion conditions for the constant pressure Langendorff preparation. Optimal perfusion pressure and optimal buffer temperature were determined. Seven different filling pressures were chosen: 30, 35, 40, 45, 50, 55 and 60 cm of H_2O . Four temperature settings on the Haake® constant temperature water recirculating pump were chosen: 36°, 37°, 38° and 39°C. Hearts were perfused at each temperature setting and the perfusion pressures were changed by altering the height of the movable reservoir. The performance of the hearts at various combinations of perfusion pressure and temperature was determined by monitoring the heart rate and coronary flow.

The effect of different filling pressures and perfusion temperatures on the heart rate is shown in Fig. 4. Hearts developed a basal rate of about 200 beats/min, when perfused at 38°C of the pump setting (At a 38°C pump setting the buffer had a temperature of 37.5°C in the reservoir and a range of 36.5°-37°C at the tip of the perfusion cannula.) The basal developed rate at different filling pressures at 38°C was significantly different from the rate

developed at the corresponding filling pressures at 36°, 37° and 39°C ($P < 0.05$) (Fig. 4).

The effect of different filling pressures and perfusion temperatures on the coronary flow rate is shown in Fig. 5. At 38°C the coronary flow rate developed at different filling pressures was significantly different from the coronary flow rate developed at the other three temperatures ($P < 0.05$). When the perfusate was delivered at a filling pressure of 40 cm of H_2O , at 38°C the coronary flow rate was 5.2 ± 0.3 mls/min. Under these conditions the heart rate developed was 195 ± 7 beats/min (Fig. 4), and the hearts developed a basal tension of about 6.0 gms. These conditions were chosen as the optimal conditions of perfusion and throughout the study, hearts were perfused at 40 cms of H_2O filling pressure and at a pump setting of 38°C.

ISOPROTERENOL DOSE-RESPONSE CURVE

A dose response curve to DL-isoproterenol hydrochloride was performed, in order to verify whether or not the hearts perfused under the above determined conditions would respond appropriately to bolus injections of isoproterenol. A 1 mg/ml stock solution of isoproterenol

hydrochloride was prepared in 0.05 N hydrochloric acid and the stock solution was diluted to the required concentration by a series of dilutions in distilled water.

The dose response curve to isoproterenol is shown in Fig. 6. Isoproterenol produced a dose-dependent increase in the tension development of the rat Langendorff preparation. Response of the hearts to doses higher than 30 ng are not shown as hearts became arrhythmic. At the 30 ng dose, isoproterenol produced a 70 per cent increase in the tension development over controls.

TIME COURSE OF THE EFFECT OF ISOPROTERENOL ON CYCLIC AMP LEVELS AND TENSION DEVELOPMENT IN THE RAT HEART

The effect of a 20 ng dose of isoproterenol on changes of myocardial tension development and cyclic AMP content was studied and is shown in Fig. 7. Isoproterenol increased the cyclic AMP content from a basal level of 314 ± 14 f moles/mg to a maximum level of 503 ± 13 f moles/mg at 20 seconds after administration. At 60 seconds after administration, the cyclic AMP content was back to the control level. The positive inotropic effect of isoproterenol reached a maximum at 25 seconds after administration. At this time point isoproterenol produced a 55% change in the developed tension. By 60 seconds the tension development was back

to control. The cyclic AMP increasing effect of isoproterenol preceded the positive inotropic effect and this result is consistent with previous reports (McNeill, 1978). Since the hearts perfused under the above-mentioned experimental conditions responded normally to isoproterenol, these experimental conditions were considered appropriate and were used throughout the study.

EFFECT OF PROSTAGLANDIN E_1 ON THE RAT HEART

Bolus injections of prostaglandin E_1 (PGE_1) into the rat Langendorff preparation up to the highest dose of 10 μ g did not produce any effect on heart rate or tension development. One of the possible reasons for not seeing any effect was that the prostaglandin was in contact with the heart for only a short period and either this period was not long enough for the prostaglandin to produce an effect or the prostaglandin was metabolised before producing an effect.

For the above reasons PGE_1 was infused at a rate of 0.5 ml/min for about 2 minutes. When infused in a dose range of 0.03 to 5 μ g/min into the perfused rat hearts, PGE_1 still did not produce any effect on the heart rate or tension development of the Langendorff perfused hearts.

EFFECT OF PROSTAGLANDIN $F_{1\alpha}$ ON THE RAT HEART

The effect of infusion of $PGF_{1\alpha}$ in a dose range of 0.1 to 5 $\mu\text{g}/\text{min}$ is shown in Fig. 8. $PGF_{1\alpha}$ produced a dose dependent increase in tension development which was associated with a dose-dependent drop in heart rate. The increase in tension development produced by $PGF_{1\alpha}$ is secondary to the negative chronotropic effect, as the positive inotropic effect was completely abolished when the hearts were electrically paced at 6 Hz (Fig. 8).

EFFECT OF PGE_1 ON ISOLATED RAT ATRIA

PGE_1 increased the rate of right atria in a dose-dependent manner (Fig. 9). The maximum concentration administered was 10^{-4}M and at this concentration the rate was increased by about 40 beats/min. Significant increases in rate were seen at 10^{-5}M and 10^{-4}M concentrations of PGE_1 . PGE_1 did not exert any significant effect on the tension development of the left atria at any dose level studied (Fig. 10).

EFFECT OF $PGF_{1\alpha}$ ON ISOLATED RAT ATRIA

$PGF_{1\alpha}$, when administered in a dose range of 10^{-7} to 10^{-4}M significantly increased the rate of right atria in a dose-

dependent manner. The rate was increased by about 60 beats/min, when a final concentration of 10^{-4} M $\text{PGF}_{1\alpha}$ was administered (Fig. 11). $\text{PGF}_{1\alpha}$, when administered to the electrically paced left atria, increased the developed tension slightly but not significantly (Fig. 12).

Both PGE_1 and $\text{PGF}_{1\alpha}$ were equipotent in exerting the positive chronotropic effect in the right atrium. The PD_2 value for PGE_1 was 5.54 ± 0.25 and for $\text{PGF}_{1\alpha}$ 5.59 ± 0.18 . The onset of the chronotropic effect of both prostaglandins was slow and the peak effect was observed only after 5 minutes of administration.

EFFECT OF PGE_1 ON THE CYCLIC AMP CONTENT AND PHOSPHORYLASE a ACTIVITY OF THE RIGHT ATRIUM.

PGE_1 (10^{-4} M) was administered to rat right atria and the atria were frozen 5 minutes after the administration and assayed for cyclic AMP content and phosphorylase a activity. The effect of 10^{-4} M PGE_1 on the cyclic AMP content and phosphorylase a activity of the right atrium is shown in Fig. 13. Administration of 10^{-4} M PGE_1 produced an increase in the rate of about 70 beats/min. over control and a 130 per cent increase in the cyclic AMP content, whereas the phosphorylase a activity was not affected.

EFFECT OF $\text{PGF}_{1\alpha}$ ON THE CYCLIC AMP CONTENT OF THE RIGHT ATRIUM

The effect of 10^{-4}M $\text{PGF}_{1\alpha}$ on the rate and cyclic AMP content of the right atrium is shown in Fig. 14. $\text{PGF}_{1\alpha}$ produced a significant increase in the rate of about 40 beats/min over control without altering the cyclic AMP content.

EFFECT OF PGE_1 AND $\text{PGF}_{1\alpha}$ ON THE CYCLIC AMP AND CYCLIC GMP CONTENT OF THE RIGHT ATRIUM

The effect of 10^{-4}M PGE_1 and 10^{-4}M $\text{PGF}_{1\alpha}$ on the cyclic AMP and cyclic GMP contents of the right atrium is shown in Table 1. The control levels of cyclic AMP and cyclic GMP did not differ significantly from the nucleotide levels obtained after treatment with vehicle. PGE_1 (10^{-4}M) produced a 130 per cent increase in the cyclic AMP levels over control, without altering the cyclic GMP content. $\text{PGF}_{1\alpha}$ (10^{-4}M) did not alter either the cyclic AMP or the cyclic GMP content of the right atrium.

EFFECT OF PGE_1 AND $\text{PGF}_{1\alpha}$ ON THE CYCLIC AMP AND CYCLIC GMP LEVELS OF THE LEFT ATRIUM

The effect of 10^{-4}M PGE_1 and 10^{-4}M $\text{PGF}_{1\alpha}$ on the cyclic AMP and cyclic GMP contents of the left atrium is

presented in Table 2. Treatment with vehicle did not alter the control levels of cyclic AMP and cyclic GMP in the left atrium. $\text{PGE}_1 (10^{-4}\text{M})$ produced a slight but not significant increase in the cyclic AMP content of the left atrium, but did not alter the cyclic GMP content. $\text{PGF}_{1\alpha} (10^{-4}\text{M})$ did not exert any effect on cyclic AMP or cyclic GMP levels.

As both the prostaglandins were equipotent in exerting their positive chronotropic effect on the right atrium, a dose of 1 $\mu\text{g}/\text{min}$ was chosen to perform a time course study of changes in cyclic nucleotides and phosphorylase a activity after the administration of each prostaglandin to the Langendorff preparation.

EFFECT OF PGE_1 ON THE CYCLIC AMP, CYCLIC GMP CONTENTS AND PHOSPHORYLASE a ACTIVITY OF THE RAT HEART

The effect of 1 $\mu\text{g}/\text{min}$ infusion of PGE_1 on the cyclic AMP, cyclic GMP content and phosphorylase a activity of the rat heart at various time points is presented in Table 3. PGE_1 significantly elevated the cyclic AMP content of the rat heart by about 57 per cent over control at 30 seconds after starting the infusion. The cyclic AMP content was still elevated by 50 per cent above control at the 60 seconds time point. PGE_1 did not

affect either cyclic GMP content or phosphorylase a activity. The phosphorylase a activity was unaffected even at 2 minutes after starting the infusion.

EFFECT OF $\text{PGF}_{1\alpha}$ ON CYCLIC AMP, CYCLIC GMP CONTENTS AND PHOSPHORYLASE a ACTIVITY OF THE RAT HEART

The effect of 1 $\mu\text{g}/\text{min}$ infusion of $\text{PGF}_{1\alpha}$ on the cyclic nucleotide content and phosphorylase a activity of the rat heart at various time points is presented in Table 4. $\text{PGF}_{1\alpha}$ did not produce any change in cyclic AMP and cyclic GMP levels or phosphorylase a activity within one minute after starting the infusion.

Figure 4

Effect of changing filling pressures on rate of isolated rat hearts perfused at different temperatures.

X-axis represents filling rpressure in cm of H_2O , which is the difference in height (in cm) between the heart and the perfusate level in the reservoir.

Y-axis represents absolute heart rate in hearts/minute.

Hearts were perfused at each combination of perfusate temperature and filling pressure and the heart rates were measured. Vertical bars represent standard error of the mean. Numbers in parenthesis denote sample size and asterisks represent significant difference at $P < 0.05$ from heart rates developed at other temperatures under the same filling pressure.

Figure 5

Effect of changing filling pressures on coronary flow rate of isolated rat hearts perfused at different temperatures.

X-axis represents filling pressure in cm of H_2O .

Y-axis represents coronary flow in ml/min.

Hearts were perfused at each combination of filling pressure and perfusate temperature and the coronary flow rates were determined.

Vertical bars represent standard error of the mean.

Numbers in parenthesis denote sample size.

Asterisks represent significant difference at $p < 0.05$ from coronary flow rates developed at other temperatures under the same filling pressure.

Figure 6

Effect of isoproterenol on the force development of the rat Langendorff preparation.

X-axis represents concentration of isoproterenol in nanograms.

Y-axis represents change in contractile force in gm.

Isoproterenol was administered by bolus injection to the rat Langendorff preparation and the change in force development was recorded.

Vertical bars represent standard error of the mean.

Numbers in parenthesis denote sample size.

Figure 7

Effect of isoproterenol on changes in cyclic AMP content and contractile force with time in isolated perfused rat hearts.

X-axis represents concentration of cyclic AMP in femtomoles/mg wet tissue and percent change in tension development.

Y-axis represents time in seconds.

20 ng of isoproterenol was administered to rat hearts by bolus injection and hearts were frozen at various time points and were assayed for cyclic AMP content. The change in tension development at various time points was also measured.

Vertical bars represent standard error of the mean.

Numbers in parenthesis denote sample size.

Solid line represents changes in cyclic AMP content and dotted line represents changes in tension development.

Figure 8

Effect of prostaglandin $F_{1\alpha}$ on the rat Langendorff preparation.

Prostaglandin $F_{1\alpha}$ was infused into both paced and unpaced rat Langendorff preparation and the changes in heart rate and tension development were measured.

X-axis represents log. dose of $PGF_{1\alpha}$ in micrograms.

Y-axis represents percent change in both heart rate and tension development from control values.

Each point represents the mean of two determinations.

Figure 9

Effect of Prostaglandin E_1 on spontaneously beating rat right atrium.

X-axis represents dose of prostaglandin E_1 in molar concentrations.

Y-axis represents absolute rate in beats/minute.

Prostaglandin E_1 was administered to spontaneously beating rat right atria and the rate was measured at five minutes following the administration.

Vertical bars represent standard error of the mean.

Numbers in parenthesis denote sample size.

Asterisks represent significant difference at $p < 0.05$ from control values.

Figure 10

Effect of prostaglandin E_1 on the tension development of the electrically paced rat left atrium.

X-axis represents dose of PGE_1 in molar concentrations.

Y-axis represents absolute tension development in gm.

Prostaglandin E_1 was administered to electrically paced rat left atria and the changes in tension development were measured at five minutes following drug administration.

Vertical bars represent standard error of the mean.

Numbers in parenthesis denote sample size.

Figure 11

Effect of prostaglandin $F_{1\alpha}$ on spontaneously beating rat right atrium.

Prostaglandin $F_{1\alpha}$ was administered to spontaneously beating rat right atria and the rate responses were measured at five minutes following the administration of the drug.

X-axis represents dose of $PGF_{1\alpha}$ in molar concentrations.

Y-axis represents absolute rate in beats/min.

Vertical bars denote standard error of the mean.

Numbers in parenthesis denote sample size.

Asterisks represent significant difference at $p < 0.05$ from control value.

Figure 12

Effect of prostaglandin F_1 on electrically paced rat left atrium.

Prostaglandin F_1 was administered to electrically paced rat left atrium and the changes in tension development were measured five minutes after drug administration.

X-axis represents dose of PGF_1 in molar concentrations.

Y-axis represents absolute tension development in gm.

Vertical bars denote standard error of the mean. Numbers in parenthesis denote sample size.

Figure 13

Effect of 10^{-4} prostaglandin E_1 on right atrial rate, cyclic AMP content and phosphorylase a activity.

Rat right atria were frozen either before or five minutes after the administration of $10^{-4}M$ prostaglandin E_1 and were assayed for cyclic AMP content and phosphorylase a activity.

Histograms represent atrial rate, cyclic AMP content and phosphorylase a activity in the control situation (0 min) and at five minutes following the administration of $10^{-4}M$ PGE_1 .

Y-axis represents absolute atrial rate in beats/min, cyclic AMP content in femtomoles/mg tissue and phosphorylase a activity as a percent of total phosphorylase activity.

Vertical bars represent standard error of the mean.

Numbers in parenthesis denote sample size.

Asterisks represent significant difference at $P < 0.05$ from control values.

Figure 14

Effect of 10^{-4} M prostaglandin $F_{1\alpha}$ on the right atrial rate and cyclic AMP content.

Rat right atria were frozen either before or five minutes after the administration of 10^{-4} M prostaglandin $F_{1\alpha}$ and were assayed for cyclic AMP content.

Histograms represent atrial rate and cyclic AMP content in the control situation (0 min) and at five minutes following drug administration.

Y-axis represents absolute rate in beats/min and cyclic AMP content in femtomoles/mg tissue.

Vertical bars represent standard error of the mean.

Numbers in parenthesis denote sample size.

Asterisks represent significant difference from control values at $P < 0.05$.

Table 1

Effect of 10^{-4} M prostaglandin E_1 and 10^{-4} M prostaglandin $F_{1\alpha}$ on right atrial cyclic AMP and cyclic GMP content.

Rat right atria were frozen at 0 time, at 5 minutes following the administration of vehicle, and at 5 minutes following the administration of either 10^{-4} M PGE_1 or 10^{-4} M $PGF_{1\alpha}$ and were assayed for cyclic AMP and cyclic GMP content.

Stock solutions of prostaglandins were made in 10% alcohol and were diluted to the required concentration with Chenoweth-Koelle buffer.

Vehicle represents the diluted 10% alcoholic solution without prostaglandins.

Cyclic AMP and cyclic GMP content are expressed in femtomoles/mg tissue.

Numbers in parenthesis denote sample size.

RIGHT ATRIUM	cyclic AMP f Moles/mg.	cyclic GMP f Moles/mg.
CONTROL	187 ± 9.0 (4)	14.9 ± 3.0 (4)
VEHICLE	178 ± 5.0 (4)	18.2 ± 1.0 (4)
10^{-4} M PGE_1	436 ± 80.0 (4)	15.7 ± 3.0 (4)
10^{-4} M $\text{PGF}_1 \alpha$	213 ± 31.0 (4)	12.1 ± 0.5 (4)

Table 2

Effect of 10^{-4} M prostaglandin E_1 and 10^{-4} M prostaglandin $F_{1\alpha}$ on left atrial cyclic AMP and cyclic GMP content.

Rat left atria were frozen at 0 time, 5 minutes after vehicle administration and at 5 minutes following the administration of either 10^{-4} M PGE_1 or 10^{-4} M $PGF_{1\alpha}$ and were assayed for cyclic AMP and cyclic GMP content.

Cyclic AMP and cyclic GMP content are represented in femtomoles/mg tissue.

Numbers in parenthesis denote sample size.

LEFT ATRIUM	cyclic AMP f Moles/mg.	cyclic GMP f Moles/mg.
CONTROL	175 ± 22.0 (4)	9.8 ± 2.0 (4)
VEHICLE	183 ± 30.0 (4)	12.0 ± 2.0 (4)
10^{-4} M PGE_1	227 ± 24.0 (4)	15.8 ± 6.0 (4)
10^{-4} M $\text{PGF}_{1\alpha}$	193 ± 27.0 (4)	10.8 ± 3.0 (4)

Table 3

Effect of prostaglandin E₁ on cyclic AMP and cyclic GMP content and phosphorylase a activity of the rat Langendorff preparation.

Isolated perfused rat hearts were frozen at various time points after receiving a 1 µg/min infusion of PGE₁. The frozen samples were assayed for cyclic AMP and cyclic GMP content and for phosphorylase a activity.

Cyclic AMP and cyclic GMP are represented in femtomoles/mg tissue. Phosphorylase a activity is expressed as a percent of total phosphorylase activity.

Numbers in parenthesis denote sample size.

Asterisks represent significant difference at $P < 0.05$ over control values.

DRUG	CYCLIC AMP (F MOLES/mg)	CYCLIC GMP (F MOLES/mg)	PHOSPHORYLASE 'a' (% OF TOTAL PHOSPHORYLASE)
Control	261 $\pm$ 16 (8)	23.0 $\pm$ 4 (8)	3.7 $\pm$ 0.7 (12)
PGE ₁ Infusion (1 μ g/min)			
15 sec	311 $\pm$ 27 (8)	20.0 $\pm$ 4 (8)	4.6 $\pm$ 1.1 (8)
30 sec	*410 $\pm$ 30 (8)	23.0 $\pm$ 5 (8)	4.1 $\pm$ 0.8 (8)
60 sec	*394 $\pm$ 41 (8)	25.0 $\pm$ 3 (8)	3.6 $\pm$ 0.7 (7)
90 sec	-	-	5.8 $\pm$ 1.4 (8)
120 sec	-	-	4.9 $\pm$ 1.2 (6)

Table 4

Effect of $\text{PGF}_{1\alpha}$ on cyclic AMP and cyclic GMP content and phosphorylase a activity of the rat Langendorff preparation.

Isolated perfused rat hearts were frozen at various time points after receiving a 1 $\mu\text{g}/\text{min}$ infusion of PGF_1 and were assayed for cyclic AMP, cyclic GMP and phosphorylase a activity.

Cyclic AMP and cyclic GMP are expressed in femtomoles/mg tissue. Phosphorylase a activity is expressed as a percent of total phosphorylase activity.

Numbers in parenthesis denote sample size.

DRUG	CYCLIC AMP (F MOLES/mg)	CYCLIC GMP (F MOLES/mg)	PHOSPHORYLASE 'a' (% OF TOTAL PHOSPHORYLASE)
Control	277 $\pm$ 15 (8)	19.0 $\pm$ 4 (8)	5.3 $\pm$ 0.4 (8)
PGF α Infusion (1 μ g/min)			
15 sec	279 $\pm$ 12 (8)	25.0 $\pm$ 5 (8)	4.9 $\pm$ 0.9 (8)
30 sec	270 $\pm$ 31 (8)	25.0 $\pm$ 6 (8)	3.8 $\pm$ 0.6 (8)
60 sec	325 $\pm$ 21 (8)	21.0 $\pm$ 4 (8)	5.1 $\pm$ 0.9 (7)

DISCUSSION

The results obtained in this study can be summarized as follows.

i) PGE_1 exerted a positive chronotropic effect on the isolated spontaneously beating right atrium and increased the cyclic AMP content without altering cyclic GMP content or phosphorylase a activity.

ii) PGE_1 had no effect on the tension development and cyclic nucleotides content of the isolated left atrium.

iii) $\text{PGF}_{1\alpha}$ increased the rate of the right atrium, but did not change cyclic AMP and cyclic GMP content

iv) $\text{PGF}_{1\alpha}$ did not affect the tension development and cyclic nucleotides level of the left atrium.

v) PGE_1 increased the cyclic AMP content of the rat Langendorff preparation, without altering heart rate or myocardial contractile force or phosphorylase a activity. PGE_1 did not produce an effect on the cyclic GMP content.

vi) $\text{PGF}_{1\alpha}$ produced a positive inotropic effect in the rat isolated perfused heart, but this effect is secondary to a negative chronotropic effect of $\text{PGF}_{1\alpha}$. $\text{PGF}_{1\alpha}$ had no effect on the cyclic nucleotides content or phosphorylase a activity.

Table 5

Summary of the effects of PGE_1 and PGF_1 on rate, force, cAMP, cGMP and phosphorylase a of isolated atria and Langendorff preparation.

↑ represents an increase

→ represents no change

↓ represents a decrease

Asterisks represent statistical significance at $P < 0.05$ over the controls.

	RIGHT ATRIUM			LEFT ATRIUM	LANGENDORFF PREPARATION				
DRUG	RATE	cAMP	PHOS a	FORCE	RATE	FORCE	cAMP	cGMP	PHOS a
PGE ₁	*↑	*↑	→	→	→	→	*↑	→	→
PGF _{1α}	*↑	→		↑	↓	↑	→	→	→

The above summarized results agree with some of the previous studies and do not agree with certain others. Our results support the observations made earlier by Keely (1977, 1979) and Reese et al. (1978), that PGE_1 selectively increased myocardial cyclic AMP content without altering myocardial contractile force or phosphorylase activity in the rat heart. PGE_1 might be selectively increasing a pool of cyclic AMP and specifically activating a cyclic AMP dependent protein kinase in the cardiac cell. The protein kinase thus activated was not apparently involved in the mediation of the positive inotropic effect or in the stimulation of phosphorylase activity. Recently, Corbin et al. (1977) and Corbin and Keely (1977) showed experimental evidence for the existence of two isozymes of cyclic AMP dependent protein kinase in the heart. These two isozymes have different elution patterns from a DEAE-cellulose column and different species distribution. In the physiological situation type II isozyme is membrane-bound and type I is dissociated from the membrane. In the presence of high cyclic AMP concentration the catalytic subunit of isozyme II is dissociated from its regulatory subunit and when the high cyclic AMP concentration is removed, the catalytic subunit is reassociated with the regulatory subunit. Hayes et al. in 1979 showed that activation of cyclic

AMP dependent protein kinase isozyme II is involved in the mediation of the positive inotropic effect and in the stimulation of phosphorylase activity produced by isoproterenol. These authors showed that isoproterenol produced a positive inotropic effect, activated phosphorylase a, increased the soluble protein kinase activity, increased cyclic AMP content and decreased particulate protein kinase content in the perfused rabbit hearts. PGE_1 , on the other hand, only elevated soluble protein kinase activity without increasing particulate cyclic AMP content or decreasing the particulate protein kinase content. The above results suggest that β -adrenergic stimulation produced a positive inotropic effect and activated the phosphorylase system by elevating cyclic AMP content and decreasing particulate protein kinase activity. Protein kinase isozyme II is particulate bound and when the cyclic AMP content is increased the active catalytic subunit of the isozyme II is dissociated from the regulatory subunit which is bound to the membrane. A decrease in the particulate protein kinase activity produced by isoproterenol is due to the dissociation of active catalytic subunit from the particulate bound protein kinase in the presence of elevated cyclic AMP content. Isoproterenol activated both the protein kinase isozyme II and soluble protein kinase produced a positive inotropic effect and activated

phosphorylase a. PGE₁ activated soluble protein kinase only and did not produce a positive inotropic effect or activation of phosphorylase a. The soluble protein kinase may be protein kinase isozyme I and PGE₁ may selectively activate isozyme I in a separate cell compartment and did not effect force development or phosphorylase activity.

The suggestion that a specific effect of PGE₁ occur on protein kinase in a separate compartment is illustrated in Fig. 15. According to this scheme the β -adrenergic receptor as well as histamine and glucagon receptors are situated in a compartment of the cardiac cell. Stimulation of β -adrenergic receptor activates both particulate protein kinase (Protein kinase isozyme II) and soluble protein kinase, through elevation of cyclic AMP levels thus producing a positive inotropic effect and phosphorylase activation. The PGE-type receptor is situated in a different compartment and stimulation of this receptor leads to activation of soluble protein kinase (perhaps protein kinase isozyme I) only and does not produce positive inotropy or phosphorylase a stimulation. The activated protein kinase in this compartment may be involved in the mediation of some other physiological responses, the nature of which is not clear at this stage.

Figure 15

Scheme representing the compartmentalization of cyclic AMP-dependent protein kinase isozymes I and II in cardiac cells. The dashed line represents hypothetical compartments within the cell separating the adenylate cyclases associated with the PGE and the α -adrenergic receptors.

A.C - Adenylate cyclase

ATP - Adenosine triphosphate

cAMP - Adenosine 3':5'-monophosphoric acid

act PK I - Activated protein kinases isozyme I

act PK II - Activated protein kinase isozyme II

Phos b and Phos a - Phosphorylase b (inactive)

and Phosphorylase a (active)

Phos kinase - Phosphorylase b kinase

S.R - Sarcoplasmic reticulum

EPI - Epinephrine.

$\text{PGF}_{1\alpha}$ does not possess this selective effect of PGE_1 . There are two possibilities for the lack of specificity of $\text{PGF}_{1\alpha}$. The concentration of $\text{PGF}_{1\alpha}$ used in this study might not be sufficient enough to mimic PGE_1 at the PGE -type receptor in the heart or there might not be a PGF receptor in cardiac muscle. It is also apparent that cyclic GMP is not involved in the mediation of responses to $\text{PGF}_{1\alpha}$ in our studies.

Our observations showing the effects of PGE_1 on isolated rat atria conflict with the observations made by Kangasaho et al. (1978). Kangasaho et al. (1978) showed that PGE_1 produced a positive inotropic effect in spontaneously beating rat atria, increased the atrial cyclic AMP content and did not effect the rate. However, our results showed that PGE_1 increased the rate and cyclic AMP content without changing force of contraction in the rat right atria. One of the factors responsible for these differences may be the experimental conditions employed. Kangasaho et al. (1978) used Tyrode's solution at 30°C to superfuse the atria and we used Chenoweth Koelle buffer at 37°C in our experiments.

$\text{PGF}_{1\alpha}$ produced a positive chronotropic effect in the right atrium and produced a negative chronotropic

effect in the Langendorff preparation. These opposing effects of $\text{PGF}_{1\alpha}$ on the right atrium and whole heart may be due to the fact that PGF compounds decrease the conduction velocity through the A-V node in mammalian hearts as reported by Forster et al. (1976). $\text{PGF}_{1\alpha}$ might be producing a positive chronotropic effect by its direct action on the pacemaker tissue in the isolated atria and producing a negative chronotropic effect in the Langendorff preparation due to the action on A-V node where PGF compounds decrease conduction velocity.

In summary, we confirmed that PGE_1 increases myocardial cyclic AMP content without altering either myocardial contractile force or phosphorylase a activity. PGE_1 also increased atrial cyclic AMP and the rate of spontaneously beating right atria. $\text{PGF}_{1\alpha}$ did not affect myocardial cyclic AMP levels or phosphorylase a activity. $\text{PGF}_{1\alpha}$ increased the rate of isolated right atria, but not atrial cyclic AMP levels. Both prostaglandins had no effect on myocardial cyclic GMP levels.

We believe that the specific effect of PGE_1 on the isolated rat heart is due to its ability to increase cyclic AMP in a specific compartment of cardiac cells and activate a cyclic AMP-dependent protein kinase, which

possibly is not involved in the mediation of positive inotropic and glycogenolytic effects. This assumption is in agreement with the experimental evidence presented by Hayes et al. (1979).

$\text{PGF}_{1\alpha}$ does not seem to possess this specific effect of PGE_1 on rat heart. Cyclic GMP is not involved in the mediation of effects of either PGE_1 or $\text{PGF}_{1\alpha}$ on rat heart.

BIBLIOGRAPHY

- Bergström, S., Duner, H., Euler, U.S. Von., Pernow, B. and Sjövall, J. (1959). Observations of the effects of infusion of prostaglandin E in man. *Acta. Physiol. Scand.* 45: 145.
- Bergström, S. and Sjövall, J. (1960). The isolation of prostaglandin E from sheep prostate glands. *Acta. Chem. Scand.* 14: 1701-1705.
- Bergström, S., Ryhage, R., Samuelsson, B. and Sjövall, J. (1962). The structure of prostaglandin E, F₁ and F₂. *Acta. Chem. Scand.* 16: 501-502.
- Bergström, S. and Euler, U.S. Von. (1963). The biological activity of prostaglandins E₁, E₂ and E₃. *Acta. Physiol. Scand.* 59: 493.
- Bergström, S., Carlson, L.A. and Orö, L. (1964). Effect of prostaglandins on catecholamine induced changes in the free fatty acids of plasma and in blood pressure in the dog. *Acta. Physiol. Scand.* 60: 170.

- Berti, F., Lentati, R. and Usardi, M.M. (1965). The species specificity of prostaglandin E_1 effect on isolated hearts. *Med. Pharmacol. Exp.* 13: 233.
- Bloor, C.M. and Sobel, B.D. (1970). Enhanced coronary blood flow following prostaglandin infusion in the conscious dog. *Circulation* 41/42: Suppl III, 123.
- Bourne, H.R., Lichtenstein, LM. and Melmon, J. (1972). Pharmacological control of allergic histamine release in vitro: Evidence for an inhibitory role of 3':5'-Adenosine monophosphate in human lenkocytes. *J. Immunol.* 108: 695.
- Brunt, M.E. and McNeill, J.H. (1978). The effect of glucagon on rat cardiac cyclic AMP, phosphorylase a and force of contraction. *Arch. Int. Pharmacodyn.* 233: 42-52.
- Butcher, R.W. and Baird, C.E. (1968). Effect of prostaglandins on Adenosine 3':5'-monophosphate levels in fat and other tissues. *J. Biol. Chem.* 243: 1713.
- Chenoweth, M.B. and Koelle, E.S. (1946). An isolated heart perfusion system adapted to the determination of non gaseous metabolites. *J. Lab Clin. Med.* 31: 600-608.

- Cheung, W.Y. and Williamson, J.R. (1965). Kinetics of cyclic adenosine monophosphate changes in rat heart following epinephrine administration. *Nature*. 207: 979-981.
- Corbin, J.D. and Keely, S.L. (1977). Characterization and regulation of heart adenosine 3':5'-monophosphate-dependent protein kinase isozymes. *J. Biol. Chem.* 252: 910-918.
- Corbin, J.D., Sugden, PH., Lincoln, T.M. and Keely, S.L. (1977). Compartmentalization of adenosine 3':5'-monophosphate and adenosine 3':5'-monophosphate-dependent protein kinase in heart tissue. *J. Biol. Chem.* 252: 3854-3861.
- Cori, G.T. and Cori, C.F. (1940). The kinetics of enzymatic synthesis of glycogen from glucose-1-phosphate. *J. Biol. Chem.* 135: 733-756.
- Curnow, R.T. and Nuttal, F.Q. (1971). Effect of prostaglandin E₁ (PGE₁) on glycogen metabolism in rat liver and heart in vivo. *Fed. Proc.* 30: 625.
- Curnow, R.T. and Nuttal, F.Q. (1974). Effect of prostaglandin E₁ administration on the heart glycogen synthase and phosphorylase systems. *Prostaglandins* 6(2): 115-123.

- DeDeckere, E.A.M., Vergroesen, A.J. and Tenhoor, F. (1979).
PGF_{2α} and PGI₂ in the isolated perfused rat heart.
Eur. J. Pharmacol. 55: 345-346.
- DuCharme, D.W., Weeks, J.R. and Montgomery, R.G. (1968).
Studies on the hypertensive effect of prostaglandin
F_{2α}. J. Pharmacol. Exp. Ther. 160: 1-10.
- Dusting, G.J., Moncada, S. and Vane, J.R. (1977). Pros-
tacyclin (PGX) is the endogenous metabolite responsible
for relaxation of coronary arteries induced by aradri-
donic acid. Prostaglandins 13: 3.
- Endoh, M. (1976). Effects of prostaglandin E₁ on the posi-
tive inotropic actions of noradrenaline, nerve stimula-
tion and calcium in the isolated blood perfused papillary
muscle of the dog. Eur. J. Pharmacol. 39: 259-265.
- Euler, U.S. Von. (1936). On the specific vasodilating and
plain muscle stimulating substances from accessory
genital glands in man and certain animals (Prostaglandin
and vesiglandin) J. Physiol. (London) 88: 213-234.
- Ferreira, S.H. and Vane, J.R. (1967). Prostaglandins: Their
disappearance from and release into circulation. Nature
(London) 216: 868.

- Fiske, C.H. and Subbarow, Y. (1925). The colorimetric determination of phosphorus. *J. Biol. Chem.* 66: 375.
- Flower, R.J. and Blackwell, G.J. (1976). The importance of phospholipase A₂ in prostaglandin biosynthesis. *Biochem. Pharmacol.* 25: 285.
- Förster, W., Borbola, J., Papp, J.G., Schrör, K. and Szekeres, L. (1974). Cardiac effects of prostaglandins E₂ and F_{2α}. *Arch. Int. Pharmacodyn.* 211: 133-140.
- Goldblatt, M.W. (1933). A depressor substance in seminal fluid. *J. Soc. Chem. Ind. (London)* 52: 1056.
- Goldblatt, M.W. (1935). Properties of human seminal plasma. *J. Physiol. (London)* 84: 208.
- Hadhazy, P., Illes, P. and Knoll, J. (1973). The effects of PGE₁ on responses to cardiac vagus nerve stimulation and acetylcholine release. *Eur. J. Pharmacol.* 23: 251-255.
- Hamberg, M. and Samuelsson, B. (1973). Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. *Proc. Nat. Acad. Sci.* 70: 899.

- Hammarström, S. and Falardeau, P. (1977). Resolution of prostaglandin endoperoxide synthetase and thromboxane synthetase of human platelets. *Proc. Nat. Acad. Sci.* 72: 3691.
- Hayes, J.S., Brunton, L.L. and Mayer, S.E. (1979). Hormonally specific activation of particulate cAMP-dependent protein kinase. *Fed. Proc.* 38(3): 594.
- Hedquist, P. and Brundin, J. (1969). Inhibition by prostaglandin E_1 of noradrenaline release and of effector response to nerve stimulation in the cat spleen. *Life Sciences.* 8: 389.
- Hedquist, P. (1970). Studies on the effect of prostaglandins E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissues. *Acta. Physiol. Scand.* 79 suppl. 345: 1.
- Hedquist, P., Stjärve, L. and Wennmalm, A. (1970). Inhibition by prostaglandin E_2 of sympathetic neurotransmission in the rabbit heart. *Acta. Physiol. Scand.* 79: 139-141.
- Henry, P.D. Dobson, J.G. and Sobel, B.D. (1975). Dissociations between changes in myocardial cyclic adenosine monophosphate and contractility. *Circ. Res.* 36: 392-400.

- Higgins, C.B., Vatner, S.F., Franklin, D., Patrick, T. and Braunwald, E. (1971). Effects of prostaglandin A₁ on the systemic and coronary circulation in the conscious dog. *Circulation Research*. 28: 638-648.
- Hintz, T.H. and Kaley, G. (1977). Prostaglandins and the control of blood flow in the canine myocardium. *Circulation Research*. 40: 313-320.
- Johnson, R.A., Mortar, D.R., Kinner, J.H., Gorman, R.R., McGaire, J.C., Sun, F.F., Whittaker, N., Bunting, S., Salmon, J., Moncada, S. and Vane, J.R. (1976). The chemical structure of prostaglandin X (Prostacyclin). *Prostaglandins*, 12: 915.
- Junstad, M. and Wennmalm, A. (1974). Release of prostaglandin from the rabbit isolated heart following vagal nerve stimulation or acetylcholine infusion. *Br. J. Pharmacol.* 52: 375-379.
- Kangasaho, M., Metsä-Ketelä, T. and Vapaatalo, H. (1978). Effects of prostaglandins on rat cardiac adenylate cyclase. *Eur. J. Pharmacol.* 52: 93-98.

- Kaumann, A.J. and Birnbaumer, L. (1974). Prostaglandin E₁ action on sinus pacemaker and adenylyl cyclase in kitten myocardium. *Nature (London)* 251: 515-517.
- Keely, S.L. (1977). Activation of cAMP-dependent protein kinase without a corresponding increase in phosphorylase activity. *Res. Com. Chem. Path. Pharmacol.* 18(2): 283-290.
- Keely, S.L. and Corbin, J.D. (1977). Involvement of cAMP-dependent protein kinase in the regulation of heart contractile force. *Am. J. Physiol.* 233: H269-H275.
- Keely, S.L. (1979). Prostaglandin E₁ activation of heart cAMP-dependent protein kinase: Apparent dissociation of protein kinase activation from increases in phosphorylase activity and contractile force. *Mol. Pharmacol.* 15: 235-245.
- Keely, S.L. and Eiring, A. (1979). Involvement of cAMP-dependent protein kinase in the regulation of heart contractile force. II. *Am. J. Physiol.* 236(1): H84-H91.
- Klein, I. and Levey, G.S. (1971). Effect of prostaglandins on guinea pig myocardial adenylyl cyclase. *Metabolism* 20(9): 890-896.

- Koss, M.C., Gray, J.W., Davison, M. and Nakano, J. (1973).
Cardiovascular actions of prostaglandins E_1 and $F_{2\alpha}$
in the cat. Eur. J. Pharmacol. 24: 151.
- Kraemer, R.J., Phernetton, T.M. and Folts, J.D. (1976).
Prostaglandin like substances in coronary venous blood
following myocardial ischemia. J. Pharmac. Exp. Ther.
199: 611-619.
- Kuehl, F.A. and Humes, J.L. (1972). Direct evidence for a
prostaglandin receptor and its application to prostag-
landin measurements. Proc. Nat. Acad. Sci. 69: 480-
484.
- Kuehl, F.A., Cirillo, V.J., Ham, E.A. and Humes, J.L.
(1973). The regulatory role of the prostaglandins on
the cyclic 3':5'-AMP system. In: Advances in the Bio-
sciences; Volume 9: International Conference on
Prostaglandins, Eds.: Bergström, S. and Bernhard, S.
(Publr. Pergamon press, Vieweg.) pp. 155.
- Kuehl, F.A. (1974). Prostaglandins, cyclic nucleotides
and cell function. Prostaglandins. 5: 325-340.

- Kurzrok, R. and Leib, C.C. (1930). Biochemical studies of human semen II. The action of semen on human uterus. Proc. Soc. Exp. Biol. Med. 28: 268-272.
- Langendorff, O. (1895). Pflüger's Arch. Ges. Physiol. 61: 291-332.
- Levey, G.S. and Klein, I. (1973). Solubilized myocardial adenylate cyclase: activation by prostaglandins. Life Sci. 13: 41-46.
- McNeill, J.H. and Brody, T.M. (1966). The effect of anti-histamines, cocaine and reserpine on amine induced rat cardiac phosphorylase activation. J. Pharmacol. Exp. Ther. 152: 478-487.
- McNeill, J.H., Verma, S.C. (1974). Blockade by burimamide of the effects of histamine and histamine analogs on cardiac contractility, phosphorylase activation and cyclic adenosine monophosphate. J. Pharmacol. Exp. Ther. 188: 180-188.
- McNeill, J.H. (1978). The effect of dobutamine on rat cardiac cyclic AMP, phosphorylase a and force of contraction. Res. Com. Chem. Path. Pharmacol. 20(3): 597-600.

Moncada, S., Gryglewski, R., Bunting, S. and Vane, J.R.

(1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation . Nature (London) 263: 663.

Moncada, S. and Vane, J.R. (1979). Prostacyclin formation and effects. In chemistry, biochemistry and pharmacological activity of prostanoids. Eds: Roberts, S.M. and Scheinmann, F., pp. 258-273. Pergamon press, Oxford.

Murad, F., Chi, Y.M., Rall, T.W. and Sutherland, E.W. (1962). Adenylcyclase: III. The effect of catecholamines and choline esters on the formation of Adenosine 3':5'-phosphate by preparations from cardiac muscle and liver. J. Biol. Chem. 237: 1233-1238.

Nakano, J. and McCurdy, J.R. (1967). Cardiovascular effects of prostaglandin E_1 . J. Pharmacol. Exp. Ther. 156: 538.

Nakano, J. and McCurdy, J.R. (1968). Hemodynamic effects of prostaglandin E_1 , A_1 and $F_{2\alpha}$ in dog. Proc. Soc. Exp. Biol. Med. 128: 39.

- Namm, D.H. and Meyer, S.E. (1968). Effect of epinephrine on cardiac 3':5'-AMP, phosphorylase kinase and phosphorylase. *Mol. Pharmacol.* 4: 61-69.
- Namm, D.H., Mayer, S.E., Maltbie, M. (1968). The role of potassium and calcium ions in the effect of epinephrine on cardiac cyclic adenosine 3':5'-monophosphates phosphorylase kinase and phosphorylase. *Mol. Pharmacol.* 4: 522-530.
- Naylor, W.G. (1977). Cyclic nucleotides and the heart. In *Advances in drug research*. Vol. 12, pp. 39-51. Eds. Simmonds, A.B. Academic Press. New York.
- Needleman, P., Kauffman, A.H., Douglas, J.R., Johnson, E.M. and Marshall, G.R. (1973). Specific stimulation and inhibition of renal prostaglandin release by angiotensin analog. *Am. J. Physiol.* 224: 1415-1419.
- Needleman, P., Key, S.L., Denny, S.E., Isakson, P.C. and Marshall, G.R. (1975). The mechanism of modification of bradykinin-induced coronary vasodilation. *Proc. Natl. Acad. Sci. U.S.A.* 72: 2060-2063.
- Needleman, P. (1976). The synthesis and function of prostaglandins in the heart. *Fed. Proc.* 35: 2376.

- Needleman, P. and Kaley, G. (1978). Cardiac and coronary prostaglandin synthesis and function. N. Engl. J. Med. 298: 1122-1128.
- Nutter, D.O. and Crumly, H. (1970). Coronary myocardial responses to prostaglandins E_1 and A_1 . Circulation 42: 124.
- Nutter, D.O. and Crumly, H. (1972). Coronary myocardial effects of the prostaglandins. Cardiovascular Res. 6: 217-225.
- Nutter, D.O. and Ratts, T. (1973). Direct actions of prostaglandins E_1 , A_1 and F_2 on myocardial contraction. Prostaglandins 3(3): 323-336.
- Oien, H.G., Humes, J.L. and Kuehl, F.A. (1973). Structural requirements for the binding of prostaglandins and analogs to a lymphocyte receptor preparation. Fed. Proc. 32: 803.
- Parratt, J.R. and Marshall, R.J. (1978). Are prostaglandins involved in the regulation of coronary blood flow? Acta. Biol. Med. Germ. 37: 747-754.
- Piccinini, F. (1965). Boll. Soc. It. Biol. Sper. 41: 1322-1324.

- Powell, W., Hammerström, S. and Samuelsson, B. (1973). In. Proc. 9th Int. Congr. Biochem. p. 404.
- Ramwell, P.W. and Shaw, J.E. (1966). Prostaglandin release from tissues by drug, nerve and hormone stimulation. In: Proc. 2nd Nobel Symposium. Eds. Bergström, S. and Samuelsson, B. (Publr. Interscience, London), p. 283.
- Ramwell, P.W., Leovey, E.M.K. and Sintetos, A.L. (1977). Regulation of arachidonic acid cascade. Biol. Reprod. 16: 70.
- Reese, J.B., Hayes, J.S., Brunton, L.L., Brown, J.H. and Mayer, S.E. (1978). Activation of protein kinase by PGE_1 without activation of PK substrates or the inotropic state. The Pharmacologist 20(3): 232.
- Robison, G.A., Butcher, R.W., Oye, I., Morgan, H.E. and Sutherland, E.W. (1965). The effect of epinephrine on adenosine 3':5'-phosphate levels in isolated perfused rat heart. Mol. Pharmacol. 1: 168-177.
- Schrör, K. and Moncada, S. (1979). Effects of prostacyclin on coronary circulation, heart rate and myocardial contractile force in isolated hearts of guinea pig and rabbit - comparison with prostaglandin E_2 . Prostaglandins.

- Sobel, B.E. and Robison, A.K. (1969). Activation of guinea pig myocardial adenylate cyclase by prostaglandins. *Circulation*. 40 Suppl. III, 189.
- Steinberg, D., Vaughan, M., Nestel, P.J. and Bergstrom, S. (1964). Effects of the prostaglandins on hormone-induced mobilization of free fatty acids. *J. Clin. Invest.* 43: 1533-1540.
- Steiner, A.L., Pagliari, A.S., Chase, L.R. and Kriphis, D.M. (1972). Radioimmunoassay for cyclic nucleotides II: Adenosine 3':5'-monophosphate in mammalian tissue and body fluids. *J. Biol. Chem.* 247: 1114-1120.
- Su, J.Y., Charles, B.H. and Friedman, W.F. (1973). Chronotropic and inotropic effects of prostaglandins E_1 , A_1 , and F_1 on isolated mammalian cardiac tissue. *Proc. Soc. Exp. Biol. Med.* 143: 1227.
- Sutherland, E.W. and Rall, T.W. (1958). Fractionation and characterization of a cyclic adenosine ribonucleotide formed by tissue particles. *J. Biol. Chem.* 232: 1077-1091.
- Sutherland, E.W., Robison, G.A. and Butcher, R.W. (1968). Some aspects of biological role of adenosine 3':5'-monophosphate. *Circulation*, 37: 279-306.

- Ten Hoor, F. and Vergroesen, A.J. (1975). Prostaglandins and the heart. *J. Mol. Cell. Cardiol.* 7(8): 535-541.
- Venter, J.C., Ross, J. and Kaplan, N.O. (1975). Lack of detectable change in cyclic AMP during the cardiac inotropic response to isoproterenol immobilized on glass beads. *Proc. Nat. Acad. Sci. U.S.A.* 72: 824-828.
- Vergroesen, A.J., DeBoer, J. and Gottenbos, J.J. (1967). Effects of prostaglandins on perfused isolated rat hearts In Nobel Symposium 2, Prostaglandins, pp. 211-218. Stockholm: Bergstrom and Samuelsson Eds., Almquist and Wicksel, Stockholm.
- Vergroesen, A.J. and DeBoer, J. (1968). Effects of prostaglandins E_1 and $F_{1\alpha}$ on isolated frog and rat hearts in relation to the potassium-calcium ratio of the perfusion fluid. *Eur. J. Pharmacol.* 3: 171-176.
- Weeks, J.R. (1969). The prostaglandins: Biologically active lipids with implications in circulatory physiology. *Circulation Research* 24 Suppl. 1, 123.
- Weeks, J.R., Sekhar, N.C. and DuCharme, D.W. (1969) Relative activity of prostaglandins E_1 , A_1 , E_2 and A_2 on lipolysis, platelet aggregation, smooth muscle and cardiovascular system. *J. Pharm. Pharmac.* 21: 103.

- Weeks, J.R. (1978). the general pharmacology of prostacyclin PGI_2 (PGX): A new prostaglandin especially active in cardiovascular system. Acta. Biol. Med. Germ. 37: 707-714.
- Wennmalm, A. (1971). Studies on mechanisms controlling the secretion of neurotransmitters in the rabbit heart. Acta. Physiol. Scand. 82 Suppl. 365: 1-36.
- Wennmalm, A. (1975). Prostaglandin release and mechanical performance in the isolated rabbit heart during induced changes in the internal environment. Acta. Physiol. Scand. 93: 15.
- Wennmalm, A. (1976). Endogenous prostaglandins as regulators of autonomic neurotransmission in the rabbit heart. Acta. Biol. Med. Germ. 35: 1127-1133.
- Wennmalm, A. (1978). Prostaglandin-mediated inhibition of noradrenaline release: III. Separation of prostaglandins released from stimulated hearts and analysis of their neurosecretion inhibitory capacity. Prostaglandins 15(1): 113-121.

- Wolf, S.M. and Shulman, N.R. (1969). Adenyl cyclase activity in human platelets. Biochem. Biophys. Res. Comm. 35: 265.
- Wollenberger, A., Ristau, O. and Schoffa, G. (1960). Pflügers. Arch. Ges. Physiol. 270: 399-412.
- Zehl, U., Ritter, Ch. and Förster, W. (1976). Influence of prostaglandins upon adenosine release and of adenosine upon prostaglandin release in the isolated rabbit heart. Acta. Biol. Med. Germ. 35: 77-82.