SOME ACTIONS OF PROSTAGLANDINS ON THE HEART

by

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ABSTRACT

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SOME ACTIONS OF PROSTAGLANDINS ON THE HEART

The role of prostaglandins (PGs) in influencing myocardial and coronary performance was studied in isolated hearts.

PGE2 and PGF2α induced rhythm instabilities in isolated rat hearts at low concentrations while at higher levels the dysrhythmic activity declined. These effects were prevented by either copper or chloroquine.

Comparative effects of PGE2, PGD2, PGF1$_\alpha$ and PGF2$_\alpha$ were studied on rat hearts. PGF2α had potent inotropic and coronary constricting actions. PGD2 was also a potent coronary constrictor but had no inotropic effects. PGE2 had slight inotropic actions and constricted the coronaries at low concentrations and dilated them at high ones. All PGs studied decreased the heart rate. It was also found in these studies that the cardiac effects of PGs can be influenced by the sex of the animal or by gonadectomy.

Studies with prostacyclin (PGI2) indicated that it possesses diverse actions. At critical low concentrations PGI2 induced arrhythmias and caused coronary constriction in rat and rabbit hearts. PGI2 also possessed chronotropic and inotropic actions in rat but not rabbit hearts.

Under hypoxic perfusion it was found that the coronary arteries of rat hearts constricted after an initial dilating response. This phenomenon was not seen in hearts from very young animals or in hearts pretreated with blockers of PG biosynthesis or antagonists of PG action. Also, progesterone was able to prevent the constricting phase while testosterone and estradiol were not effective.

These studies point to some undesirable actions of PGs which may play a role in the development of coronary heart disease.

RESUME

M. Karmazyn Ph.D. Physiologie

QUELQUES ACTIONS DES PROSTAGLANDINES SUR LE COEUR

Le rôle des prostaglandines (PGs) dans des coeurs isolés de rats a été étudié en ce qui a trait à leur influence sur la performance du myocarde et des artères coronaires.

La PGE2 et la PGF2α à faible concentration dans des coeurs de rats isolés produisaient des irrégularités rythmiques tandis que à concentrations plus élevées l'activité dysrythmique était diminuée. Ces effets étaient empêchés soit par le cuivre ou la chloroquine.

Nous avons comparé les effets de la PGE2, la PGD2, la PGF1α et la PGF2α dans le coeur des rats. La PGF2α avait de puissantes actions inotropiques et constrictait les vaisseaux coronaires. La PGD2 était aussi un puissant constricteur mais n'avait aucun effet inotropique. La PGE2 avait une légère action inotropique et constrictait à faibles concentrations les coronaires et les dilatait à concentrations élevées. Les PGs étudiées diminuaient les battements cardiaques. Ces études ont démontré que les effets cardiaques peuvent être influencés par le sexe de l'animal ou par la gonadectomie.

Les études faites avec la prostacycline (PGI2) ont indiqué que celle-ci a des actions diverses. A des concentrations critiquement faibles, la PGI2 a induit des arrythmies et a causé une constriction coronaire dans les coeurs de rats et de lapins. La PGI2 possédait aussi des actions chronotropiques et inotropiques dans le coeur des rats mais non de lapins.

Sous une perfusion hypoxique, nous avons trouvé que les artères coronaires des coeurs de rats se dilataient pour ensuite se constricter. Ce phénomène n'a pas été observé dans les coeurs de très jeunes animaux ou dans les coeurs auparavant traités avec les bloqueurs de la biosynthèse ou avec les antagonistes de l'action des PGs. Aussi, la progesterone était capable d'empêcher la phase de constriction. Cependant, la testosterone et l'estradiol n'étaient pas efficaces.

Ces études suggèrent que les prostaglandines ont des actions indésirables qui peuvent jouer un rôle dans le développement des maladies cardiaques coronaires.

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CLAIMS TO ORIGINALITY

These may be summarized as follows:

1. Prostaglandins are known to be antiarrhythmic agents. This is the first report indicating that these substances, at physiological concentrations can induce dysrhythmic activity.

2. The actions of prostaglandins on isolated heart preparations have been studied with high unphysiological levels. This report describes complete dose-response studies with prostaglandins on parameters of cardiac and coronary function. It also describes for the first time the actions of a newly-discovered prostaglandin, prostacyclin, on isolated hearts. Although regarded as a coronary dilatator, this report presents evidence that prostacyclin possesses diverse cardiac actions including coronary vasoconstriction.

3. Sexual variation in the heart response to prostaglandins has not been documented. This is the first report indicating that gender can influence the heart responses to exogenous prostaglandins *in vitro*. This presentation also indicates for the first time, that gonadectomy can modify the cardiac responses to prostaglandins.

4. The response of the coronary vessels to hypoxia is considered to be vasodilatation. This report details evidence that if prolonged, hypoxia can result in coronary artery constriction in hearts from mature but not young animals. Evidence is presented for the possible involvement of prostaglandins in this response. Prevention of this phenomenon through pharmacological manipulation is also described for the first time.

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INTRODUCTION

Since their initial discoveries the prostaglandins have been studied extensively for their physiological actions. Their cardiac effects however, are poorly understood. As will become evident in the Literature Review the prostaglandins possess a myriad of cardiac actions. Generally they are considered to be coronary dilating, chronotropic, inotropic and antiarrhythmic agents.

There are various possible reasons for the diverse reports regarding cardiac prostaglandin actions. One of these is that some prostaglandins possess biphasic actions, ie., opposite actions at different concentrations. Sexual variation in the cardiac response to prostaglandins has not been reported and this may be another possible cause in the contradictory results.

Although the prostaglandins are considered antiarrhythmic agents, there is much indirect evidence suggesting prostaglandin involvement in arrhythmogenesis (see Discussion).

Various workers have proposed that coronary artery spasm may be an important contributor to the development of coronary heart disease. Yet it is believed that the primary coronary response to reduced oxygen levels is dilatory.

This study was undertaken therefore, to observe the direct actions of prostaglandins on isolated hearts using a wide range of prostaglandin concentrations. The main parameters that were measured include coronary perfusion pressure, contractile force and heart rate. Also, using a very sensitive system, the effects of prostaglandins on rhythm stability were studied.

Special attention was paid to prostacyclin, since this may be the main cardiac prostaglandin. In some experiments, prostaglandin effects were studied in hearts from male and female normal and gonadectomized animals.

The coronary artery response to prolonged hypoxia in isolated rat hearts was also studied. A possible role of prostaglandins was examined using various agents that inhibit prostaglandin biosynthesis or antagonize their action. Due to the resistance of pre-menopausal woment to coronary heart disease, the effects of various gonadal steroid hormones on the coronary artery response to hypoxia was also studied.

LITERATURE REVIEW

1. The Prostaglandins: A General Introduction

The prostaglandins (PGs) are a group of closely related 20 carbon fatty acid derivatives with a wide range of physiological actions. Kurzrok and Lieb (1930), Euler (1934, 1935) and Goldblatt (1933, 1935) were the first to describe the existence of a substance in the seminal fluid of a variety of animals which was able to stimulate the activity of smooth muscle and to lower the blood pressure of experimental animals. Euler (1936) was the first to show that the biological activity was associated with a new lipid soluble acid fraction which he termed prostaglandin and vesiglandin. Since this early discovery the presence of PGs have been confirmed in most body tissues.

Subsequently, nine prostaglandin families have been identified. These are labelled as PGA, PGB, PGC, PGD, PGE, PGF (α and β), PGG, PGH and PGI according to the configuration of the five-membered ring. Each PG is further identified by the subscripts 1-3 depending on the number of specifically located double bonds on the fatty acid chain. The structures of various PGs as well as their fatty acid precursors are shown in figure 1. The principal pathway for PG synthesis has been described by different workers (Bergstrom 1967, Van Dorp 1967, Samuelsson 1972) and is shown in figure 2. In mammals the most abundant precursor of PGs is arachidonic acid (5,8,11,14-eicosatetraenoic acid) which produces PGs of the "two" series. The main precursors of PGs of the "one" and "three" series are 8,11,14-eicosatrienoic acid and 5,8,11,14,17-eicosapentaenoic acid respectively. The PG precursors are cleaved from

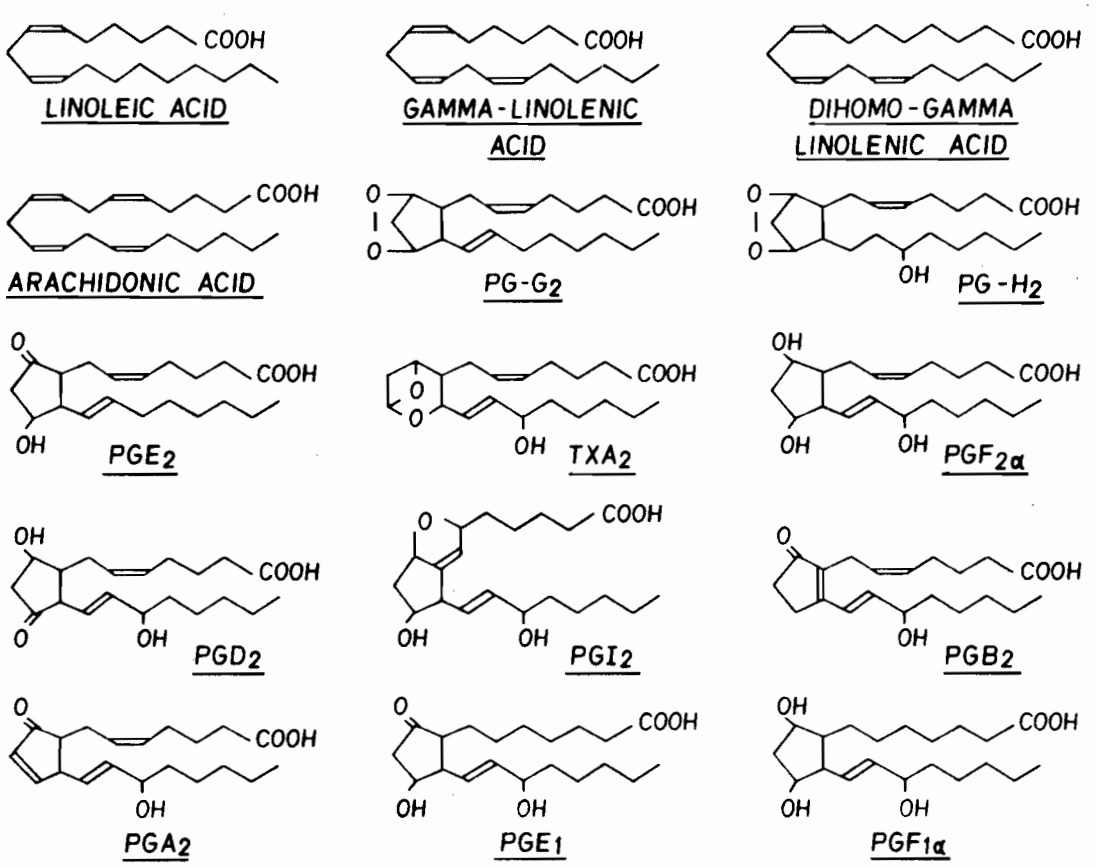

Figure 1. Structures of various prostaglandins and fatty acid precursors

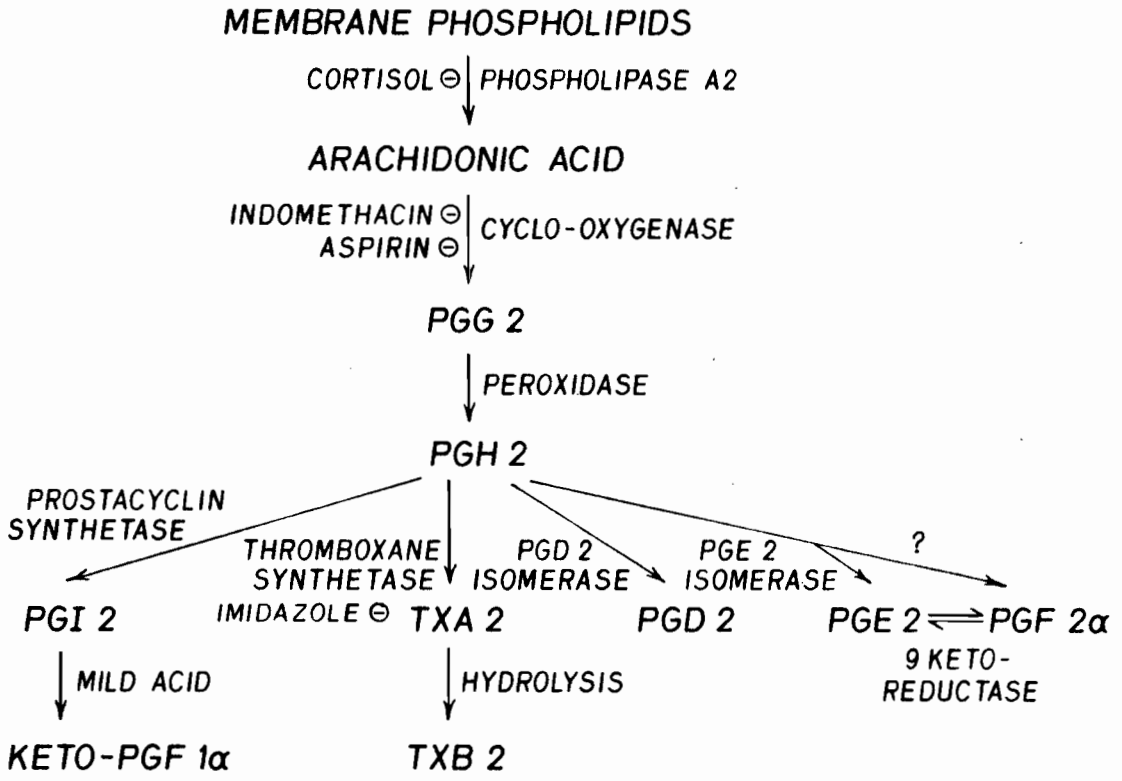

FIG. 2 PRINCIPAL PATHWAY FOR THE SYNTHESIS OF THE '2' TYPE OF PROSTAGLANDINS.

membrane-bound phospholipids by a group of enzymes known as acyl hydrolases of which phospholipase A2 (which removes arachidonic acid) is the most important. A multienzyme system which has not been fully described and known as PG synthetase or cyclo-oxygenase converts arachidonic acid (or other precursors) to the biologically active endoperoxides (PGG and PGH). These substances can then be converted to the various PGs or to thromboxane A2 (TXA2). This latter substance is biologically very active and unstable and is readily converted to the stable and weakly active TXB2. Various steps in the PG synthesis pathway can be inhibited by different agents. Cortisol can block the action of phospholipase A2 (Hong and Levine 1976) while non-steroidal anti-inflammatory agents (eg indomethacin or aspirin) can inhibit the PG synthetase system (for review of drugs that inhibit PG synthesis see Flower 1974). Selective inhibition of specific pathways can be brought about by a number of agents. The synthesis of PGI2 (prostacyclin) from endoperoxides can be blocked by 15-hydroperoxyarachidonic acid (Dusting *et al* 1977a). Similarly TXA2 production can be selectively inhibited by a number of agents including imidazole, benzydamine, or N-0164 (Moncada *et al* 1977a, Needleman *et al* 1977a).

The synthesis of PGs can be increased by a number of stimuli. In the heart a wide variety of agents can have this effect as will be discussed in the next section. In a wide variety of other tissues various naturally occurring agents can stimulate PG production. Some of these include bradykinin (McGiff *et al* 1976, Hong *et al* 1976), catecholamines (Takeguchi *et al* 1971, Danon *et al* 1975), thrombin

(Feinstein *et al* 1977), angiotensin (Alexander *et al* 1976, Kondo *et al* 1977), serotonin (Takeguchi *et al* 1971), vasopressin (Danon *et al* 1975, Karmazyn *et al* 1978), myo-Inositol (Karmazyn *et al* 1977) and prolactin (Horrobin *et al* 1974, Rillema 1975).

PGs are widely distributed in mammalian tissues. In humans PGs have been isolated from the kidney (Vance 1973), lung (Anggard 1965, Karim *et al* 1967), thymus (Karim *et al* 1967), thyroid (Karim *et al* 1967), nerve tissue (Karim *et al* 1967), skin (Greaves *et al* 1971) as well as in various types of body fluids (for summary of the occurrence of PGs in human tissues and fluids see Karim and Rao 1975). The most common type of PG found in body tissues and fluids are the "2" type probably because arachidonic acid is the most common fatty acid in the body. PGs are probably formed at their site of action due to local stimuli and are probably not stored in the body. Most PGs are inactivated as they pass through the pulmonary circulation (Ferreira and Vane 1967). On the other hand PGI2 is not affected in this manner (Armstrong *et al* 1978). The PGs exert a wide variety of effects on most physiological parameters which is beyond the scope of this review. The interested reader is therefore referred to Karim and Rao 1975.

2. *Identification of Cardiac Prostaglandins and Stimulation of Cardiac Prostaglandin Release*

The first report of the identification of PGs in mammalian hearts was presented by Karim and coworkers (1967). They demonstrated the presence of PGs in human tissues including the heart using a bioassay method. The hearts were obtained from patients within 12 hours of death. In two samples

studied PGE2 was detected at 2.25 ng/g tissue and 3.5 ng/g tissue. The presence of other PGs was not observed. Karim *et al* (1968) have also observed the presence of a variety of PGs in the hearts of various animal species. The concentrations they found (in ng/g heart tissue) was for the cat 3.7 F2α , rat 3.8 E2, chicken 3.5 F2α and 51.0 E2. PGs were not detected in the dog, guinea pig and rabbit hearts. The characterisation of the myocardial PG synthetase system was performed by Limas and Cohn (1973a). They demonstrated that an active PG synthesis system in the canine myocardium was located in the microsomal fraction. In this report PG synthesis was found to be highly resistant to inhibition by aspirin (up to 150 ug/ml) or to stimulation by phospholipase A2 (up to 10 U/ml). In another report the same workers (Limas and Cohn 1973b) demonstrated an active presence of myocardial PG dehydrogenase in the canine heart. This enzyme inactivates PGE and PGF by oxidizing the 15-hydroxy group of these substances. This dehydrogenase is NAD^+ dependant and requires an intact sulfhydryl group. The enzyme activity is enhanced by cyclic adenosine monophosphate and inhibited by calcium chloride. In a recent report however, Needleman (1976) found little PG dehydrogenase activity in isolated rabbit hearts. Compared to other tissues in the rat Pace-Asciak and Rangaraj (1977) have found that the heart produces small amounts of PGs. The main PGs found in the heart in order of amount were 6 keto-PGF1α>PGE2>TXB2>PGD2>PGF2α . A new PG was was recently found to be the main PG released from isolated rat and rabbit hearts (De Deckere *et al* 1977, Isakson *et al* 1977,

Dembincka-Kiec *et al* 1977) as well as in arterial walls (Raz *et al* 1976, Moncada *et al* 1976a, 1976b, Gryglewski *et al* 1976) including human arteries (Moncada *et al* 1977). This substance known as prostacyclin or PGI2 has powerful effects in inhibiting platelet aggregation (Bunting *et al* 1976). Prostacyclin is rapidly converted to 6 keto-PGF1α which could explain the predominance of the latter compund in the heart (Pace-Asciak and Rangaraj 1977).

Although the existence of PGs in the heart was first demonstrated over 10 years ago, various stimulants of cardiac PG release (presumably by increased synthesis) have been identified only recently. Kent and co-workers (1973) were one of the first groups to show increased PG release from the heart as a result of manipulation using either intact dogs or heart-lung preparations. Occlusion of the left main coronary artery for 10, 15 or 20 seconds, or inducing hypoxia ,resulted in an increased coronary blood flow (after removing occlusion) accompanied by an increased appearance of PGE in the coronary sinus effluent. The dilatation due to hypoxia, the reactive hyperaemia after removal of occlusion, or the increase in PGE levels were blocked by indomethacin or meclofenamate. In isolated rabbit hearts production of ischemia had variable effects on PG release (Minkes *et al* 1973). Wennmalm (1975a) reported that ischemia actually reduced PG outflow from rabbit hearts. Hypoxia however, was a potent stimulant of PG release (Wennmalm 1975a, 1975b). In a series of recent reports Block *et al* (1975), Needleman *et al* (1976a) and Needleman (1976) all showed that either hypoxia, anoxia or ischemia stimulated the release of PGs from rabbit hearts. In experiments using anaesthetized open-chest dogs Kraemer and colleaues (1976) showed increased PGE2, PGA2 (which may have been measured due to artifacts of extraction methods) and arachidonic acid levels in the

coronary outflow after occlusion of the left anterior descending artery. Pretreatment with indomethacin prevented the rise in PG but not in arachidonic acid levels. Two other groups of workers showed increased cardiac PG production in intact dogs as a result of ischemia. Berger *et al* (1976) measured PGs in aortic, cardiac vein and coronary sinus blood during coronary occlusion. PGF and PGE levels after occlusion were high in the coronary sinus but not in the aorta. PGF levels were not increased in the cardiac vein (which drains blood from non-ischemic regions) but PGE levels rose in blood from both the ischemic and non-ischemic areas. The authors suggested that local availability of PGs may influence the cardiac responses to ischemia. Ogletree *et al* (1977) also demonstrated increased PGF2α levels in the coronary sinus after ischemia in anaesthetized dogs. This release was blocked by indomethacin. Very low PGE levels were found in these studies however. The first report of increased PG production in humans as a result of myocardial ischemia was demonstrated by Berger *et al* (1977). In 11 of 12 patients with coronary heart disease, angina induced by atrial pacing significantly increased PGF levels in the coronary sinus blood but not in the aorta. PGE and PGA levels in the coronary sinus were slightly but insignificantly increased. After recovery the PG levels declined to normal values.

Minkes *et al* (1973) and Needleman *et al* (1974) reported that purine nucleotides (ADP and ATP) increased the release of a prostaglandin-like substance from the rabbit heart. Norepinephrine, bradykinin, adenosine, angiotensin I or angiotensin II had no effect. However Pham-Huu-Chanh *et al* (1973). did demonstrate increased PG release from isolated rabbit hearts after the addition of norepinephrine (10 µg/ml). This effect was not blocked by

phentolamine or propranolol. Similarly Junstad and Wennmalm (1973) and Wennmalm (1975a) showed that norepinephrine infusion at 5 µg/ml also increased PG (mostly PGE2) outflow from isolated rabbit hearts. This was not prevented by either phenoxybenzamine, propranolol or the destruction of adrenergic nerves. Cholinergic nerve stimulation or acetyl choline infusion also produced an increase in the outflow of a prostaglandin-like substance in isolated rabbit hearts (Junstad and Wennmalm 1974, Wennmalm 1975a). These effects as well as the cardiac effects of vagal stimulation or acetyl choline infusion were blocked by atropine.

The role of angiotensin II was again studied this time by Limas (1974) who showed that rat heart slices incubated with angiotensin II demonstrated increased PG synthetase activity. Omission of tyrosine from the incubating medium inhibited this effect suggesting that the PG stimulation is through the ability of angiotensin II to enhance the release of newly synthesized norepinephrine. Rats pretreated with 6-hydroxydopamine had depleted PG synthetase levels and showed a decreased angiotensin II-induced stimulation. Norepinephrine was also able to stimulate PG synthesis on its own, a phenomenon not blocked by propranolol. The studies of Minkes *et al* (1973) and Needleman *et al* (1974) have used angiotensin II concentrations of 10^{-10}M. Limas (1974) however showed that the threshold for angiotensin II induced stimulation was 10^{-9}M (maximum effect at 10^{-6}M) which may explain the discrepancies between these reports.

Anaphylaxis has recently been shown to be a potent stimulus of PG release from isolated guinea pig hearts (Liebig *et al* 1975). Anaphylaxis was induced by direct injection of ovalbumin into the heart. PGF2α levels

were greatest while only trace amounts of PGE were observed. The effect was increased by theophylline, reduced with propranolol and prevented with indomethacin. Indomethacin however did not affect the typical anaphylactic reaction (eg. release of slow reacting substance of anaphylaxis or histamine). Levi *et al* (1976) showed that isolated hearts obtained from sensitized guinea pigs exhibited tachycardia, arrhythmias and coronary constriction as well as increased histamine release. In the presence of indomethacin (5 µg/ml) anaphylactic histamine release increased. By itself indomethacin had no effect on cardiac function including coronary flow rate. Indomethacin also did not modify the chronotropic and inotropic actions of exogenous histamine. Increased $PGF2\alpha$ levels were observed in the anaphylactic hearts. Anhut *et al* (1978) have recently demonstrated that TXB2 (indicating TXA2 production) is released in large amounts after antigenic challenge with ovalbumin in isolated guinea pig hearts. PGD2 and $PGF2\alpha$ were released in lesser amounts. Isoproterenol decreased the TXB2 or PG release from the anaphylactic hearts. The increased release of PG compounds was associated with significant decreases in coronary flow.

A wide variety of other chemical factors can stimulate PG release from the heart. Moretti *et al* (1976) demonstrated increased PG release after the addition of blood plasma to isolated rabbit hearts. This phenomenon was associated with increased conversion of labelled arachidonic acid to PGE2 and $PGF2\alpha$. Unsaturated fatty acids can stimulate PG release from isolated guinea pig hearts (Mentz and Forster 1977). The order of potency in stimulating PG efflux was arachidonic acid>linoleic acid>linolenic acid. Nicotine has recently been found to stimulate cardiac PG release (Wennmalm and Junstad 1976a,Wennmalm 1977). In the first study a corresponding increase in norepinephrine release was also seen after the addition of nicotine (8 µg/ml).

Table 1. STIMULANTS OF CARDIAC PROSTAGLANDIN RELEASE

STIMULANT	SPECIES	PROSTAGLANDINS RELEASED	REFERENCE
Anoxia	Rabbit	E2	Block *et al* 1975
	"	I2	De Deckere *et al* 1977
	Rat	I2	"
Hypoxia	Rabbit	E	Wennmalm *et al* 1974
	"	E2	Wennmalm 1975
	"	PLS	Needleman *et al* 1975
Ischemia	Rabbit	PLS	Needleman *et al* 1975
	Dog	E,F	Berger *et al* 1976
	"	A2,E2	Kraemer *et al* 1976
	"	F2α,E2	Ogletree *et al* 1977
	Man	F	Berger *et al* 1977
Norepinephrine	Rabbit	PLS	Pham-Huu-Chanh 1973
Acetylcholine/ Vagal Stim	Rabbit	PLS	Junstad *et al* 1974
Angiotensin	Rabbit	PSA	Limas 1974
Nicotine	Rabbit	E	Wennmalm and Junstad 1976a
	"	PLS	Wennmalm 1977
Anaphylaxis	Guinea Pig	F2α	Liebig *et al* 1975
	"	F2α,D2,TXB2	Anhut *et al* 1978

PLS: Prostaglandin-like substance
PSA: Prostaglandin synthetase activity

Fasting or diabetes seem also to be related to increased cardiac PG production (Stam and Hulsmann 1977). An enhanced coronary flow and PG-like outflow occurs in hearts removed from fasted or diabetic (streptozotocin-induced) rats. Both effects were reduced by indomethacin.

Cardiac overload has also been shown to cause an increase in PG synthesis (Limas *et al* 1974). In this report, left ventricular hypertrophy was produced by aortic constriction. Within 5 minutes intramyocardial cyclic adenosine monophosphate (cAMP) and PG levels were elevated. Both the PG and the cAMP increases were prevented by pretreatment with indomethacin.

Isolated bovine coronary artery strips have been shown to produce a PGE-like substance (Kalsner 1975, 1976). These studies also demonstrated that the outflow of PGs can be increased by hypoxia (Kalsner 1976) and reduced by aspirin or indomethacin (Kalsner 1975, 1976).

Various changes in the cardiac internal environment including acidosis, hyperthermia, hyperosmolarity, hyperkalemia or hypercalcemia all were found to have no effect on PG release from rabbit hearts (Wennmalm 1975a).

3. *Prostaglandins and Cardiac Electrical Activity*

PGs are generally considered to be antiarrhythmic agents and the first report to describe this was by McQueen and Ungar (1969). Numerous other workers have since demonstrated the antiarrhythmic properties of PGs. Intravenous injection of PGE1 (0.5 to 4 µg/kg) or continuous infusion (0.5 to 4 µg/kg/min) supressed dysrhythmic activity as a result of coronary ischemia in anaesthetized dogs (Zijlstra *et al* 1972). Similar results were

reported by Kelliher and Glenn (1973) who showed that PGE1 (50 µg/kg) injection raised the threshold for ouabain induced arrhythmias in anaesthetized cats. The effects of PGF2α on various models of experimental arrhythmias was examined by Forster *et al* (1973). This PG was effective in increasing survival rates in calcium chloride-induced arrhythmias in rats as well as barium chloride-induced arrhythmias in cats. In another report the same group (Mann *et al* 1973) found that intravenous infusion of PGF2α (10 to 40 µg/min) had antiarrhythmic properties in patients with constant extrasystoles. However it was less effective in patients exhibiting tachyarrhythmias. In a second patient study PGF2α had variable effects in preventing or reversing extrasystoles (Mann 1976). Most favourable results were seen with patients who previously suffered a myocardial infarction and were on glycoside therapy. Kaiser and Schoelkens (1974) reported that PGE2 had the strongest antiarrhythmic properties in cats (1 µg/kg), guinea pigs (5 µg/kg) and dogs (2 to 10 µg/kg). In a variety of experimental models however PGF2α ,PGE2, PGA2, PGA1, and PGE1 all demonstrated similar efficacy (Mest *et al* 1975). Kelliher and coworkers (1975) compared the effects of PGs with the antiarrhythmic agents quinidine and lidocaine on pacemaker automaticity in isolated hearts with atrio-ventricular block. PGA1 was the only PG which affected ventricular rate while both PGA1 (1 µg/ml) and PGF2 (1 µg/ml) decreased atrial rate as well as ventricular asystole or ventricular fibrillation.

Possible antiarrhythmic properties of PG precursors were recently examined by Mest *et al* (1977). In ouabain-induced arrhythmias in cats, rabbits and guinea pigs arachidonic acid infusion (1 mg/kg/min) for 3 to

5 minutes had antiarrhythmic effects. Linoleic acid was one-half as effective. In rabbits arachidonic and linoleic acids were effective in abolishing barium chloride induced arrhythmias in most of the animals tested. Two other fatty acids, linolenic and oleic had little effect.

A possible undesirable effect of PGs on cardiac rhythm was recently reported by Burt *et al* (1977). In this report a patient undergoing therapeutic abortion with PGF2α administration developed cardiac arrhythmias and hypokalemia. These arrhythmias were corrected within 12 hours by lidocaine and potassium chloride infusion.

There have been various studies determining the effects of PGs on the cardiac action potential. Kobayashi *et al* (1971) using isolated guinea pig hearts demonstrated that PGE1 (5 µg, direct injection) produced shortening of phase 2 (plateau phase) of the action potential and increased the resting potential. However, Smejkal and coworkers (1970) using an isolated trabecula of the frog atrium showed that PGE1 from 35 ng/ml to 3.5 µg/ml increased the duration and amplitude of the action potential. The ionic basis of cardiac PGE1 action was recently studied on frog atrial muscle (Mironneau and Grosset 1976). The authors reported that PGE1 (up to 350 ng/ml) increased the amplitude and duration of the action potential while increasing the force of contraction. In voltage clamp experiments the same authors reported that PGE1 prolonged the slow inward current and the delayed outward current indicating that calcium influx and the release of intracellular calcium may be involved in the positive inotropic actions of PGE1 on the frog atrium (Mironneau and Grosset 1976).

A biphasic actions of PGs on the cardiac action potential was reported by Kecskemeti *et al* (1973). Low PGE2 concentrations (5 ng/ml) increased the maximum rate of depolarization and overshoot of the transmembrane potential of isolated cat atrial and rat ventricles and produced no effect in the guinea pig atria. At higher PGE2 concentrations (20 ng/ml) opposite effects were seen. Similarly at high PGE2 concentrations the resting potential was decreased in all tissues. In another report by the same authors (Kecskemeti *et al* 1976) using isolated cat and guinea pig atria reported that PGE2 as well as PGE1 at 100 ng/ml decreased the resting potential and this effect was not dependant on extracellular sodium. When potassium permeability was increased with carbaminoyl, PGE2 did not decrease the resting potential indicating that the depolarizing effect at high concentrations of PGE2 may be dependant on changes in potassium permeability. In a recent report Scholkens and Kaiser (1977) demonstrated biphasic actions of PGE2 (1 pg/ml to 0.1 µg/ml) on isolated guinea pig papillary muscle. Up to 35 pg/ml PGE2 prolonged the refractory period while at higher concentrations the effect disppeared.

Administration of PG synthesis precursors has also been shown to have electrophysiological actions on cardiac muscle. Arachidonic acid (0.3 to 30 µg/ml) increased the rate of rise and amplitude of the action potential of isolated atrial and papillary muscle and canine Purkinje fibers (Szekeres *et al* 1976). Slight increases in membrane resting potential and prolongation of the action potential were also seen. These effects of arachidonic acid were blocked by indomethacin (21 µg/ml). In contrast to

previous report, action potentials were shortened by the administration arachidonic acid (1 µg/ml to 50 µg/ml) in right ventricular strips of the guinea pig (Mentz *et al* 1976b). Similar results were seen with oleic, linoleic and linolenic acid at similar concentrations. In this same report PGE1 (10 ng/ml to 1 µg/ml) had little effect but PGF2α at similar concentrations prolonged and raised the action potential. These workers reported that the refractory period was shortened by all the fatty acids but not by any of the PGs.

4. *Inotropic Actions of Prostaglandins*

The effects of PGs and their related substances on heart rate and force of contraction are extremely varied as they may depend on the type of PG studied, method of study, species or sex of the animal. Euler (1936) was the first to demonstrate that a PG extract from the seminal fluid had no inotropic or chronotropic effects on isolated rabbit hearts or on a cat heart-lung preparation. Since these studies most of the currently known PGs have been examined for their cardiac actions both in the *in vivo* and *in vitro* situations.

a. In vivo inotropic actions

PGA1

Intracoronary injection of PGA1 (0.1 to 12 µg) in dogs was found to increase the left ventricular contractile force (Nutter and Crumly 1970). These actions of PGA1 seemed to be mediated through β adrenergic receptors as β receptor blockade attenuated the cardiac stimulating actions of this substance (Higgins *et al* 1972). However in anaesthetized normal dogs PGA1 (0.1 to 12.5 µg) intracoronary injections did result in an augmented contractile force (Higgins *et al* 1972).

Table 2.EFFECTS OF PROSTAGLANDINS ON THE CARDIAC CONTRACTILE FORCE IN DIFFERENT SPECIES

Prostaglandin	Species	Method	Effect	Reference
A1	Dog	Intracoronary	↑	Nutter & Crumly 1970
	"	Isolated Heart	→	Su *et al* 1973
	Cat	"	→	"
	Rabbit	Isolate Atria	→	Nutter & Ralls 1973
C2	Cat	Intravenous	→	Jones *et al* 1974
	Dog	"	↑	"
E1	Dog	Intravenous	↑	Nakano & McCurdy 1967
	"	Intracoronary	↑	Hollenberg *et al* 1968
	Cat	Intravenous	↑	Momma *et al* 1976-77
	Rat	Isolated Heart	↑	Berti *et al* 1965
	Guinea pig	"	↑	"
	Rabbit	"	→	"
	Cat	"	→	Su *et al* 1973
	Dog	"	→	"
	Frog	Isolated Atria	↑	Mironneau & Grosset 1976
E2	Cat	Intravenous	→	Jones *et al* 1976
	Dog	"	↑	"
	Rabbit	Isolated Heart	→	Hedqvist & Wennmalm 1971
	Human	Isolated Atrial Tissue	→	Levy & Killebrew 1971
	Rat	Isolated Atria	↑	Levy 1973
	Guinea pig	"	↑	Szekeres *et al* 1976
F1α	Dog	Intracoronary	→	Hollenberg *et al* 1968
F2α	Dog	Intracoronary	→	Hollenberg *et al* 1968
	"	"	→	Chiba *et al* 1972
	Rabbit	Isolated Heart	→	Hedqvist & Wennmalm 1971
	Cat	"	→	Su *et al* 1973
	Dog	"	→	"
	Guinea pig	Isolated Atria	↑	Nutter & Ralls 1973
	Toad	Isolated Ventricles	↓	"

↑ Increased contractile force
↓ Decreased contractile force
→ No effect

PGC2

PGC2 (0.06 to 5.5 µg/kg/min) infused intravenously had no myocardial actions in the intact cat while at infusion rates of 0.07 to 1 µg/kg/min it did demonstrate positive inotropic actions in the dog (Jones *et al* 1974).

PGE1

Nakano and McCurdy (1967) demonstrated that PGE1 injection into dogs (0.25 to 4 µg/kg iv) decreased arterial pressure, left atrial pressure and left ventricular end diastolic volume while on the other hand increasing the cardiac output and myocardial contractile force. Propranolol did not block the inotropic actions of PGE1 suggesting that this action was not centrally mediated. Direct intracoronary infusion of PGE1 (2 to 8 µg/min) in dogs increased the contractile force (Hollenberger *et al* 1968). Nutter and Crumly had similar results with single injections of PGE1. In these experiments (Nutter and Crumly 1970) intracoronary PGE1 injections (0.1 to 12 µg) resulted in a greatly enhanced myocardial contractile force. Similarly, in anaesthetized open-chest dogs PGE1 intracoronary injection (0.1 to 12.5 µg) again was found to have positive inotropic actions (Higgins *et al* 1972). However PGE1 did not have inotropic actions when injected into the canine sinus node artery in concentrations ranging from 0.5 to 5 µg (Chiba *et al* 1972). Cardiac responses to PGE1 can be modified in the cat by making the animal hypoxic (Momma *et al* 1976-1977). In this study direct PGE1 infusion (2 µg/kg/min) caused an increase in the myocardial tension. In cats made hypoxic by breathing a 10% O_2/90% N_2 mixture PGE1 had completely opposite actions.

PGE2

In vivo inotropic actions of PGE2 have not been extensively reported. It has no effects at doses from 0.3 to 2.8 µg/kg/min iv in the intact cat but lower levels (0.07 to 1 µg/kg/min) have inotropic actions in the dog (Jones *et al* 1974). Also, PGE2 intracoronary injection (2 µg/kg) increase the myocardial contractile force in dogs, an effect which can be blocked by hexamethonium or propranolol (Slotkoff *et al* 1976).

PGF1α

PGF1α (4 to 12 µg/min) intracoronary infusion in dogs was found to be without effect on the cardiac contractile force (Hollenberg *et al* 1968).

PGF2α

As with PGF1α,PGF2α (4 to 12 µg/min) when injected into the coronary artery has no effect on the contractile force of the dog heart (Hollenberger *et al* 1968). Similar results were shown by Higgins (1972) with PGF2α concentrations of 0.1 to 12.5 µg injected into the coronary artery of the dog. A single bolus injection of PGF2α (0.5 to 5 µg) into the canine sinus node artery produced no inotropic actions (Chiba *et al* 1972).

b. In Vitro Actions

PGA1

PGA1 (1 µg/ml) was found not to have inotropic actions on isolated canine or cat hearts (Su *et al* 1973). Also, it did not have inotropic actions on rabbit atria but did increase the force of contraction of guinea pig atria (Nutter and Ralls 1973). In the toad ventricle there was also no inotropic effect.

PGE1

The addition of PGE1 to the perfusion medium increased the contractile force of the isolated frog, rat and guinea pig heart but not of the rabbit heart (Berti *et al* 1965). Further studies with PGE1 confirmed the inotropic actions on rat hearts (Vergroesen *et al* 1967). These same workers also showed that in potassium-intoxicated frog and rat hearts PGE1 (2 µg/ml) increased the force of contraction but had no effect on normally perfused hearts (Vergroesen and de Boer 1968). In this same report PGE1 was able to restore function of potassium-arrested rat hearts. In a canine-heart-lung preparation PGE1 injection into the left atrium was shown to increase the strength of ventricular contraction (Katori *et al* 1970). In the rabbit heart PGE1 (26 to 99 ng/ml) had only slight inotropic actions (Wennmalm and Hedqvist 1970). In another study PGE1 (1 to 500 ng/ml) had no inotropic effects on isolated rabbit hearts (Hedqvist and Wennmalm 1971). PGE1 (100 ng/ml) did not have inotropic actions on either isolated cat or dog hearts (Su *et al* 1973). In the guinea pig atria PGE1 (10 µg/ml) increased the force of contraction while no effects were seen on rabbit atria (Nutter and Ralls 1973). At similar concentrations PGE1 increased the force of contraction of the toad ventricle (Nutter and Ralls 1973). Similarly, Baysal and Vural (1974) observed that PGE1 increased the contractile force of frog ventricular strips, an effect not blocked by either propranolol, phenoxybenzamine or ouabain. In the isolated blood-perfused papillary muscle of the dog PGE1 (1 ng to 1 µg) injected directly into the papillary muscle artery increased the developed tension and this effect could not be reduced

by β receptor blockade (Endoh 1976). Similar inotropic actions of PGE1 were demonstrated by Mironneau and Grosset (1976) using isolated frog atrial muscle. In isolated rat heart muscle cell cultures PGE1 (7 ng/ml to 66 µg/ml) addition was associated with an increased force of contraction (Warbanow 1976).

PGE2

Hedqvist and Wennmalm (1971) found that PGE2 (1 ng/ml to 0.5 µg/ml) had no effect on isolated rabbit hearts. Levy and Killebrew (1971) used a variety of cardiac tissue to examine the actions of PGE2. These included electrically driven or spontaneously beating rabbit right or left atrial preparations as well as human atrial appendages. In the rabbit atrial preparations PGE2 (0.93 ng/ml) had a biphasic effect; first decreasing and then increasing the force of contraction with time. No effects were seen on human atrial tissue. Cholinergic or β adrenergic blocking agents did not modify the PGE2 actions although reserpine pretreatment decreased the negative inotropic effects. Bretylium attenuated the positive inotropic response and diphenylhydramine decreased both the negative and positive inotropic effects of PGE2. In guinea pig atrial preparations PGE2 (0.07 to 7 µg/ml) increased the strength of contraction (Szekeres *et al* 1976). Levey (1973) observed that PGE2 (0.2 to 2 µg/ml) had similar inotropic actions on atria of either normal or spontaneously hypertensive rats.

PGF2α

PGF2α in concentrations ranging from 1 ng/ml to 0.5 µg/ml had no effect on the contractile force of isolated rabbit hearts (Hedqvist and Wennmalm 1971). Similarly, inotropic actions of PGF2α (1 µg/ml) were not observed on isolated cat or dog hearts (Su *et al* 1973). However PGF2α

did increase the contractile force of the isolated guinea pig but not rabbit atrium at 10 µg/ml (Nutter and Ralls 1973). Similar effects on the guinea pig atria were reported by Szekeres *et al* (1976). Conversely, in the toad ventricle PGF2α decreased the force of contraction (Nutter and Ralls 1973).

PG Precursors

A number of recent studies have reported effects of PG precursors on cardiac action. In right ventricular strips of the guinea pig heart oleic acid and linoleic acid (1 to 50 µg/ml) decreased the force of contraction while linolenic acid and arachidonic acid had less inhibitory effects (Mentz *et al* 1976b). In a similar study using isolated guinea pig hearts virtually the same effects with linoleic acid and oleic acid ie., cardiodepression were seen (Mentz *et al* 1976a). However in this report arachidonic acid increased the contractile force while linolenic acid had no effect. In guinea pig atrial preparations arachidonic acid (0.3 to 30 µg/ml) increased the rate and force of contraction (Szekeres *et al* 1976).

Phospholipase A2

This enzyme was found to increase the force of contraction of isolated guinea pig atria at a concentration of 1 µg/ml (Giessler *et al* 1976)

5. *Chronotropic Actions of Prostaglandins*

a. In Vivo Chronotropic Actions

PGA1

In the dog intracoronary injection of PGA1 (0.1 to 12 µg) did not have any chronotropic actions (Nutter and Crumly 1970). However in conscious

Table 3. EFFECTS OF PROSTAGLANDINS ON HEART RATE IN DIFFERENT SPECIES

Prostaglandin	Species	Method	Effect	Reference
A1	Dog	Intracoronary	→	Nutter & Crumly 1970
	"	Intravenous	↑	Higgins *et al* 1973
	"	Isolated Heart	→	Su *et al* 1973
	Cat	"	↑	"
	Guinea Pig	Isolated Atria	↑	Nutter & Ralls 1973
B1 & B2	Dog	Intravenous	↑	Greenberg *et al* 1973
E1	Dog	Intravenous	↑	Carlson & Oro 1966
	"	Intracoronary	→	Nutter & Crumly 1970
	Man	Intravenous	↑	Bergstrom *et al* 1959
	Guinea pig	Isolated Heart	↑	Berti *et al* 1965
	Frog	"	↑	"
	Rabbit	"	→	"
	Rat	"	→	Vergroesen & de Boer 1968
	Cat	Isolated Atria	↑	Kaumann & Birnbaumer 1974
E2	Man	Intravenous	↑	Karim *et al* 1971a
	"	Oral	→	Karim *et al* 1971b
	Rat	Isolated Atria	↑	Levy 1973
	Guinea pig	"	↑	Szekeres *et al* 1976
F2α	Man	Intramuscular	↓	Karim *et al* 1971a
	"	Oral	→	Karim *et al* 1971b
	Dog	Intracoronary	→	Chiba *et al* 1972
	"	Isolated Heart	↑	Su *et al* 1973
	Cat	"	→	"
	Guinea pig	Isolated Atria	↑	Nutter and Ralls 1973
	Rabbit	"	→	"

↑ Increased heart rate
↓ Decreased heart rate
→ No effect

dogs PGA1 injected intravenously (0.01 to 1 µg/kg) increased the cardiac output and heart rate while at the same time lowering the blood pressure (Higgins *et al* 1973).

PGB1 and PGB2

The actions of these PGs were studied by Greenberg *et al* (1973) in the intact dog. In doses of 4.5 to 45 µg/kg (iv) both substances particularly PGB2 decreased the heart rate.

PGE1

PGE1 infusion (0.12 to 12 µg/kg/min) to anaesthetized dogs resulted in tachycardia with a drop in blood pressure (Carlson and Oro 1966). Reserpine pretreatment prevented the rise in heart rate. In another study intracoronary injection of PGE1 (0.1 to 12 µg) had no effect on altering the heart rate (Nutter and Crumly 1970). In the intact cat PGE1 (6 µg/kg) was found to increase the heart rate and decrease the systemic arterial pressure (Koss *et al* 1973). In humans PGE1 was able to induce a tachycardia when infused into male subjects (Bergstrom *et al* 1959, 1965).

In the mouse PGE1 injection (2.5 to 20 µg/kg) has been shown to reduce the bradycardia induced by vagal stimulation (Feniuk and Large 1975) or the tachycardia produced by sympathetic stimulation (Feniuk 1976).

PGE2

In man rapid injection of PGE2 (100 µg) or slow infusion (0.8 µg/kg/min) decreased the blood pressure as well as heart rate (Karim *et al* 1971a). Oral ingestion however had no cardiovascular effects (Karim *et al* 1971b).

PGE2 has similar effects as PGE1 in reducing cardiac responses to either sympathetic or parasympathetic stimulation in the mouse (Feniuk 1976, Feniuk and Large 1975).

PGF2α

A single injection of PGF2α (1 mg) increased the blood pressure and decreased the heart rate in man, the cardiac effects probably being reflex in origin (Karim *et al* 1971a). As with PGE2 oral ingestion of PGF2α had no cardiac effects (Karim *et al* 1971b). In the dog PGF2α did not have any chronotropic actions when injected into the sinus node artery at doses of 0.5 to 5 µg (Chiba *et al* 1972). In the cat PGF2α injection (10 µg/kg to 15 µg/kg) decreased the blood pressure and reduced the heart rate (Koss *et al* 1973).

PGF2α was found to be without effect in modifying the cardiac responses to vagal stimulation in the mouse (Feniuk and Large 1975).

b. In Vitro Chronotropic Actions

PGA1

PGA1 did not have any chronotropic actions on canine hearts but did increase the rate of isolated cat hearts (Su *et al* 1973). The threshold concentration for PGA1-induced tachycardia was about 1 µg/ml. PGA1 (10 µg/ml) also increased the rate of isolated guinea pig atria while having no effect on rabbit atria (Nutter and Ralls 1973).

PGE1

Berti *et al* (1965) reported that PGE1 increased the rate of contraction of guinea pig and frog hearts while having no effect on rabbit or cat hearts. Vergroesen and de Boer (1968) reported no chronotropic actions of PGE1 (2 µg/ml) on rat or frog hearts. However in hearts that were potassium-intoxicated PGE1 did increase the heart rate. In another report PGE1 had no chronotropic effects on frog ventricular strips

(Baysal and Vural 1974). PGE1 injected into the left atrium had no effect on heart rate in an isolated heart-lung preparation (Katori *et al* 1970). Similarly in the isolated dog heart PGE1 was without chronotropic actions while it did increase the rate of isolated cat hearts at 100 ng/ml (Su *et al* 1973). The rate of isolated guinea pig but not rabbit hearts was increased by 10 µg/ml PGE1 (Nutter and Ralls 1973). In the isolated guinea pig atria PGE1 increased the rate of contraction and this effect could be attenuated by the addition of the H2 receptor blocker metiamide (Susskand *et al* 1975) or by morphine (Susskand *et al* 1976). In isolated kitten atria PGE1 (7 µg/ml) increased the rate of contraction (Kaumann and Birnbaumer 1974). These effects were associated with increased adenyl cyclase activity in the myocardial membranes. β receptor blockade with propranolol had no effect either on the chronotropic action of PGE1 or on its ability to stimulate adenyl cyclase. In isolated rat heart muscle cell cultures PGE1 (7 ng/ml to 70 µg/ml) was found to have no inotropic actions (Warbanow 1976).

PGE2

Levy (1973) compared the effects of PGE2 (0.2 to 2 µg/ml) on isolated rat atria from normal or spontaneously hypertensive animals. PGE2 exhibited similar chronotropic actions in both groups. This PG also produced increased rates of isolated guinea pig atria at concentrations from 7 ng/ml to 7 µg/ml (Szekeres *et al* 1976). The chronotropic actions of PGE2 on the guinea pig atria can be blocked by the addition of morphine (Susskand *et al* 1976).

PGF2α

PGF2α (1 µg/ml) was found to increase the rate of contraction of isolated cat but not dog hearts (Su *et al* 1973). Similarly chronotropic effects of PGF2 were seen with guinea pig atria but not with rabbit atria at concentrations between 7 ng/ml and 10 µg/ml (Nutter and Ralls 1973, Szekeres *et al* 1976).

A summary of the chronotropic actions of PGs is presented in table 3.

6. *The Influence of Prostaglandins on the Actions of various Cardio-Modulating Agents In Vitro*

Besides the direct effects of PGs on cardiac performance, these agents have been reported to modify the actions of externally applied stimuli. Hedqvist and coworkers (1970) have shown that PGE2 (30 ng/ml) can inhibit sympathetic neurotransmission in the perfused rabbit heart. The outflow of norepinephrine was significantly decreased while PGE2 reduced only slightly the chronotropic and inotropic actions of externally added norepinephrine. Similar results were obtained with PGE1 (30 ng/ml to 0.1 µg/ml) (Wennmalm and Hedqvist 1970). In this report PGE1 also decreased norepinephrine outflow during sympathetic stimulation and induced a marked increase in the inotropic actions and a slight decrease in the chronotropic actions of sympathetic stimulation. PGE1 also inhibited the chronotropic effects of exogenous norepinephrine but had no effect in modifying the norepinephrine-induced inotropic actions. In another report Hedqvist and Wennmalm (1971) again demonstrated that the outflow of norepinephrine during sympathetic stimulation as well as the chronotropic and inotropic actions can be inhibited by PGE1 and PGE2 (1 ng/ml to 0.5 µg/ml). However, PGF2α at similar concentrations was without effect. None of the PGs modified the responses to exogenous nor-

epinephrine. The above reports as well as that of Samuellson and Wennmalm (1971) suggested a possible role of endogenously synthesized PGs to limit transmitter release in the heart. PGE1 has also been shown to modify cholinergic stimulation in isolated guinea pig atria (Hadhazy *et al* 1973). Concentrations from 2.5 to 5 ng/ml reduced the bradycardia response to vagal stimulation. This effect was associated with a marked decrease in the acetylcholine release from the heart.

In a series of experiments de Boer and coworkers (1973) demonstrated diverse PG interactions with various agents in isolated frog hearts. The following were the main findings (PG concentrations from 34 ng/ml to 3.4 µg/ml);

1. PGE1 opposed the depressant effects of potassium
2. PGE1 had no direct effects in the absence of calcium
3. PGE1 had no effect on potassium-induced depression in the presence of ergotamine
4. PGE1 antagonized megnesium-induced activation of phosphodiesterase
5. PGE1 antagonized the cardiotonic actions of nicotine
6. PGE1 counteracted propranolol and monoiodoacetic acid-induced cardiodepression
7. PGE1 counteracted zinc-induced cardiodepression
8. PGE2 was less potent than PGE1 in reversing the potassium or magnesium-induced cardiac depression. Other effects of PGE2 were not reported.

Antonaccio and Lucchesi (1970) have shown that PGE1 potentiates the positive inotropic actions of glucagon. In the isolated blood perfused

papillary muscle of the dog PGE1 (1 ng to 1 µg) enhanced the positive inotropic responses to norepinephrine as well as to calcium (Endoh 1976). However, Hadhazy (1976) has shown that PGE1 (0.1 ng/ml to 1 µg/ml) has very little effect on the positive inotropic response to the elevation of external calcium. In rat heart cell cultures PGE1 (0.34 µg/ml) can restore spontaneous activity of potassium-arrested cells, an effect not blocked by propranolol (Warbanow 1976). PGE2 has been shown to potentiate the stimulating actions of isoprenaline in guinea pig hearts from normal animals but not in those from reserpine pretreated ones (Krebs and Schror 1976). A PGE2 concentration of 50 ng/ml was used in this study.

In isolated rabbit hearts inhibition of PG synthesis by indomethacin did not influence the cardio-stimulating effects of exogenous norepinephrine (Horrobin *et al* 1974). Indomethacin has also been reported to decrease the positive inotropic actions of histamine in isolated guinea pig hearts (Zehl and Forster 1976).

7. *Actions of Prostaglandins on the Coronary Circulation*

The possible roles of endogenously released PGs as a result of various stimuli has been described in a previous section (p 9). This sectionwill be concerned mainly with the effects of exogenously added PGs in a variety of experimental designs. Also the effects of agents which modify PG synthesis or action will be discussed. Basically, the coronary actions of these substances have been studied in the *in vivo* situation by injection or infusion as well as *in vitro* using isolated heart or coronary artery strip preparations.

Table 4 . EFFECTS OF PROSTAGLANDINS ON CORONARY RESISTANCE IN DIFFERENT SPECIES

Prostaglandin	Species	Method	Effect	Reference
A1	Dog	Intracoronary	↓	Nakano 1968
	"	"	↓	Rowe & Afonso 1971
	Rat	Isolated Heart	↓	Vergroesen *et al* 1967
E1	Dog	Intracoronary	↓	Nakano 1968
	"	"	↓	Nutter & Crumly 1972
	Rat	Isolated Heart	↓	Mantagazza 1965
	Cat	"	↓	"
	Rabbit	"	↓	"
	Guinea Pig	"	↓	"
E2	Dog	Intracoronary	↓	Rowe & Afonso 1974
	"	Isolated Artery	↑↓	Toda *et al* 1975
	Rat	Isolated Heart	↓	Vergroesen *et al* 1967
	Ox	Isolated Artery	↑	Needleman *et al* 1977
	Man	"	↑	"
F1 α	Dog	Intracoronary	→	Hollenberg *et al* 1968
	"	"	→	Nutter & Crumly 1972
	"	Heart-Lung	↓	Katori *et al* 1970
	Rat	Isolated Heart	→	Vergroesen *et al* 1967
	"	"	↓	Willebrands & Tasseron 1968
F2 α	Dog	Intracoronary	→	Nakano 1968
	"	Intravenous	→	Bloor & Sobel 1970
	"	"	↓	Maxwell 1969
	"	Heart-Lung	↓	Katori *et al* 1970
	Cat	Isolated Artery	↑	Ogletree & Lefer 1977
H1	Ox	Isolated Artery	↑	Needleman *et al* 1977
H2	Ox	Isolated Artery	↓	Needleman *et al* 1977
	Pig	"	↑	Svenson & Hamberg 1976
H3	Ox	Isolated Artery	↓	Needleman *et al* 1977
I2	Ox	Isolated Artery	↓	Dusting *et al* 1977a
	Pig	"	↑	Dusting *et al* 1977b
TXA2	Pig	Isolated Artery	↑	Ellis *et al* 1976

a. In Vivo Coronary Actions

PGA1

Nakano (1968) demonstrated that intraaortic administration of PGA1 (0.01 µg/kg) to dogs produced a decreased coronary vascular resistance resulting in increased coronary blood flow. In a similar report Nutter and Crumly (1970) demonstrated increased coronary dilatation in open-chest dogs after intracoronary injection of PGA1 (0.1 to 12.5 µg). Also, PGA1 infused through a left atrial catheter at 0.3 to 3 µg/kg/min increased the coronary blood flow in dogs (Bloor and Sobel 1970). In dogs on a right heart by-pass in which heart rate and cardiac output was kept constant PGA1 infusion (1 to 5 µg/kg) decreased the coronary vascular resistance while at the same time decreasing greatly the systemic vascular resistance; the net result being an actual reduction in coronary blood flow (Barner *et al* 1972). Recently Boroyan (1976) reported a slight coronary dilatation after PGA1 intracoronary injection (0.1 to 10 µg/kg) in dogs.

PGE1

Maxwell (1967) reported that intravenous PGE1 infusion (1.5 µg/kg/min) into anaesthetized dogs increased the coronary blood flow, cardiac output and heart rate. Similar results were observed by Nakano and McCurdy (1967) after PGE1 injection in doses from 0.25 to 4.0 µg/kg. The coronary effects of PGE1 were not blocked by propranolol. Intracoronary PGE1 infusion at 0.5 µg/kg/min also produced coronary dilatating actions in dogs (Glaviano and Masters 1968). Intraaortic administration of PGE1 (0.01 µg/kg) produced increases in the coronary blood flow in dogs (Nakano 1968).

Hollenberg and coworkers (1968) demonstrated dilatating actions of PGE1 after intracoronary infusion (2 to 8 µg/min) in dogs. Similarly, Nutter and Crumly (1970) using open-chest dogs reported potent coronary relaxing actions of PGE1 after direct coronary injections at doses between 0.1 and 12.5 µg. In conscious dogs PGE1 infused through a left atrial catheter (0.3 to 3 µg/kg/min) increased the coronary blood flow (Bloor and Sobel 1970). PGE1 (0.1 to 12.5 µg) reduced the coronary vascular resistance in dogs with either beating, fibrillating or hypocalcemic-arrested hearts (Nutter and Crumly 1972). Boroyan (1976) compared the effects of a variety of PGs on the coronary vessels in dogs. PGE1 (0.1 to 10 µg/kg) injected into the coronary artery was the most potent dlitatating agent. The coronary dilatating actions of PGE1 are probably not mediated via β receptors as propranolol or sotalol had no significant effect in preventing the PGE1 coronary actions (Rowe and Afonso 1974). Also, the actions of PGE1 on the coronary vessels were found not to be influenced by indomethacin (Mentz *et al* 1976). However Ishihara *et al* (1974) reported that PGE1 coronary effects can be prevented by aspirin. PGE1 has also been shown to be a possible cardio-protective agent during coronary occlusion (Ogletree and Lefer 1978). In this report PGE1 administration (1 µg/kg) to anaesthetized cats prevented S-T segment elevation in the electrocardiogram and also the increase in plasma creatinephosphokinase levels which occured after coronary occlusion.

PGF1α

Hollenberg *et al* (1968) found no effects of PGF1α after intracoronary injection in dogs. A lack of PGF1α coronary actions in the dog was also reported by Boroyan (1976).

PGF2α

PGF2α was without coronary effects after injection into the coronary artery of anaesthetized dogs (Hollenberg *et al* 1968). In conscious dogs PGF2α had no effect on either the coronary blood flow or on the reactive hyperaemia following coronary artery occlusion (Bloor and Sobel 1970).

Boroyan (1976) also reported no effect of PGF2α after intracoronary injection in dogs. The author thus concluded that the keto group at the C-9 position of the cyclopentane ring of the PG molecule may be important for PG action on the coronary vessels. Maxwell (1969) and Mentz *et al* (1976a) however did report that after infusion of high levels of PGF2α (15 to 20 µg/kg/min) into anaesthetized dogs an increased in coronary blood flow was observed. These effects were not blocked by indomethacin (Mentz *et al* 1976a). PGF2α was found to have no beneficial effect on electrocardiogram changes or changes in plasma creatinephosphokinase levels after coronary artery occlusion in the cat (Ogletree and Lefer 1978).

TXA2

The potent coronary constrictor TXA2 when injected into rabbits at a single dose of 800 ng was able to induce a myocardial infarction (Shimamoto *et al* 1977). This effect was prevented by phthalazinol.

PG Precursors

PG precursors and other unsaturated fatty acids have been studied on dog hearts for their coronary actions (Mentz *et al* 1976a). Linolenic acid (40 to 80 mg), arachidonic acid (5 to 10 mg) and linoleic acid (40 to 80 mg) all increased the coronary blood flow. On the other hand, oleic acid (80 mg) had only slight coronary dilatating actions. The effects

of oleic acid were not influenced by indomethacin. However indomethacin did inhibit the coronary dilatating actions of linoleic, arachidonic and linolenic acids (Mentz *et al* 1976a).

b. In Vitro Coronary Actions

PGA1 and PGA2

Strong and Bohr (1967) found that in isolated canine coronary arteries 4 PGs in order of potency PGA1>PGE2>PGE1>PGF1α had constricting actions between 10 pg/ml and 10 µg/ml. PGA2 also has been shown to have constrictor actions in the isolated guinea pig heart preparation (Schror and Krebs 1977).

PGE1

The coronary actions of PGE1 have been extensively studied in a variety of preparations. It has been shown to increase the coronary blood flow in isolated hearts from different animal species including the cat, rabbit, guinea pig and the rat (Berti *et al* 1965, Mantegazza 1965). In the isolated rat heart similar actions were reported by Strong and Bohr (1967) and Hulsmann (1976). PGE1 also increased the coronary flow rate in the canine heart-lung preparation (Katori *et al* 1970). In isolated dog coronary arteries PGE1 (7ng/ml to 3.4 µg/ml) consistently caused relaxation (Toda *et al* 1975). PGE1 (50 ng/ml to 10 µg/ml) also relaxed isolated bovine and human coronary arteries (Kulkarni *et al* 1976, Needleman *et al* 1977b). Isolated cat coronary arteries responded in a biphasic manner to PGE1 (Ogletree and Lefer 1977). Up to 50 ng/ml PGE1 constricted the arteries while higher concentrations relaxed them. Suprisingly, PGE1 consistently constricted

the isolated porcine coronary artery (Needleman *et al* 1977b). PG/adenosine interaction was studied by Blass *et al* (1976). In this report the adenosine antagonist aminophylline inhibited in a non-competitive manner PGE1-induced coronary flow increases in isolated rabbit hearts.

PGE2

PGE2 (10 pg/ml to 10 µg/ml) was reported to constrict the isolated canine coronary artery (Strong and Bohr 1967). In another report using the isolated canine coronary artery PGE2 had biphasic actions (Toda *et al* 1975). At concentrations lower than 3 µg/ml coronary contraction was seen while higher PGE2 levels relaxed the vessels. In the isolated cat coronary arteries PGE2 at concentrations from 1 to 150 ng/ml consistently caused coronary constriction (Ogletree and Lefer 1977). PGE2 (50 ng/ml to 10 µg/ml) has also been shown to contract the isolated bovine, canine, porcine and human coronary artery (Kulkarni *et al* 1976, Needleman *et al* 1977b). Similarly Kalsner (1975) showed that PGE2 (3 ng/ml to 0.3 µg/ml) had constricting actions on the isolated bovine coronary artery. In guinea pig hearts PGE2 was found to decrease the coronary vascular resistance in concentrations from 10 pg/ml to 50 ng/ml (Krebs and Schror 1975, 1976). These effects were not modified by reserpine pretreatment.

PGE3

PGE3 (200 ng to 10 µg) addition was reported to contract the isolated bovine and porcine coronary artery (Needleman *et al* 1977b).

PGF1α

At high concentrations (1 µg/ml) PGF1α constricted the isolated canine coronary artery (Strong and Bohr 1967). It was without coronary

actions on isolated rat hearts (Vergroesen *et al* 1967)

PGF2α

Kalsner (1975) reported constricting actions of PGF2α (70 ng/ml to 700 ng/ml) on the isolated bovine coronary artery. Similar results were obtained by Ogletree and Lefer (1977) using a cat coronary artery preparation with PG concentrations of 1 to 150 ng/ml.

TXA2

TXA2, a product of PG biosynthesis released from platelets has been reported to be a potent coronary constrictor. In isolated swine coronary arteries TXA2 was 2 to 10 times more potent than the endoperoxide PGH2, 9 to 102 times stronger than PGE2 and 26 to 308 times more potent than PGF2 in its constrictor actions (Svenson and Hamberg 1976). A similar report by Ellis *et al* (1976) also demonstrated the constricting actions of TXA2 usung the same preparation.

Endoperoxides

PGH1 contracted the bovine coronary artery (100 ng to 2 μg) while PGH2 and PGH3 at similar concentrations relaxed them (Needleman *et al* 1977b). However all the endoperoxides constricted the porcine artery. These diverse effects may be due to conversion of the endoperoxides to their respective PGI compound. PGI2 for instance has been shown to dilate bovine coronary vessels while contracting porcine arteries.

Other agents

Moretti *et al* (1976) have recently suggested a possible role of PGs in the regulation of coronary flow. In this report the addition of blood plasma to the buffer perfusing rabbit hearts resulted in coronary constriction. The vasoconstrictor response to the plasma was associated

with a 4-fold increase in PG outflow from the hearts. In the absence of plasma ,changes in coronary perfusion pressure from 40 mm Hg to 100 mm Hg resulted in coronary dilatation, indicating an absence of auto-regulation by the coronary vasculature. In the presence of plasma auto-regulation was restored andan increase in perfusion pressure resulted in coronary constriction. When PG synthesis was inhibited by either indo-methacin or eicosatetraenoic acid all the effects of plasma were attenuated.

Most direct studies with indomethacin indicate coronary constrictor actions. In hearts removed from reserpinized guinea pigs indomethacin (0.17 µg/ml) increased the coronary pressure and this effect was abolished by the presence of 5 ng/ml PGE2 (Krebs *et al* 1976). The constrictor effects of indomethacin are not due to changes in myocardial activity and are not blocked by either phenoxybenzamine or propranolol according to Schror *et al* (1976).

A summary of the coronary actions of various PGs is given in table 4.

8. *Actions of Prostaglandins and Related Substances on Various Agents Affecting Coronary Vascular Resistance*

Besides having direct effects on the coronary circulation various PGs and related substances have been shown to modify the effects of some physiological and pharmacological factors affecting coronary per-formance. PGA1 and PGE1 have both been reported to inhibit or reverse the reactive hyperaemia following coronary artery occlusion in the dog (Bloor and Sobel 1970).

PGE2 was able to antagonize the coronary dilatating actions of isoprenaline in isolated guinea pig hearts in a report by Krebs and

Schror (1976). In this report isoprenaline decreased the coronary vascular resistance in hearts from normal animals in the absence of PGE2 but increased the coronary resistance slightly in the presence of 50 ng/ml PGE2. In hearts from reserpinized animals coronary resistance decreased when isoprenaline was added alone or in the presence of PGE2.

PGs can also affect metabolically-induced alterations in coronary performance (Sunahara and Talesnik 1974, Sen *et al* 1976). In the first report PGE1 infusion (2.5 µg/min) in the isolated perfused cat heart reduced coronary dilatation due to norepinephrine, isoprenaline and dimethylphenylpiperazinium addition (Sunahara and Talesnik 1974). In isolated rat hearts challenged with norepinephrine or calcium chloride, increases in coronary flow rate and cAMP levels were significantly reduced in the presence of 5 ng/ml PGE2 (Sen *et al* 1976). As would be expected inhibitors of PG biosynthesis had converse effects on such changes in the coronary resistance. Increases in coronary flow in isolated rat hearts due to norepinephrine or calcium administration or electrically-induced tachycardia were significantly augmented in the presence of either aspirin or indomethacin (Talesnik and Sunahara 1973).

Indomethacin was found to be ineffective in influencing coronary dilatation by a variety of vasoactive agents including substance P, eledoisin, adenosine and nitroglycerin (Regoli *et al* 1977). On the other hand, the coronary dilatating effects of bradykinin in isolated rabbit hearts were reduced by the presence of 1 µg/ml indomethacin (Regoli *et al* 1977).

9. *Metabolic and Biochemical Aspects of Cardiac Prostaglandin Action*

Although the PGs have been found to have a myriad of actions on cardiac muscle and the coronary arteries their basic mechanisms of action are currently little understood. They have been found however to alter myocardial metabolism in a number of ways. In anaesthetized dogs PGE1 infusion (1.5 µg/kg) increased myocardial oxygen extraction rate while at the same time increasing the heart rate and coronary blood flow (Maxwell 1967). PGF2α however had little effect on myocardial oxygen metabolism (Maxwell 1969). Glaviano and Masters (1968) reported that intracoronary infusion of PGE1 (0.5 µg/kg/min) into dogs reduced the coronary perfusion pressure while decreasing the uptake of free fatty acids by the heart. Also glucose uptake rose significantly leading to increased myocardial triglyceride levels. In isolated rat hearts PGE1 and PGF1α (0.5 and 0.025 µg/ml respectively) increased glucose oxidation in the heart (Willebrands and Tasseron 1968). Both PGs increased the rate of oxidation of palmitic acid. Also both agents increased oxygen consumption of the isolated rat heart (Willebrand and Tasseron 1968). In anaesthetized dogs intracoronary PGE1 infusion (0.5 µg/kg/min) reduced arterial levels and myocardial extraction ratio of free fatty acids while increasing these values for glucose (Glaviano and Masters 1971). Other effects observed in this study were increased myocardial triglyceride levels, decreased lipolysis and decreased myocardial uptake of oxygen. PGE1 infusion into rats was found not to modify myocardial glycogen synthetase or phosphorylase activity or cardiac glycogen levels (Curnow and Nuttall

1971). Myocardial oxygen uptake in dogs was found to be inhibited by PGA1 injection (1 to 5 µg/kg) (Barner *et al* 1972). At the same time these studies also reported decreased myocardial lactate and pyruvate uptake. Rowe and Afonso (1974) found that intracoronary administration of PGE1 or PGE2 did not affect left ventricular oxygen consumption in anaesthetized dogs. A recent report using isolated rabbit hearts demonstrated that very high PGF2α concentrations (2.5 to 40 µg/ml) induced various histological and histochemical effects (El Zay *et al* 1977). The histological alterations included shortening, thickening and irregular appearances of the cardiac myofibrils. Also, in histochemical studies ATPase and succinic dehydrogenase activity were increased while alkaline phospatase activity was decreased. PGF2α addition was also associated with glycogen granule depletion.

There has been some evidence presented recently suggesting a possible role of cyclic nucleotides in the cardiac actions of PGs. Sobel and Robison (1969) found that the addition of either PGE1 or PGF1α (0.35 ng/ml) stimulated adenyl cyclase and cAMP activity in guinea pig hearts without altering phosphodiesterase levels. Similar results were reported by Klein and Levey (1971) who showed that PGE1 (3.5 pg/ml to 0.35 ng/ml) increased cAMP levels in the guinea pig myocardium which was independant of changes in phosphodiesterase activity. PGE2 and PGA1 had similar effects while PGF1α and PGF2α were inactive. Propranolol did not block the PG effects but did inhibit norepinephrine-stimulated cAMP increases suggesting that separate receptor sites exist for PGs and norepinephrine. In another report all PGs studied at 35 ng/ml including PGE1, PGE2, PGA1

PGF1α and PGF2α activated solubilized adenyl cyclase of the guinea pig and cat myocardium (Levy and Klein 1973). PGE1 however, exhibited a biphasic action first increasing at 0.35 ng/ml but starting to decrease adenyl cyclase activity at 35 ng/ml. In cultured rat heart cells PGE1 and PGF1α (35 pg/ml) increased cAMP levels but PGE2 and PGF2α had no effect (Moura and Simpkins 1975). PGs probably do not have any significant direct effect on norepinephrine release or uptake by the heart according to studies by Wennmalm and Junstad 1976). These authors reported that in isolated rabbit hearts increasing PG synthesis by hypoxia did not alter norepinephrine release after sympathetic stimulation. Similarly in rabbit hearts PGE2 (3.5 ng/ml to 35 µg/ml) had no effect either on the biosynthesis or the metabolism of norepinephrine (Pham-Huu-Chanh *et al* 1976). Norepinephrine uptake was however slightly inhibited.

PGs may also be exerting their cardiac effects by modification of calcium transport. A number of workers have demonstrated that PGs can alter myocardial handling of calcium ions. In the frog heart PGE1 (0.4 µg/ml) increased the rate of calcium flux whereas total myocardial calcium content and the net amount of cellular exchangeable calcium was not affected (Piccinini *et al* 1969). Sabatini-Smith (1970) has demonstrated that PGE1 and PGF2α (1 µg/ml) enhanced calcium uptake by isolated guinea pig atria and fragmented sarcoplasmic reticulum. However total atrial tissue calcium content was not affected by the PGs. In isolated sarcoplasmic reticulum of rabbit ventricles, incubation with either PGE1 or PGF2α (1 µg/ml) also resulted in increased calcium accumulation (Sabatini-Smith 1971). In cultured

rat myocardial cells Moura and Simpkins (1976) demonstrated that PGE1 and PGF1α (35 pg/ml) increased significantly the size of the calcium pool. On the other hand similar concentrations of PGE2 and PGF2α had no effect.

10. Prostacyclin (PGI2)

The recent discovery of a new PG called prostacyclin or PGI2 has aroused much interest among cardiac and PG researchers as it seems to be the main PG released from rat and rabbit hearts (De Deckere *et al* 1977, Isakson *et al* 1977). This release from isolated hearts can be increased by hypoxia (De Deckere *et al* 1977). Isolated arteries have very significant PGI2 synthesis systems (Raz *et al* 1977, Moncada *et al* 1976a, 1976b, Gryglewski *et al* 1976, Moncada *et al* 1977c). Moncada *et al* (1977b) have shown that more than 60% of PGI2 synthesis activity is located in the non-endothelial part of blood vessels. Similarly the ability of rat and rabbit aortas to generate PGI2 in the sub-endothelium has been shown by Hornstra *et al* (1978). These authors also demonstrated that endothelial disruption causes the release of large amounts of PGI2. The possible role of PGI2 in the maintenance of cardiac function is currently uncertain as it seems to possess diverse actions. It is known to be a powerful inhibitor of platelet aggregation (Bunting *et al* 1976) and this effect may be due to is ability to increase platelet cAMP levels (Gorman *et al* 1977, Tateson *et al* 1977).

The coronary actions of PGI2 seem to be more complex. At relatively high concentrations (2.5 ng/ml to 1 μg/ml) it relaxes strips from bovine coronary arteries (Dusting *et al* 1977a, Omini *et al* 1978).

In isolated porcine coronary arteries PGI2 (0.4 to 5 µg/ml) has constrictor properties (Dusting *et al* 1977b). Isolated cat coronary arteries have been shown to contract slightly in the presence of 1.0 nM PGI2 while higher concentrations had relaxing effects (Lefer *et al* 1978).

In the *in vivo* situation PGI2 intravenous infusion (50 to 500 ng/kg/min) into anaesthetized dogs increased coronary flow (Armstrong *et al* 1977). Direct intracoronary injection (50 to 1000 ng) also produced coronary dilating actions without altering heart rate (Armstrong *et al* 1977). In another report intravenous injection of PGI2 (0.25 to 5.0 µg/kg) into dogs produced a slight decrease in myocardial contractile force as well as exerting hypotensive actions (Fitzpatrick *et al* 1978). Coronary actions were not reported in this study. Decreases in blood pressure were also observed after PGI2 infusion (1.0 ng/kg/min) into anaesthetized cats although significant cardiac actions were not seen (Lefer *et al* 1978).

MATERIALS AND METHODS

A. GENERAL CONSIDERATIONS

1. Animals

The animals used in this study were all obtained from the Canadian Breeding Laboratories, St. Constant, Quebec. Rats of the Wistar strain were used as well as New Zealand rabbits in some experiments. All animals were kept on a 12 h light/12 h dark photoperiod and given access to water and Purina rabbit or rat chow *ad libitum*. Weight and sex of the animals will be described in the individual sections below.

2. Prostaglandins

The prostaglandins (except for the prostacyclin) used in this study were kindly donated by Dr. J. Pike of the Upjohn Company, Kalamazoo, Michigan, USA. All prostaglandins were disolved in absolute ethanol and kept at -20°C until the day of the experiment in aliquots of 1 mg/ml. The concentrations were then diluted in saline and added to the perfusing buffer in the desired concentrations. Dilution time took approximately 30 seconds so that degradation,particularly of prostacyclin was not a problem. For example, to obtain the lowest concentration desired (1 pg/ml), one µl of the stock (1 µg) was dissolved in 10 ml of saline to give a concentration of 100 ng/ml. Ten µl of this (1 ng) was then added to 1 liter of perfusing buffer to yield a final concentration of 1 pg/ml.Fresh dilutions were prepared daily. The prostacyclin was provided by Dr. KC Nicolaou of the Department of Chemistry, University of Pennsylvania, Philadelphia, Pa., USA. Sodium iodide (10^{-3}M) and sodium ethoxide (10^{-3}M) were added to the stock solutions to improve stability. The solvents were always tested in control experiments.

3. Heart Perfusion

The isolated rat or rabbit heart perfused retrogradely through the

coronary arteries was used in this study (Langendorff 1895). The procedure for removing and mounting the heart in a Langendorff preparation was as follows; the abdominal cavity was opened by making a transverse incision. The diaphragm was cut and lateral incisions were made along both sides of the rib cage. The rib cage was folded back and the heart exposed. The pericardium and all connective tissue was pulled away from the heart with the fingers. The heart was then picked up and cut free. Using fine tip forceps the heart was immediately picked up by the aorta and mounted on a grooved perfusion cannula placed in a theromoregulated chamber (37°C) and readied for perfusion. The tranfer period took an average of 30-45 seconds. The perfusion medium was a Krebs-Henseleit buffer (Table 5). The hearts were perfused by a constant flow method using a Watson-Marlow peristaltic pump. Flow rate was adjusted before starting the experiment to give an initial coronary perfusion pressure of 75-100 mm Hg (10-13.3 kPa). The perfusion buffer was continuously gassed with a 95%O_2-5% CO_2 mixture 30 minutes prior to and during the perfusion period. Some hearts exhibiting dysrhythmic activity were not immediately discarded but tested to determine possible anti-dysrhythmic properties of prostaglandin synthesis inhibitors or antagonists of prostaglandin actions.

4. *Measurement of Heart Rate and Electrocardiogram (EKG).*

Beat to beat changes in the spontaneously beating heart were used to indicate any alterations in rhytm stability. After the heart was mounted and perfusion began, a fine gauge needle electrode was placed in the left ventricular epicardium while a second electrode was placed around the steel perfusion cannula. The electrical activity was then "fed" into a Devices

model 4522 instantaneous ratemeter and an EKG pre-amplifier both of which were connected to a Devices M19 recorder. The ratemeter is triggered by the R wave of the QRS complex and analyzes the the duration of the R-R interval on a beat to beat basis to give the heart rate in beats per minute. Any change in R -R duration from one beat to the next is immediately indicated as a change in rate in the ratemeter recording. Thus a constant line on the calibrated chart paper will indicates no beat to beat deviations in the R-R interval and this was regarded as indicating rhythm stability. On the other hand alterations in the R-R interval from one beat to the next resulted in deflection in the ratemeter recording which was then taken as indicating rhythm instability. At the same time the EKG was obtained to observe the possible presence of gross changes in the EKG parameters such as illustrated in figure 17.

Table 5. COMPOSITION OF KREBS-HENSELEIT BUFFER

	mM	g/l
NaCl	120	7.01
NaHCO3	20	1.68
KCl	4.63	0.35
KH2PO4	1.17	0.16
CaCl2	1.25	0.14
MgCl2	1.20	0.24
Glucose	8	1.44

5. *Measurement of Contractile Force*

The contractile force was measured by attaching the apex of the heart to a force displacement transducer (Devices). The transducer was located to give a resting diastole tension of 1 gram. The recorder was calibrated to give a 1 cm pen deflection for each gram developed tension.

6. *Measurement of Coronary Perfusion Pressure*

The coronary perfusion pressure was measured via a side arm off the perfusion cannula which was connected to a physiological pressure transducer (Bell and Howell, Basingstoke, England). Because the hearts were perfused at constant flow, changes in perfusion pressure were regarded as indicating changes in the coronary vascular resistance.

All the above parameters were obtained on a Devices M19 recorder

7. *Validity of the Isolated Heart Preparation*

The isolated perfused heart preparation has been used extensively for the study of cardiac muscle since 1895. A large number of workers have demonstrated the stability and versatility of this preparation (Brodie 1903, Bleehen and Fisher 1954, Opie 1965). The availability of oxygen to the tissue when using blood-free medium may be a problem. At the flow rates used in the present experiments (calculated at 5-8 ml/min/g heart weight) oxygen was probably adequate since rat hearts perfused at similar low rates for up to 9 days were found to be without histologically detectable damage at the end of the perfusion period (Linask *et al* 1978). The electrical stability of the isolated rat heart has also been demonstrated making it suitable for pharmacological studies relating to cardiac rhythm (Mitchell and Murnaghan 1976).

TABLE 6. SOURCE AND SOLVENTS OF VARIOUS DRUGS USED IN THIS STUDY[1]

DRUG	SOURCE	SOLVENT
Aspirin	Sigma[2]	Saline
Benzydamine	Acraf[3]	Saline
Chloroquine	Sigma	Saline
Copper Sulphate	Fisher[3]	Saline
Dipyridamole	Boehringer Ingelheim[4]	Persantine[R] Vehicle
Oestradiol	Sigma	Ethanol
Imidazole	Sigma	Ethanol
Indomethacin	Sigma	1% Na_2CO_3
N-0164	Nelson[5]	Ethanol
Procaine	Sigma	Saline
Progesterone	Sigma	Ethanol
Propranolol	Ayerst[3]	Saline
Testosterone	Sigma	Ethanol

1. Exluding PGs which are described on page 46
2. St. Louis, Missouri, USA
3. Montreal, Quebec
4. Dorval, Quebec
5. Irvine California

8. Statistical Analysis

Statistical analysis was performed using Student's t-test on a Hewlett-Packard Model 10 programmable calculator.

9. Expression of Results

Results are expressed in absolute terms except in the experiment determining sexual variation in the cardiac response to prostaglandins in which case percent changes from control values are given. All data presented either in graph or table form represent mean values ± standard error of the mean. SI units for pressure changes are included in brackets.

10. Drugs

A list of drugs used as well their solvents and source of supply are given in Table 6.

B. EXPERIMENTS

The following experiments were performed;

1. Effects of Low Prostaglandin Concentrations on Rhythm Stability of Isolated Rat Hearts

Male rats (175-200 g) were anaesthetized with ether, their hearts were rapidly excised and arranged for perfusion by the Langendorff method. The EKG and heart rate were recorded as described above. Any abrupt changes in the R-R interval which may indicate an instability of the conducting system appears instantly on the ratemeter tracing as a sharp change in the rate. Within 5 minutes of setting them up most hearts reached a steady state which remained steady for at least 1 hour. Hearts which during an initial 10 minute observation period showed any sharp fluctuations of the instantaneous ratemeter recording indicating a greater than 10% beat to beat

change in the R-R interval were not tested with the prostaglandins (PGs). In order to test the possible involvement of endogenous PGs in the irregularities the following drugs were added (in separate experiments) to the buffer: 20 µg/ml indomethacin (an inhibitor of PG synthesis), 4 µg/ml chloroquine diphosphate (an antagonist of PG action) and copper sulphate at 0.5 µg/ml. PGE2 and PGF2α were each tested in five hearts. After an initial control period 1 pg/ml to 1 µg/ml (2.8×10^{-12}M to 2.8×10^{-6}M) of the PGs were added to the buffer. Each PG concentration was present for 5 minutes and there was no return to plain buffer between successive changes in concentrations. In eight hearts each typical rhythm disturbances were induced by 1 ng/ml PGE2 or PGF2α . In four hearts each 0.5 µg/ml copper sulphate or 4 µg/ml chloroquine diphosphate was added to the buffer in addition to the PG.

2. *Actions of PGs on Force of Contraction, Rate, and Coronary Perfusion Pressure in Isolated Rat Hearts*

Hearts from male rats (175-200 g) were removed and and arranged for perfusion as described. Contractile force, heart rate and coronary perfusion pressure were monitored. PGD2, PGE2, PGF1α, and PGF2α in concentrations from 10 pg/ml to 1 µg/ml (2.8×10^{-11}M to 2.8×10^{-6}M) were added in increasing dosages. Each PG was tested for its cardiac actions on ten hearts.

3. *Sexual Variation in the Response of the Isolated Rat Heart to Prostaglandins. Effect of Gonadectomy*

This study compared the effects of 3 PGs (PGE1, PGE2 and PGF2α) on male and female hearts. A possible modifying effect of gonadectomy was also examined. Heart rate, contractile force, coronary perfusion pressure and the amplitude of the R wave of the QRS complex of the EKG were measured. Thirty male and thirty female rats were gonadectomized at three weeks of age under ether anaesthesia. A similar number of animals were sham-operated and served as controls. The animals were then kept under standard conditions for three

months at which time their hearts were removed and arranged for perfusion. Each PG was tested in ten separate hearts from each group at two concentrations only (10 minutes at each concentration); 500 pg/ml (1.4×10^{-9}M) and at 50 ng/ml (1.4×10^{-7}M). Results are expressed as percent change from control values.

4. *Effects of Prostacyclin (PGI2) on Coronary Perfusion Pressure, Electrical Activity, Rate and Force of Contraction in Isolated Rat and Rabbit Hearts*

Hearts were removed from male rats (175-200g) and New Zealand rabbits (2.5-2.9 kg) and immediately arranged for coronary perfusion. Ten rat and five rabbit hearts were used to study the coronary perfusion pressure, EKG and heart rate. In a second series of experiments the contractile force of five rat and five rabbit hearts were also studied. After a control period of 15 minutes PGI2 as the sodium salt was added to the perfusion medium in concentrations from 1 pg/ml to 1 µg/ml (2.8×10^{-12}M to 2.8^{-6}M). Each concentration was perfused for 10 minutes. The PGI2 solvent containing sodium ethoxide and sodium iodide was tested in control experiments.

5. *Effect of Prostaglandin Synthesis Inhibition on Coronary Perfusion Pressure in Isolated Rat Hearts*

The effects of adding increasing concentrations the PG synthesis inhibitor indomethacin to the buffer perfusing hearts from male (175-200 g) rats was studied. Indomethacin was added in concentrations from 1.25 µg/ml to 20 µg/ml. Each concentration was perfused for 15 minutes. Seven hearts were examined.

6. Coronary Artery Response to Hypoxia in Isolated Rat Hearts

Hearts were removed from ether anaesthetized animals and arranged for coronary perfusion using either normally oxygenated (pO2 500 mm Hg) or hypoxic (pO2 30-40 mm Hg) buffer. After an initial 30 minute perfusion period with oxygenated buffer a switch was made to unoxygenated (ungassed) buffer. The pH of the ungassed medium was found to be 7.3 at the end of the perfusion period while the oxygenated buffer had a pH of 7.4. In 2 control experiments oxygenated buffer was titrated to pH 7.3 with .1 N HCl. In these hearts a slight dilatation (about 5 mm Hg) was seen throughout the 1 hour perfusion. In one series of experiments the response of hearts from young animals (30 days of age, n=6) was compared with hearts from mature rats (60 days of age, n=12). Possible pharmacological modification of the coronary response to hypoxia was studied by using the following classes of drugs:

a) PG synthesis inhibitors indomethacin (1.25 to 40 µg/ml) and aspirin (1 to 128 µg/ml).

b) Antagonists of PG action chloroquine diphosphate (1 to 16 µg/ml), procaine hydrochloride (1 to 128 µg/ml) and propranolol (1 to 16 µg/ml).

c) TXA2 synthesis inhibitors dipyridamole, benzydamine, N-0164 (sodium P-benzyl-4-[1-oxo-2(4-chloro-benzyl)3-phenyl propyl]phenyl phosphate) and imidazole (all drugs tested from 0.02 to 40.96 µg/ml).

d) Gonadal steroids testosterone propionate (1 to 32 ng/ml), oestradiol benzoate (1 to 320 ng/ml) and progesterone (1 ng/ml to 1.24 µg/ml)

All the above agents were tested for their ability to modify coronary artery responses to hypoxia as well as for their direct actions on normally perfused coronary arteries.

RESULTS

Experiment 1

Preliminary experiments

Within five minutes of setting them up most hearts perfused with Krebs-Henseleit buffer reached a steady state rate which remained steady for at least one hour (control records, figures 4 and 5). Hearts which during an initial ten minute observation period showed any sharp fluctuations of the instantaneous ratemeter recording indicating a greater than 10% beat to beat change in the QRS-QRS interval were not tested with PGs. Rat hearts are very resistant to rhythm disturbances and only two hearts in the present studies showed spontaneous irregularities in rhythm. In order to test the possible involvement of endogenous PGs in these irregularities indomethacin (100 µg/ml) was added to the buffer. In both situations indomethacin improved the irregularities. Figure 3 indicates the effect of indomethacin on spontaneously occurring rhythm instability in the rat heart.

Effects of PGs on Heart Rhythm

PGE2 and PGF2α were each tested in 5 hearts. After an initial ten minute period in which no beat to beat changes in the QRS-QRS interval of greater than 10% were noted, 1, 10, 100 pg/ml, 1, 10, 100 ng/ml and 1 µg/ml of the PG tested were added to the buffer. Each PG concentration was present for five minutes and there was no return to plain buffer between successive changes in concentrations. Preliminary experiments have shown that on returning to buffer PG-induced rhythm changes disappeared within one to five minutes. Both PGs in all hearts tested caused the appearance of abrupt beat to beat changes (table 7, figure 4 and 5). With PGE2 the instability

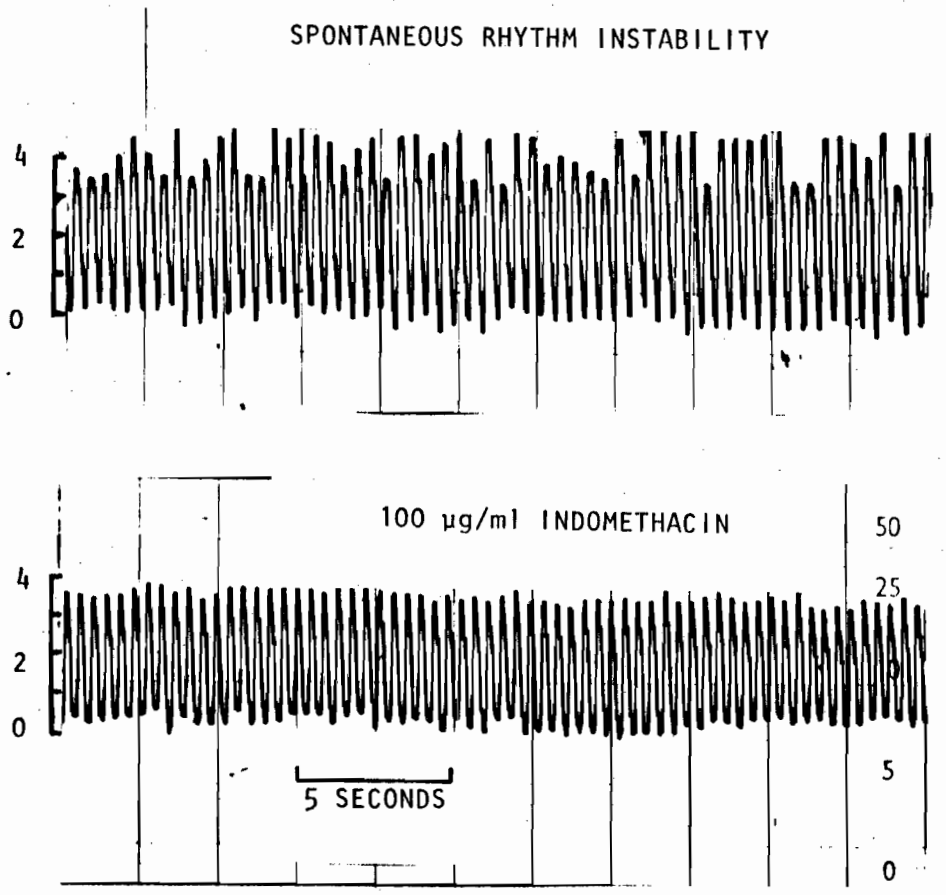

Figure 3. Effect of indomethacin on spontaneously occurring mechanical rhythm instability in the isolated rat heart.

Bottom recording indicates mechanical activity 5 minutes after the addition of indomethacin.

Scale indicates developed tension in grams.

was at its greatest at 1 pg/ml, the lowest concentration tested. With PGF2α maximum instability occurred at 10 ng/ml. Higher concentrations of both PGs restored rhythm stability (table 7, figures 4 and 5). The stabilty at higher concentrations was not a consequence of prior exposure to low concentrations because the addition of 1 µg/ml PGE2 or PGF2α immediately after the control period (2 hearts each) caused no rhythm disturbances. The disappearance of rhythm disturbances was not a consequence of duration of exposure since perfusion of 1 ng/ml of either PG for up to one hour caused continuous rhythm changes. In two hearts an attempt was made to find the minimum dilution at which PGE2 might induce rhythm changes. In one heart irregularities appeared at 10 fg/ml and in the other at 100 fg/ml.

Effects of Copper and Chloroquine on PG-Induced Rhythm Changes

In eight hearts each typical rhythm disturbances were induced by 1 ng/ml PGE2 or PGF2α . In four hearts each 0.5 µg/ml copper sulphate or 4 µg/ml chloroquine diphosphate was added to the buffer in addition to the PGs. In every case the copper or chloroquine restored a stable rhythm (table 8, figures 6 and 7). In two hearts perfused with PGF2α 20 µg/ml indomethacin failed to restore a stable rhythm. In two hearts each in which copper or chloroquine was added before PGE2, the PG failed to have an effect on rhythm.

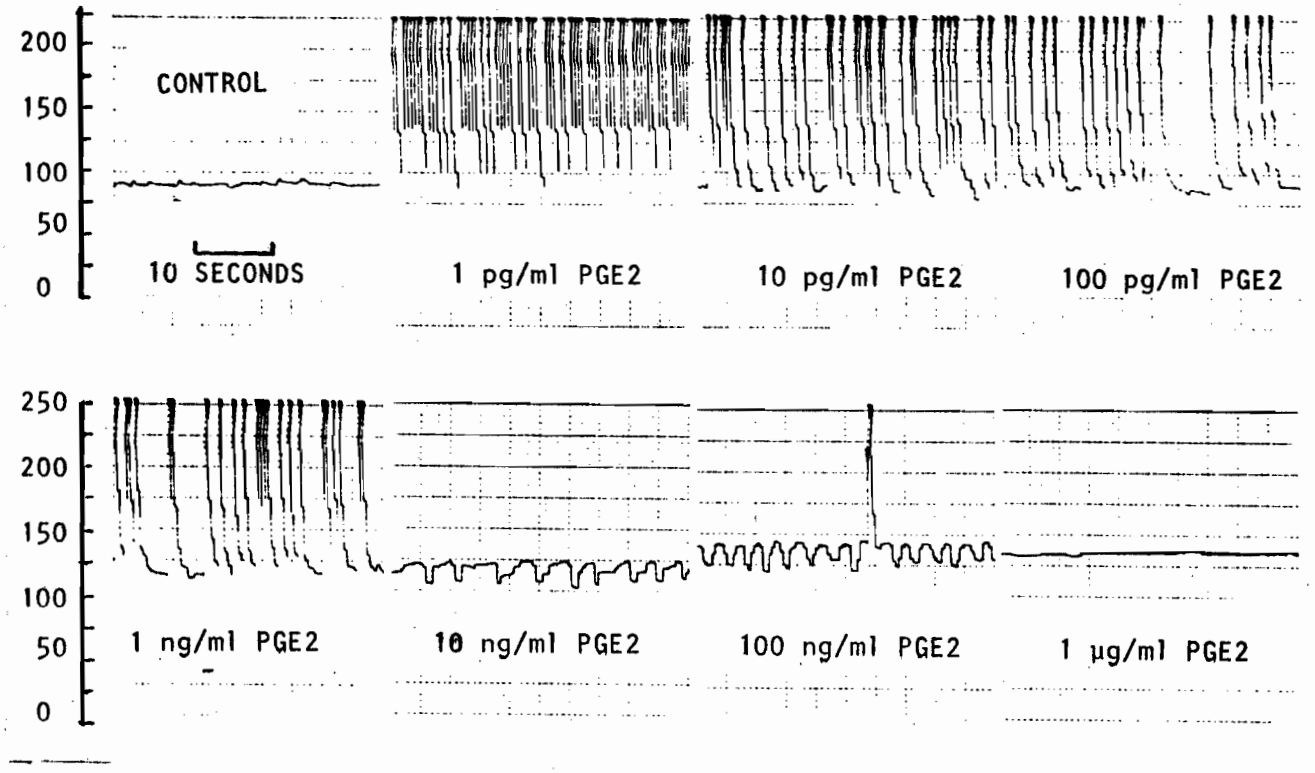

Figure 4. The effects of increasing concentrations of PGE2 on heart rate stability of isolated rat hearts . The ratemeter analyzed the R-R interval duration from the surface electrode activity to give the instantaneous rate in beats/minute. Figure indicates stable heart rate (top left) in the absence of any PG, and ratemeter deflection indicating deviations in R-R interval between beats with different concentrations of PGE2. Scale indicates heart rate in beats/minute.

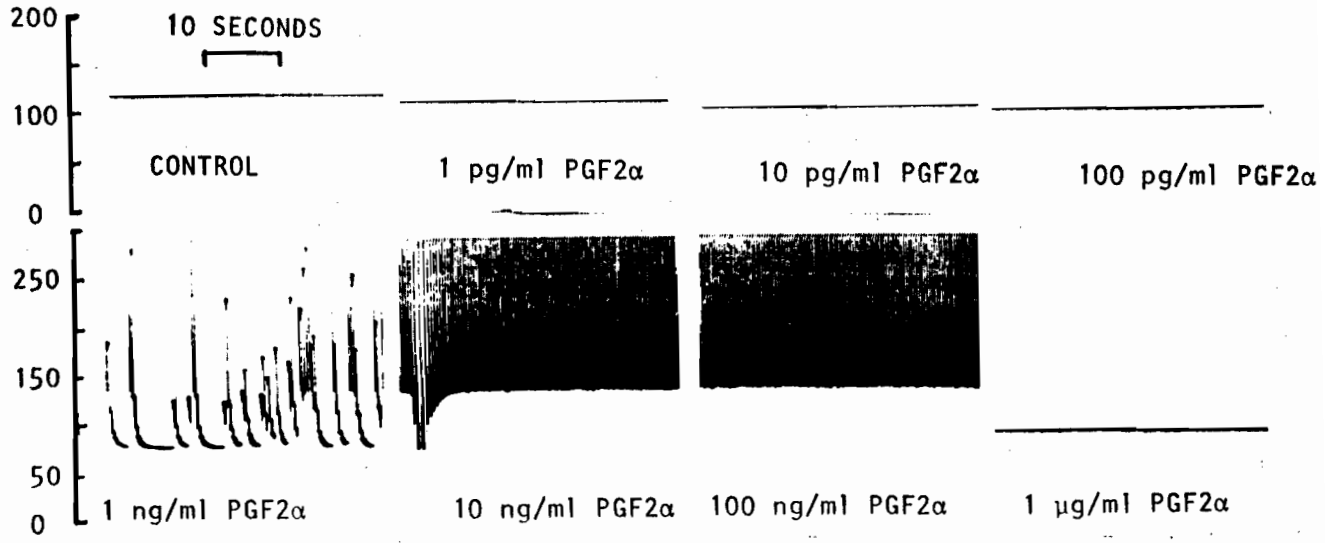

Figure 5. The effects of increasing PGF2α concentrations on heart rate stability of the isolated rat heart. The ratemeter analyzed R-R interval duration from the surface electrode activity to give the instantaneous rate in beats/minute. Figure indicates stable heart rate (top left) in the absence of any PG, and changes if any occurring after the addition of different concentrations of PGF2α . Ratemeter pen deflections indicate variations in R-R interval durations between heart beats. Scale indicates heart rate in beats/minute.

Table 7. Rhythm disturbances in hearts perfused with increasing concentrations of PGE2 or PGF2α. The period for 3 minutes to 3 minutes 30 seconds after changing to a new concentration was studied. During this period in which the behaviour of the hearts was similar to that in the preceding 3 minutes, episodes in which there was a greater than 10% change in the beat to beat duration of the QRS-QRS interval were counted. Results are expressed as the mean number of such episodes in this 30 second period ± SEM. With both PGs the mean peak number of episodes (at 1 pg/ml PGE2 and 10 ng/ml PGF2α) was highly significantly different ($P < 0.01$) from the mean number of episodes either prior to adding the PG (zero in each case) or at 1 µg/ml PG concentration.

CONCENTRATION	PGE2 (n=5)	PGF2α (n=5)
1 pg/ml	26.3 ± 2.2	0.8 ± 0.6
10 pg/ml	21.0 ± 2.5	0
100 pg/ml	17.1 ± 2.6	0.6 ± 0.4
1 ng/ml	16.3 ± 2.2	18.0 ± 6.2
10 ng/ml	10.6 ± 1.5	32.0 ± 6.4
100 ng/ml	8.0 ± 1.5	18.5 ± 4.8
1 µg/ml	2.4 ± 1.1	5.1 ± 2.8

Table 8. Effects of copper and chloroquine on rhythm instabilities induced by 1 ng/ml PGE2 or PGF2α . Records were analysed as described in table 7.

	BEFORE COPPER		WITH COPPER	BEFORE CHLOROQUINE		WITH CHLOROQUINE
		(n=4)			(n=4)	
PGE2	17.3 ± 4.1		0	17.9 ± 3.9		0
PGF2α	19.3 ± 7.2		0	16.6 ± 6.7		0

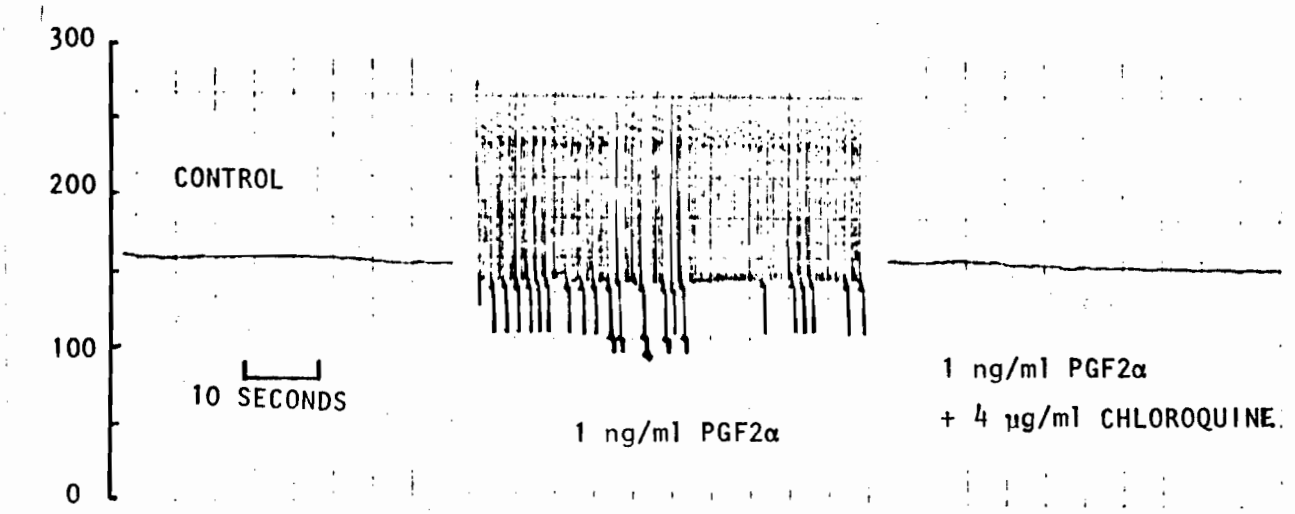

Figure 6. The effect of 1 ng/ml PGF2α and 1 ng/ml PGF2α plus 4 µg/ml chloroquine on the instantaneous ratemeter recording which is based on the R-R interval. During the control period the record was stable. The addition of the PG caused great instability in the R-R interval duration. Chloroquine completely reversed the PG effect. Each tracing was taken 2 minutes after the addition of the PG or PG plus chloroquine.

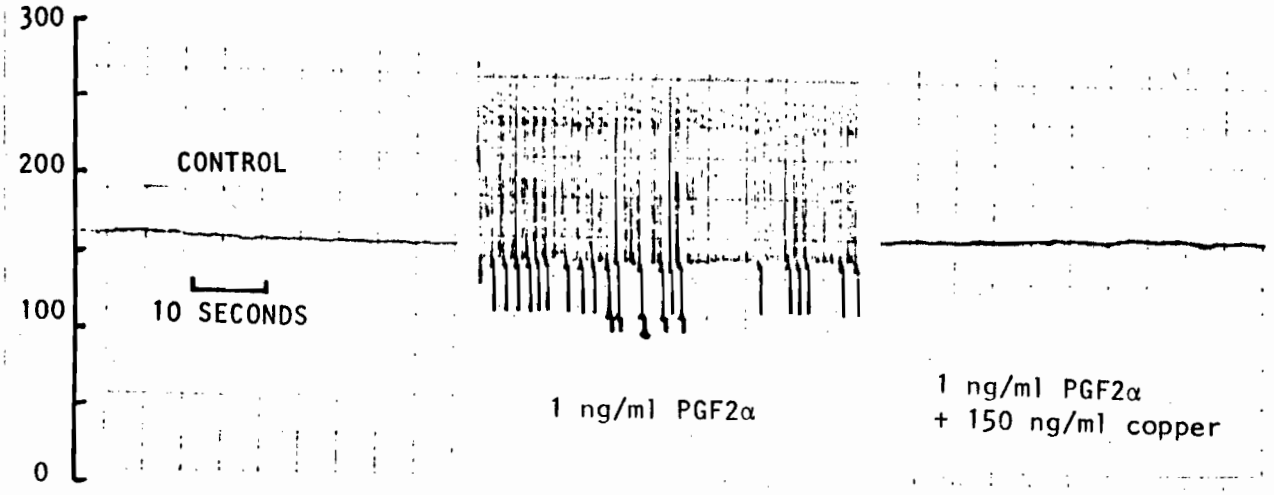

Figure 7. The effect of 1 ng/ml PGF2α and 1 ng/ml PGF2α plus 150 ng/ml copper as the sulphate on the instantaneous ratemeter recording which i based on the R-R interval. During the control period the record was stable. The addition of the PG caused great instability in the R-R intervals. Copper completely reversed the PG effect. Each tracing was taken 2 minutes after the addition of the PG or the PG plus copper. Scale indicates rate in beats/minute.

Experiment 2

The ability of PGD2, PGE2, PGF1α and PGF2α to alter myocardial contractile force, heart rate and coronary perfusion pressure was examined on isolated rat hearts (figure 8).

Coronary Perfusion Pressure

PGD2 was found to have constrictor actions at the lowest concentration studied (10 pg/ml). Significant effects were seen with 1 ng/ml PGD2 ($P < 0.05$). This concentration caused a rise in perfusion pressure 8.2 ± 0.8 mm Hg (1.09 ± 0.1 kPa, 1 mm Hg = 0.133 kPa) from control values. Further increases in PGD2 concentrations (1 µg/ml) resulted in an increase of over 20 mm Hg (2.66 kPa) from control levels ($P < 0.01$)

PGF2α also had constrictor actions at all concentrations. 10 pg/ml PGF2α in the buffer resulted in an increased perfusion pressure of 10.3 ± 2.3 mm Hg (1.4 ± 0.3 kPa, $P < 0.05$).

PGE2 up to 100 pg/ml had constrictor actions on the coronary arteries. This concentration significantly increased ($P < 0.05$) perfusion pressure by 13.7 ± 1.1 mm Hg (1.8 ± 0.1 kPa). However ,subsequent increases in PGE2 concentrations resulted in relaxing actions. At 1 µg/ml PGE2 coronary perfusion pressure declined by 8.4 ± 1.7 mm Hg (1.1 ± 0.2 kPa) from control values ($P < 0.05$).

PGF1α at all concentations tested had slight and insignificant actions on the coronary perfusion pressure.

Contractile Force

The most potent actions on the contractile force in these experiments were observed with PGF2α . Significant inotropic effects ($P < 0.05$)

were observed with 10 pg/ml of this agent while higher concentrations produced further rises in the developed tension. At 10 ng/ml of PGF2α the developed tension was measure to be more than 800 mg greater than control values ($P < 0.01$) . This represented approximately a 40% increase.

PGE2 also had inotropic actions although they were less than those observed with PGF2α . Significant effects with PGE2 ($P < 0.05$) were observed only with the highest PG concentration used (1 µg/ml).

Both PGD2 and PGF α were without significant inotropic actions. PGF1α in fact had slight but insignificant negative inotropic effects at all of the concentrations studied.

Heart Rate

All of the PGs had negative chronotropic effects. PGD2 was the least potent in this respect with no significant chronotropic actions being seen. PGE2 on the other hand, significantly reduced the heart rate at concentrations from 10 pg/ml to 1 ng/ml ($P < 0.01$ at 1 ng/ml) Higher PGE2 concentrations increased the heart rate to levels not significantly different from control. PGF2α however, decreased heart rate significantly only at very high levels ($P < 0.01$ at 1 µg/ml). Similar results were seen with PGF1α .

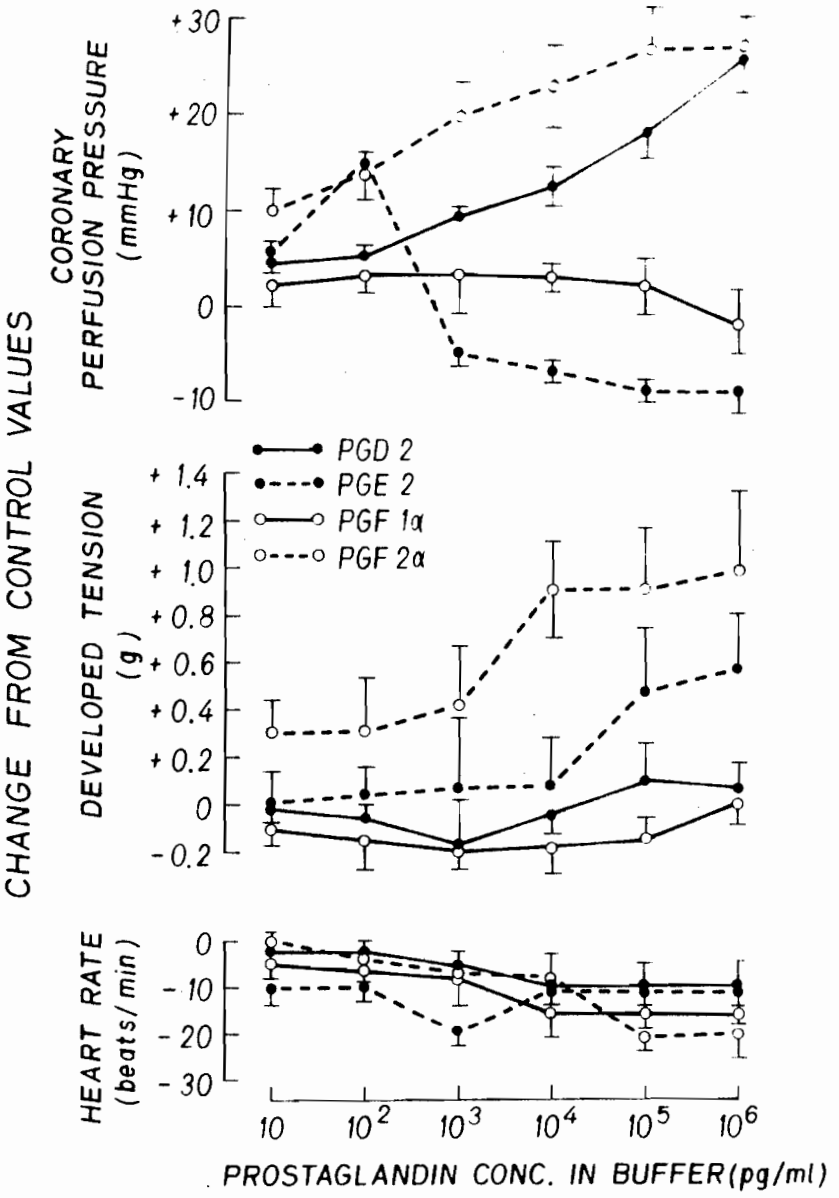

Figure 8. The effects of increasing concentrations of PGD2, PGE2, PGF1α, and PGF2α on heart rate, developed tension and coronary perfusion pressure in isolated rat hearts (n=10)

Experiment 3

The effects of PGE1, PGE2 and PGF2α on hearts from male and female normal and gonadectomized rats are shown in figures 9 to 11.

PGE1 had little effect on the heart rates (figure 9). R wave amplitude of the EKG increased with the high PGE1 level in hearts removed from normal males ($P < 0.05$). Only hearts removed from normal females exhibited a significant increase in the contractile force with 10 ng/ml PGE1 ($P < 0.05$) while hearts removed from ovariectomized females showed a decrease in contractile force ($P > 0.05$). PGE1 decreased the coronary perfusion pressure in hearts from all animals.

Very slight positive chronotropic and inotropic effects were seen with PGE2 (figure 10). Hearts from ovariectomized females exhibited a negative inotropic response ($P < 0.05$). 0.5 ng/ml PGE2 increased the perfusion pressure in hearts from male rats ($P > 0.05$) and 10 ng/ml decreased it ($P > 0.05$). PGE2 increased the perfusion pressure at both concentrations in all female hearts. Only the effects on hearts from normal females with 10 ng/ml PGE2 were significant ($P < 0.05$).

PGF2α increased the values of all parameters studied (figure 11). However hearts from castrated males were resistant to the chronotropic and coronary constricting actions of PGF2α .

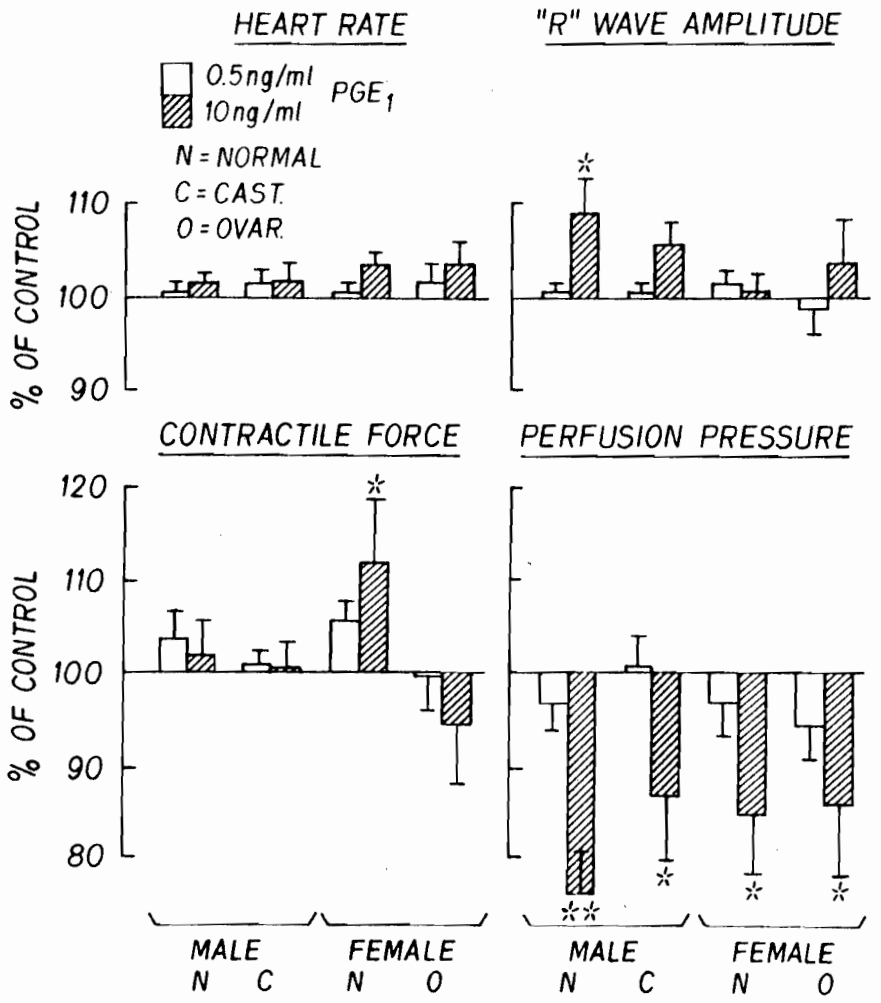

Figure 9. The effects of sex and gonadectomy on the response of isolated rat hearts to PGE1 (n=10)

* $P < 0.05$

from control values before the addition of PGE1

** $P < 0.01$

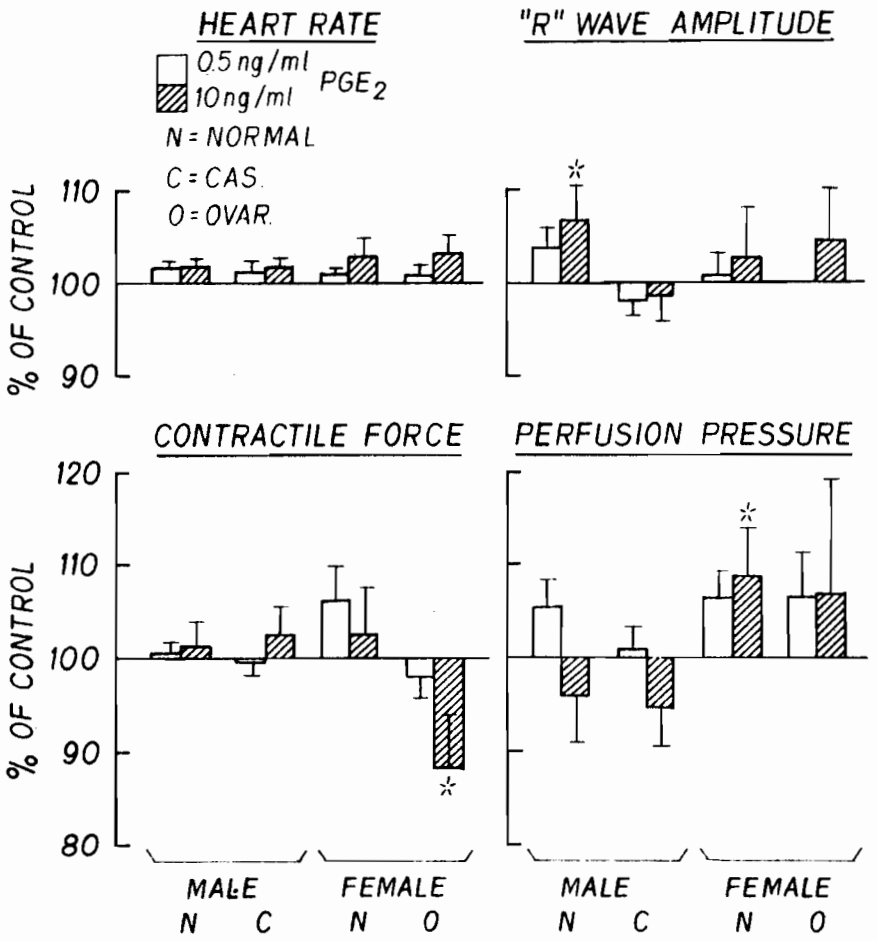

Figure 10. The effects of sex and gonadectomy on the response of isolated rat hearts to PGE2 (n=10)

* $P < 0.05$

** $P < 0.01$ from control values before the addition of PGE2

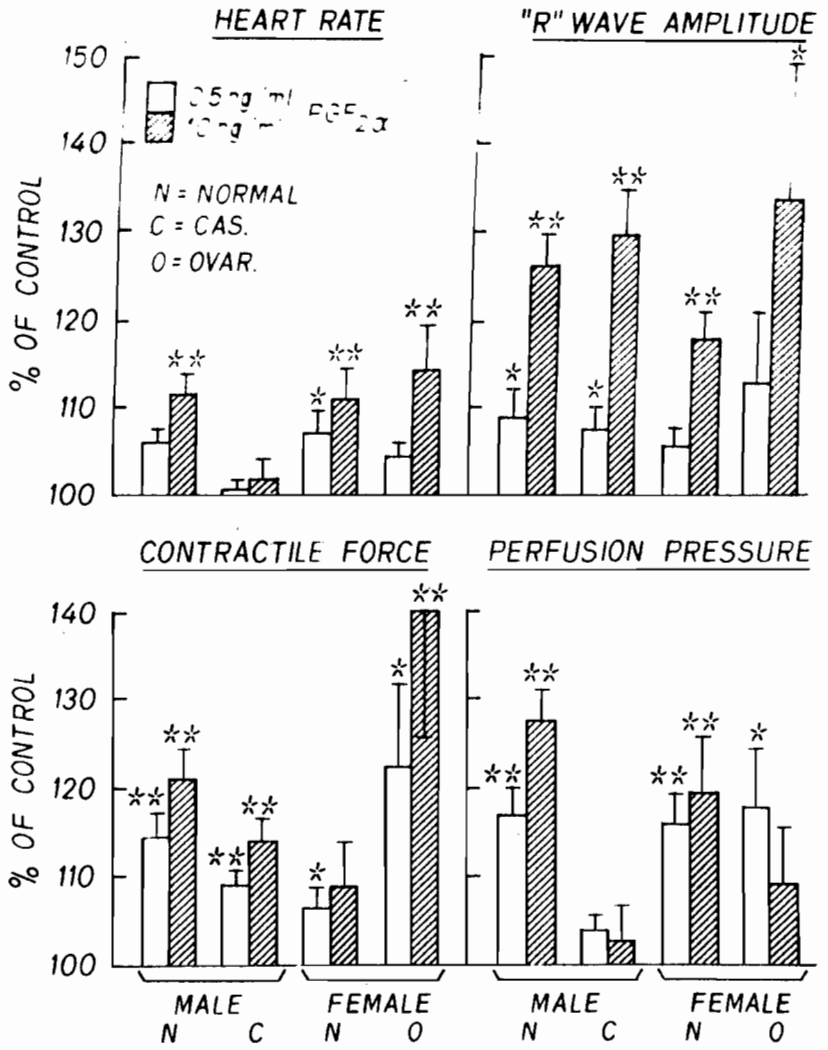

Figure 11. The effects of sex and gonadectomy on the response of isolated rat hearts to PGF2α (n=10)

* $P < 0.05$

** $P < 0.01$ from control values before the addition of PGF2α

Experiment 4

Due to the possible importance of PGI2 in the regulation of coronary and myocardial function, this agent was studied in detail for its actions on isolated rat and rabbit hearts.

Actions of PGI2 on Coronary Perfusion Pressure

The addition of 1 pg/ml PGI2 significantly increased the perfusion pressure of the isolated rat hearts (figure 13, $P < 0.01$). The perfusion pressure increased with increasing amounts of PGI2 up to 1 ng/ml (figures 12 and 13). At this concentration the mean perfusion pressure was 33.1 ± 5.3 mm Hg (4.4 ± 0.7 kPa, $P < 0.01$) above control values. Higher concentrations led to a fall in pressure back to control values. Similar results were observed with the isolated rabbit hearts (figure 13). 1 pg/ml PGI2 again significantly increased the perfusion pressure ($P < 0.05$). Maximum increases were seen with 100 pg/ml and 1 ng/ml. Both concentrations gave mean increases in coronary perfusion pressure of 22.2 ± 3.0 mm Hg (3.0 ± 0.4 kPa) from control levels ($P < 0.01$). Higher PGI2 concentrations caused perfusion pressure to decline again. 100 ng/ml decreased the perfusion pressure from control values by about 30 mm Hg (4.0 kPa, $P < 0.01$).

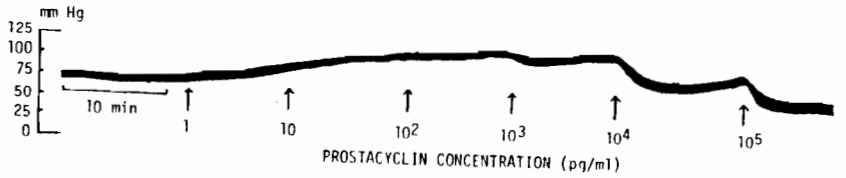

Figure 12. Example of recording showing perfusion pressure changes with increasing concentrations of prostacyclin in the isolated rat heart. Although not shown, 10^6 pg/ml caused a further decrease in the perfusion pressure.

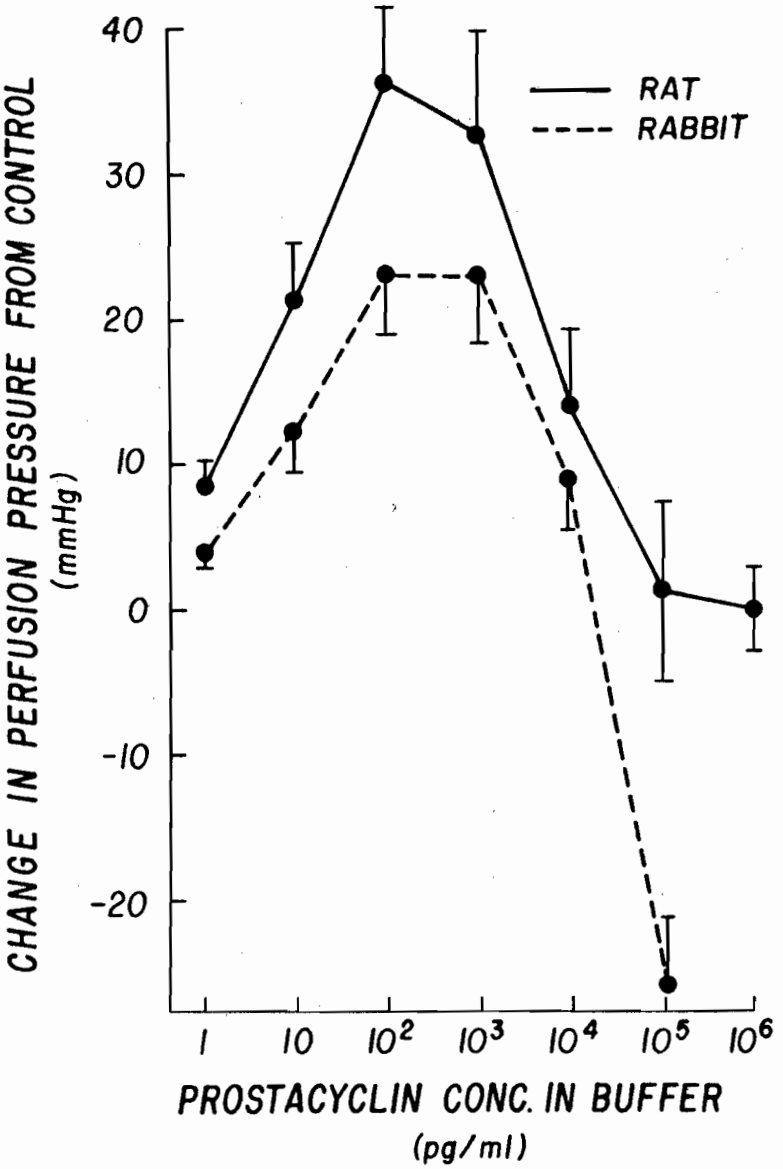

Figure 13. The effects of increasing concentrations of prostacyclin on the coronary perfusion pressure of isolated rat and rabbit hearts. (n=10 for rat hearts and n=5 for rabbit hearts).

Actions of PGI2 on the Heart Rate

In the isolated rat heart PGI2 had positive chronotropic effects (figure 14). The addition of 1 pg/ml PGI2 increased the heart rate by about 4 beats/minute from control values ($P > 0.05$). Significant effects were seen with 100 pg/ml PGI2 ($P < 0.05$). Higher concentrations caused further increases in heart rate ($P < 0.05$ at 1 to 10 ng/ml). The highest PGI2 concentration (1 µg/ml) caused an increase of over 20 beats/minute from control values ($P < 0.01$). In the isolated rabbit heart no chronotropic actions of PGI2 were observed.

Actions of PGI2 on the Cardiac Contractile Force

The addition of 1 pg/ml PGI2 to the medium perfusing the rat hearts resulted in an increased developed tension (DT) by approximately 50 mg ($P > 0.05$) (figure 15). A significant mean increase in the DT was observed with 100 pg/ml PGI2 present in the buffer ($P < 0.01$). As can be seen in figures 15 and 16, systolic tension reached a peak with 10 ng/ml PGI2. Further increases in PGI2 levels decreased DT values back to levels which were not significantly different from control ($P > 0.05$). Rabbit hearts did not demonstrate significant responses to PGI2 administration. Of the 5 hearts studied only one demonstrated a slight inotropic response to PGI2 (10 mg increase in DT at 100 pg/ml).

Thus PGI2 has actions on the coronary vessels and myocardium in the isolated rat heart. It is difficult to dissociate the coronary and myocardial actions of PGI2 using this particular experimental model. In the rabbit heart, while having no direct myocardial actions, PGI2 had significant effects on coronary resistance which may suggest that this PG has seperate actions on the coronary vasculature and the myocardium.

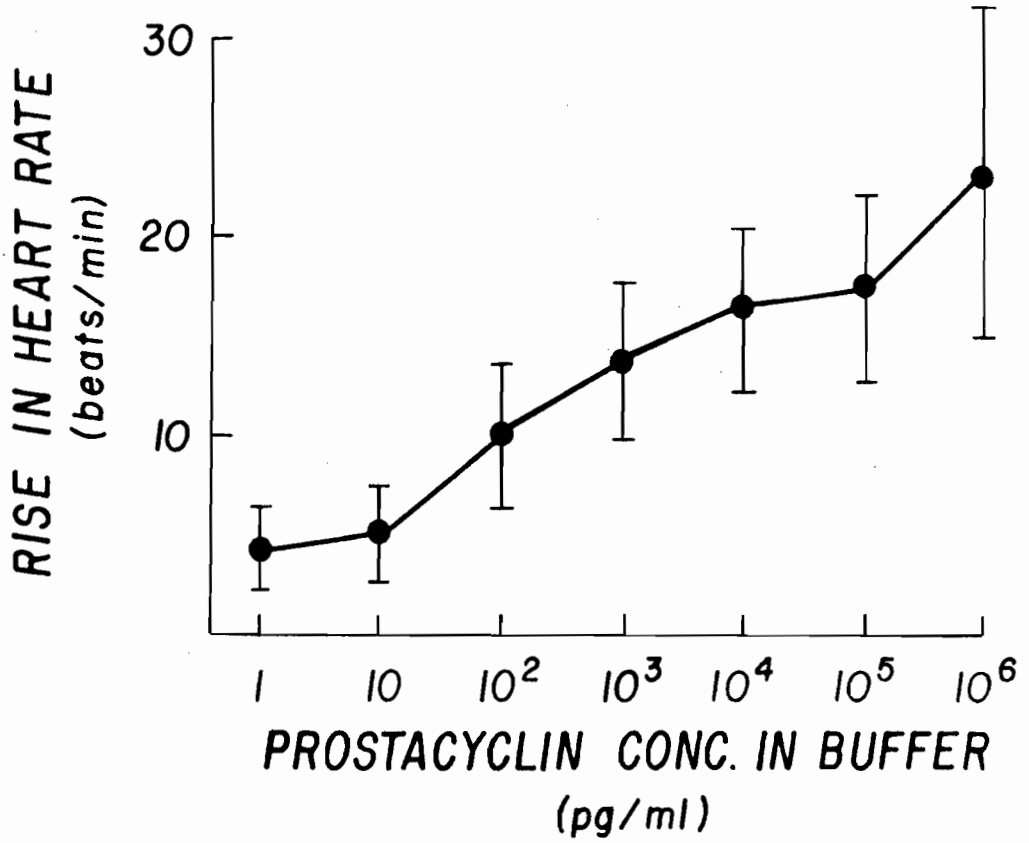

Figure 14. Increases in heart rate associated with increasing concentrations of prostacyclin in the buffer in the isolated rat heart. No effects were seen with the rabbit hearts (n=10)

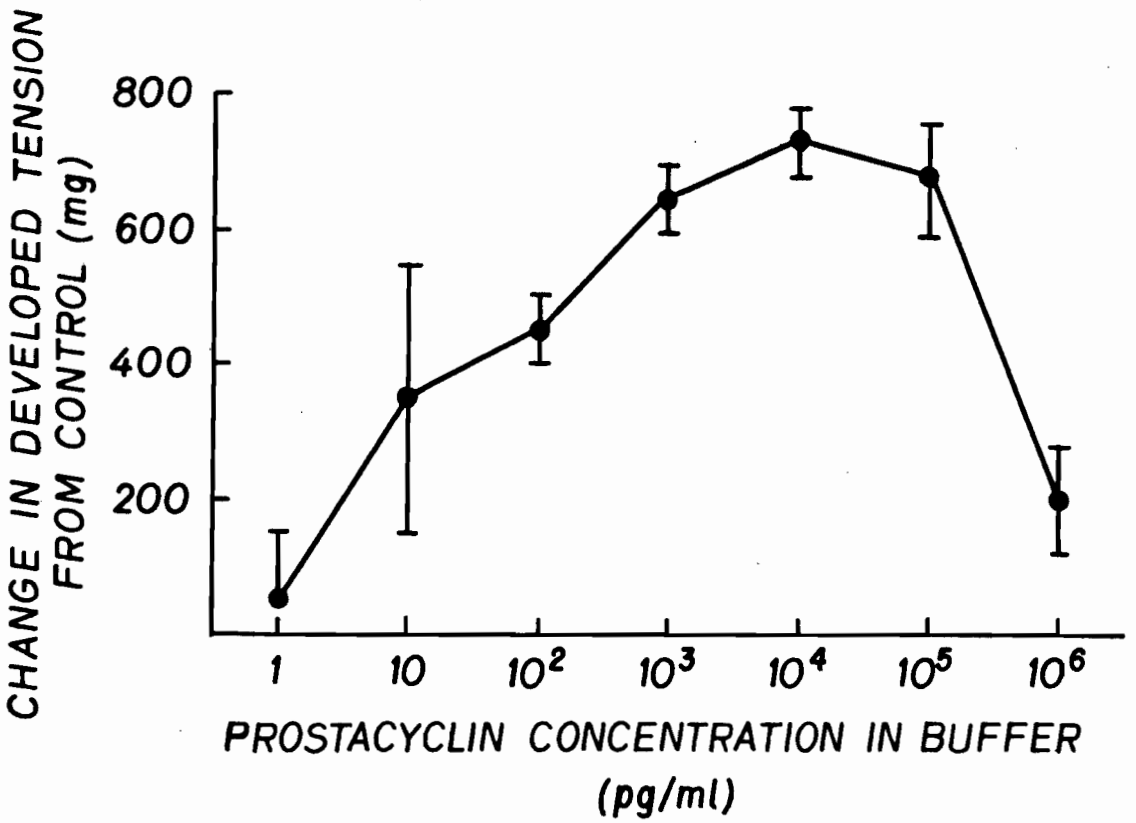

Figure 15. Changes in the contractile force associated with increasing concentrations of prostacyclin in the buffer in the isolated rat heart. No effects were seen with the rabbit hearts. (n=10)

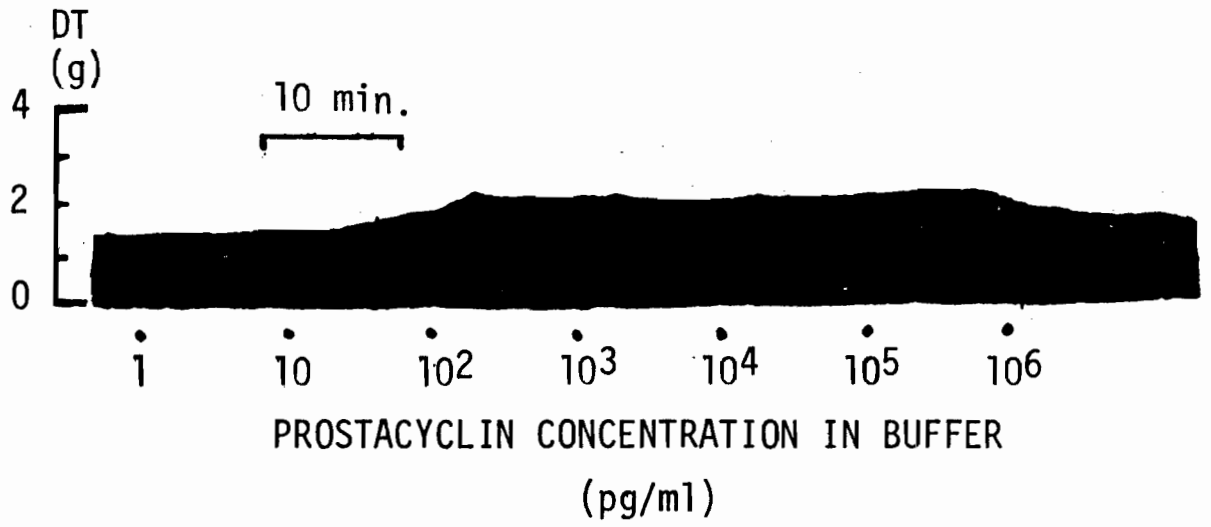

Figure 16. Example of a recording indicating the contractile force of the isolated rat heart with increasing concentrations of prostacyclin.

DT: Developed tension

Actions of PGI2 on Cardiac Electrical Activity

PGI2 did not affect the amplitude of the QRS complex in any of the hearts studied. In six of the ten rat hearts, PGI2 at concentrations of 10 pg/ml to 1 ng/ml caused transient dysrhythmic episodes usually lasting approximately one minute. Dysrhythmic activity was diagnosed if fluctuations in ratemeter recordings were observed without visible EKG disturbances or if severe deviations were seen on the EKG recordings. In the 6 hearts just referred to, alterations in rhythm stability were seen on the rate-meter recording. In four of these hearts, these were accompanied by severe dysrhythmias (tachyarrhythmias) similar to the one seen in figure 17. Two of the five rabbit hearts studied also demonstrated rhythm instability at the same PGI2 concentrations. As with the rat hearts,this rhythm instability lasted approximately one minute. No severe dysrhythmias were seen however in the rabbit hearts. It should be stressed that no dysrhythmic episodes were seen with PGI2 concentrations greater than 1 ng/ml in either rat or rabbit hearts.

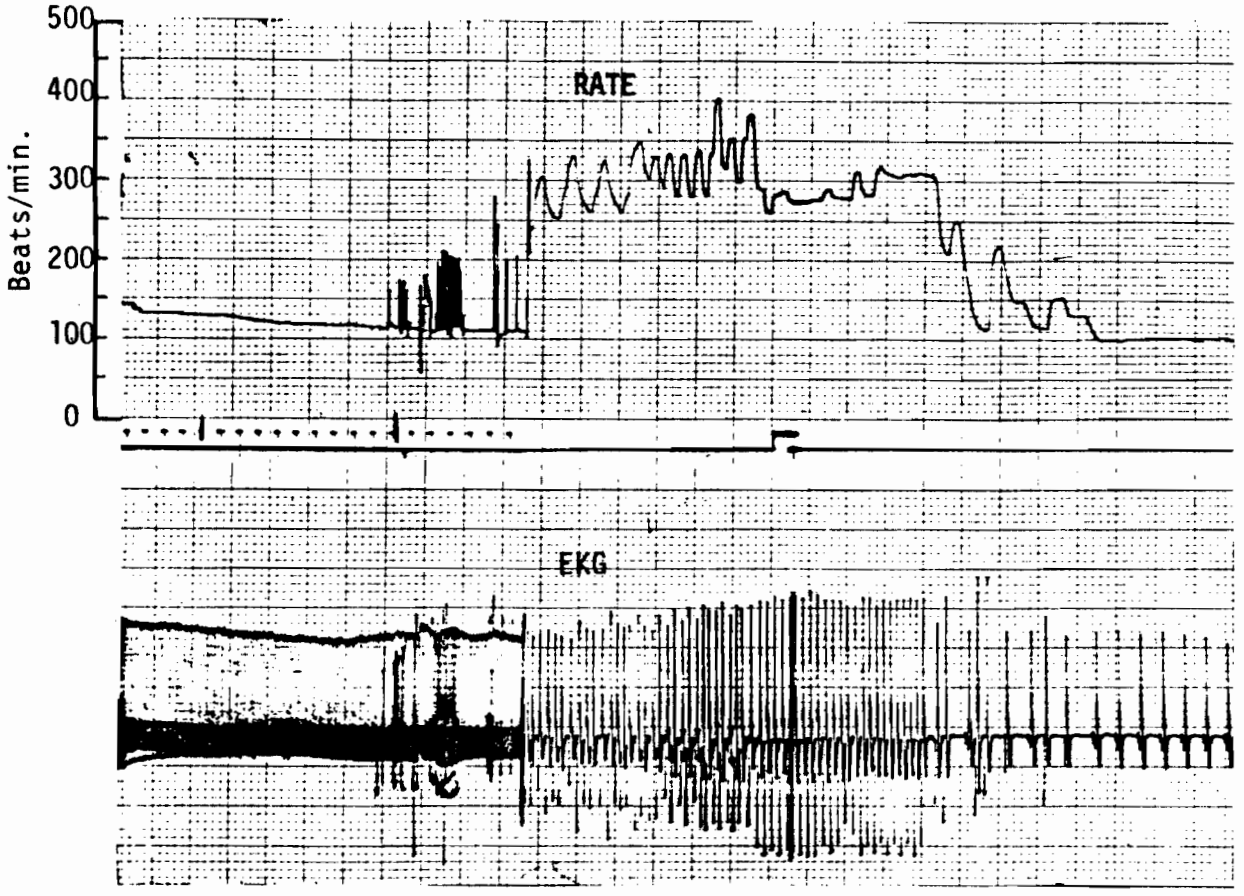

Figure 17. Recordings illustrating tachyarrhythmia in an isolated rat heart with 100 pg/ml prostacyclin present in the buffer. Top frame indicates the ratemeter recording and bottom frame the EKG. Dots indicate 1 minute intervals. Speed of the recorder was increased during the dysrhythmic episode (arrow) from 25 mm/min to 5 mm/sec.

Experiment 5

The ability of indomethacin to alter coronary resistance in isolated rat hearts was tested in seven preparations (figure 18). At 2.5 µg/ml indomethacin significantly reduced the coronary perfusion pressure from control values by 7.3 ± 1.1 mm Hg (1.0 ± 0.1 kPa, $P < 0.05$). Further increases in the indomethacin concentration resulted in a subsequent further decline in perfusion pressure. At 40 µg/ml indomethacin reduced the coronary perfusion pressure by about 25 mm Hg (3.3 kPa) from control values ($P < 0.01$).

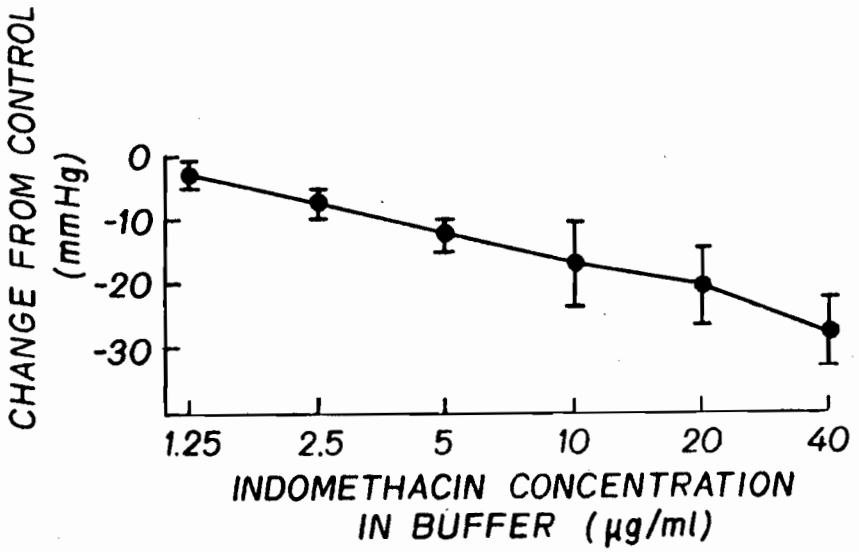

Figure 18. The effect of increasing indomethacin concentrations on the coronary perfusion pressure of isolated rat hearts. (n=7)

In two hearts the coronary effects of aspirin were examined. In concentrations from 10 to 160 µg/ml this had almost no effect on coronary perfusion pressure. The highest aspirin concentration used produced only a slight dilating effect of 4.2 ± 0.9 mm Hg (0.6 ± 0.1 kPa) which was not significantly different from control ($P > 0.05$).

Experiment 6

Effect of Hypoxia in Hearts from Young and Mature Animals

The effect of hypoxia on the perfusion pressure in hearts from young and mature rats is shown in figure 19. After an initial 30 minute perfusion with oxygenated buffer a switch was made to unoxygenated buffer. Hearts from young (30 day old) animals exhibited an immediate and significant ($P < 0.01$ at 5 minutes) drop in perfusion pressure after switching to unoxygenated buffer. This persisted throughout the perfusion period. Reoxygenation after sixty minutes resulted in an increase in perfusion pressure back to levels not significantly different from control ($P > 0.05$). Hearts from mature (3 months old) animals exhibited an initial drop in perfusion pressure ($P < 0.01$ at 5 minutes) similar to that observed in young animals. However, within 15 minutes the perfusion pressure started rising to levels above control. Maximum perfusion pressure [40 mm Hg above control (5.3 kPa), $P < 0.01$] was reached after 30-40 minutes and remained there for the balance of the hypoxic perfusion period. Results with reoxygenation at this point were variable although in 9 of the hearts perfusion pressure dropped after beginning reoxygenation. The hypoxic vasoconstriction could not be prevented by the presence of the angiotensin II antagonist saralasin (1 µg/ml) or the alpha-receptor blocker phenoxybenzamine (50 ng/ml, 2 experiments each).

Effects of PG synthesis Inhibitors on the Coronary Artery Response to Hypoxia

Indomethacin (10 µg/ml) prevented the hypoxia-induced coronary constriction (figure 20, table 7) and reversed it when added at the constrictor phase of hypoxic perfusion (figure 21). Aspirin, however had very little effect when added during the constrictor phase. When aspirin was already present (100 µg/ml) in the buffer, significant effects were observed at certain

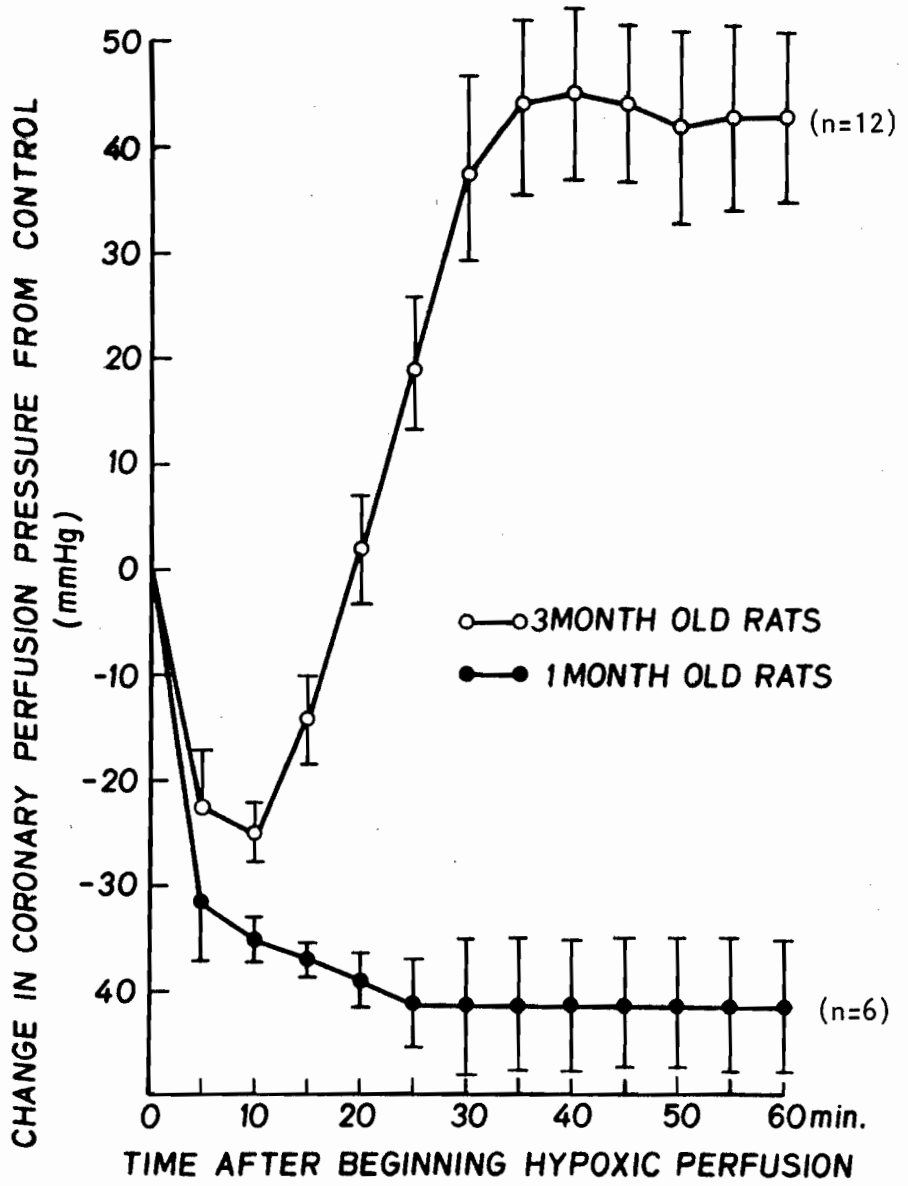

Figure 19. Effect of age on the coronary artery response to hypoxia in isolated rat hearts

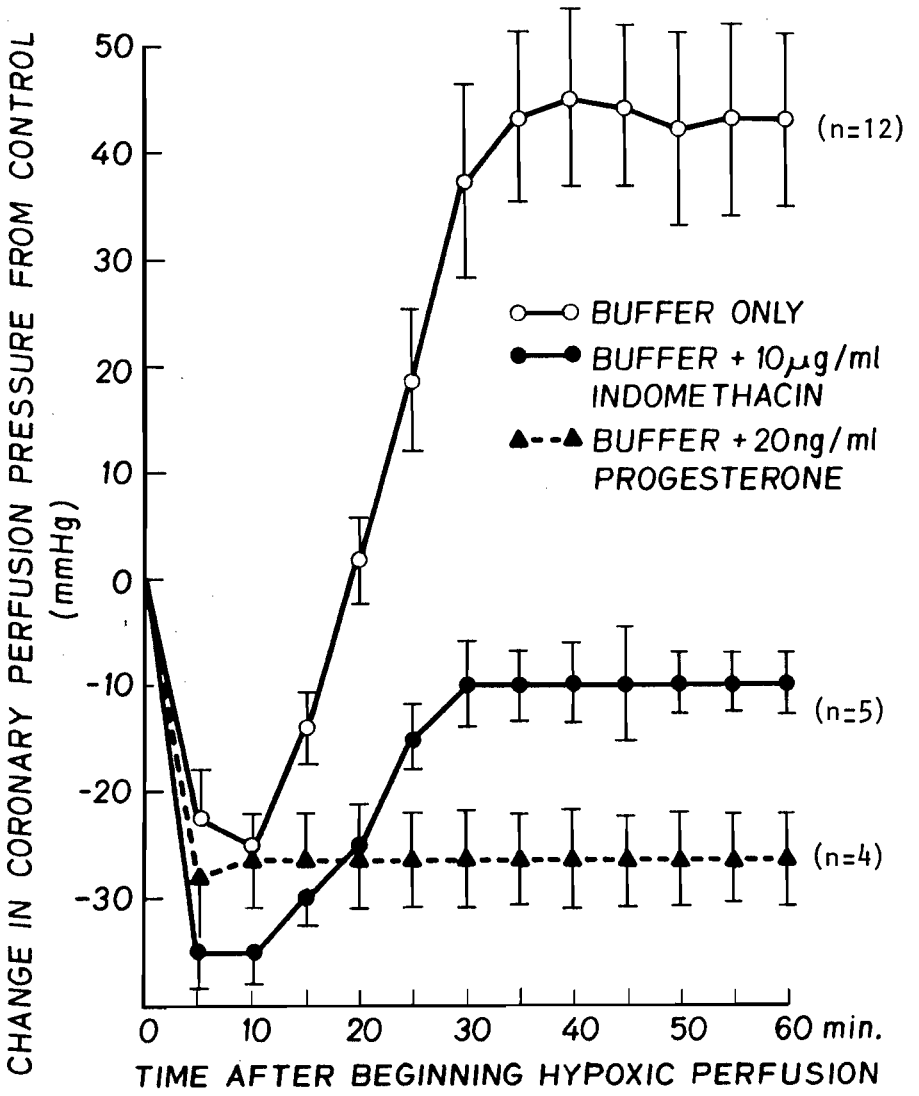

Figure 20. Effect of indomethacin and progesterone on the coronary artery response to hypoxia in isolated rat hearts

Table 9. Effects of prostaglandin synthesis inhibitors on the coronary artery response to hypoxia

TIME AFTER BEGINNING HYPOXIC PERFUSION (min)	BUFFER ONLY (n=12)	INDOMETHACIN (10 µg/ml) (n=6)	ASPIRIN (100 µg/ml) (n=4)
10	-24.2 ± 3.1	-35.3 ± 2.1*	-29.0 ± 4.6
20	1.3 ± 2.6	-26.4 ± 3.2**	- 7.8 ± 4.1
30	36.1 ± 2.6	-12.8 ± 4.3**	11.9 ± 5.6**
40	41.7 ± 9.2	-12.9 ± 4.1**	18.7 ± 9.1*
50	39.8 ± 9.7	-12.9 ± 3.4**	27.2 ± 7.0
60	40.6 ± 9.1	-13.0 ± 3.8**	31.3 ± 9.0

Values in columns represent mean changes in coronary perfusion pressure (in mm Hg) ± SEM

* Indicates significant differences ($p < 0.05$) and ** indicates higly significant differences ($p < 0.01$) from mean values of drug-free buffer at the specific time interval

Drugs were present in the buffer before commencing hypoxic perfusion

time intervals (table 7). However after 60 minutes of hypoxic perfusion the coronary perfusion pressure changes were not significantly different from values observed with drug-free buffer.

Effects of Substances that Antagonize PG Action

Three agents tested; chloroquine, procaine and propranolol were able to inhibit significantly the hypoxia-induced coronary constriction (table 8). Chloroquine was the most potent of the three drugs tested. At 4 µg/ml it was able to prevent significantly the rise in perfusion pressure. High levels of procaine (128 µg/ml) and propranolol (16 µg/ml) were required for similar effects. When added during the constricting phase of hypoxic perfusion all three drugs were able to reverse the increased coronary perfusion pressure. Again, chloroquine was the most effective in this respect (figure 21). All three drugs were able to significantly reduce the coronary perfusion pressure in normoxically perfused hearts when added at the above mentioned concentrations. The decline in perfusion pressure was for chloroquine 9.8 ± 2.1 mm Hg (1.3 ± 0.3 kPa), procaine 7.4 ± 1.0 mm Hg (1.0 ± 0.2 kPa) and propranolol 7.7 ± 1.3 mm Hg (1.0 ± 0.2 kPa) (2 hearts each, $P < 0.05$ for each drug).

Effects of TXA2 Synthesis Inhibitors

Four agents; imidazole, N-0164, benzydamine and dipyridamole were used. All four agents failed to prevent the rise in perfusion pressure observed during hypoxic perfusion although some significant differences at various time intervals were observed (table 9). When administered to normally oxygenated hearts dipyridamole, benzydamine and N-0164 increased the perfusion pressure at low concentrations and decreased it at high ones

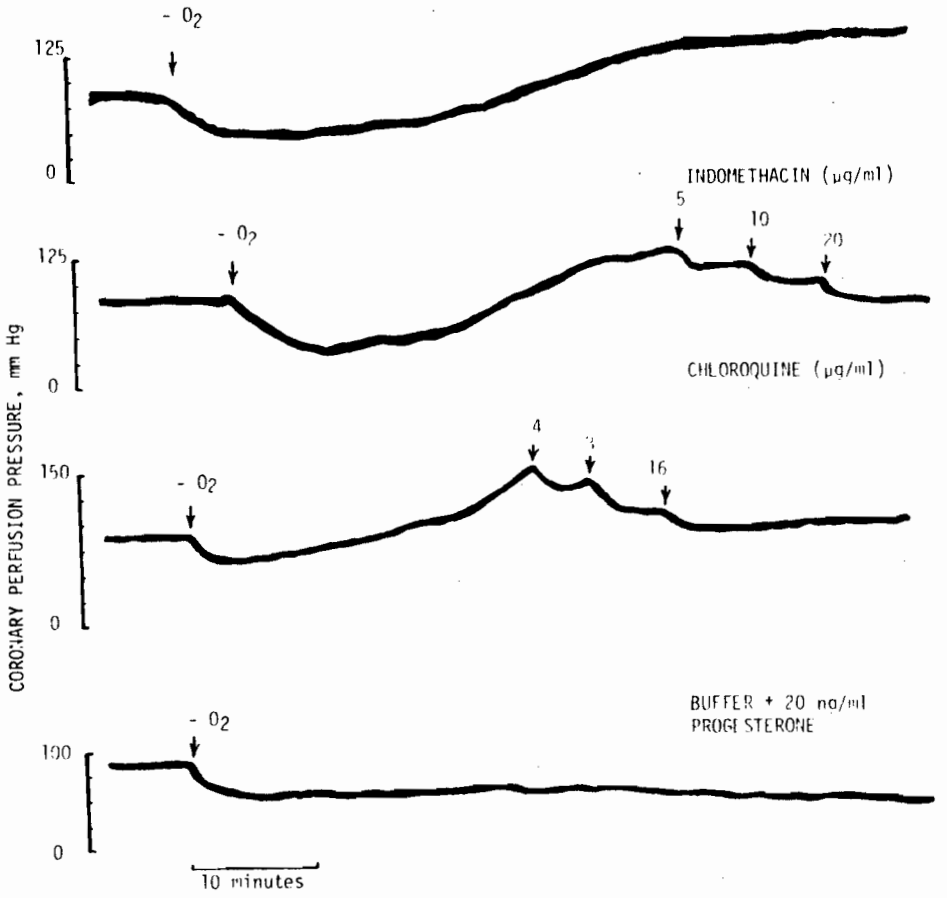

Figure 21. Coronary perfusion pressure of the isolated rat heart during hypoxia in normal buffer (top) and after the addition of indomethacin or chloroquine. Bottom recording illustrates the effect of hypoxia in the presence of progesterone

Table 10. Effects of chloroquine, procaine and propranolol on the coronary artery response to hypoxia

TIME AFTER BEGINNING HYPOXIC PERFUSION (min)	BUFFER ONLY (n=12)	CHLOROQUINE (10 µg/ml) (n=4)	PROCAINE (128 µg/ml) (n=4)	PROPRANOLOL (16 µg/ml) (n=4)
10	-24.2 ± 3.1	-19.8 ± 7.1	-21.7 ± 6.8	-24.6 ± 7.8
20	1.3 ± 2.6	-24.9 ± 6.3**	-20.5 ± 6.3**	-18.5 ± 8.3**
30	36.1 ± 9.3	-20.7 ± 9.7**	-20.5 ±11.0**	-12.3 ± 6.1**
40	41.7 ± 9.2	-19.4 ± 5.8**	-14.8 ± 7.3**	-10.0 ± 4.5**
50	39.8 ± 9.7	-20.6 ± 9.1**	-12.7 ± 5.9**	-10.0 ± 4.7**
60	40.6 ± 9.1	-22.4 ± 8.7**	-14.3 ± 7.6**	- 9.7 ± 5.8**

Values in columns represent mean changes in coronary perfusion pressure (in mm Hg) ± SEM

** Indicates highly significant differences ($p < 0.01$) from mean values of drug-free buffer at the specific time interval

Drugs were present in the buffer before commencing hypoxic perfusion

Table 11. Effects of thromboxane A2 synthesis inhibitors on the coronary artery response to hypoxia

TIME AFTER BEGINNING HYPOXIC PERFUSION (min)	BUFFER ONLY (n=12)	DIPYRIDAMOLE (0.32 µg/ml) (n=3)	BENZYDAMINE (0.16 µg/ml) (n=4)	N-0164 (0.16 µg/ml) (n=3)	IMIDAZOLE (40 µg/ml) (n=4)
10	-24.2 ± 3.1	-29.8 ± 6.6	-20.8 ± 8.3	-25.3 ± 6.1	-18.7 ± 6.2
20	1.3 ± 2.6	- 4.9 ± 7.1	2.0 ± 1.9	- 6.6 ± 2.0*	- 7.7 ± 3.2*
30	36.1 ± 9.3	29.3 ± 9.0	24.8 ± 9.5*	19.9 ± 7.3**	16.7 ± 6.1**
40	41.7 ± 9.2	42.1 ±11.7	33.8 ± 8.7*	24.7 ± 6.1**	25.8 ± 7.1**
50	39.8 ± 9.7	46.9 ± 8.4*	38.3 ± 9.0	30.6 ± 5.2	31.4 ± 7.0
60	40.6 ± 9.1	44.9 ± 9.0	42.7 ± 6.5	36.7 ± 7.1	36.9 ±10.2

Values in columns represent mean changes in coronary perfusion pressure (in mm Hg) ± SEM

* Indicates significant differences ($p < 0.05$) and ** indicates highly significant differences ($p < 0.01$) from mean values of drug-free buffer at the specific time interval

Drugs were present in the buffer before commencing hypoxic perfusion

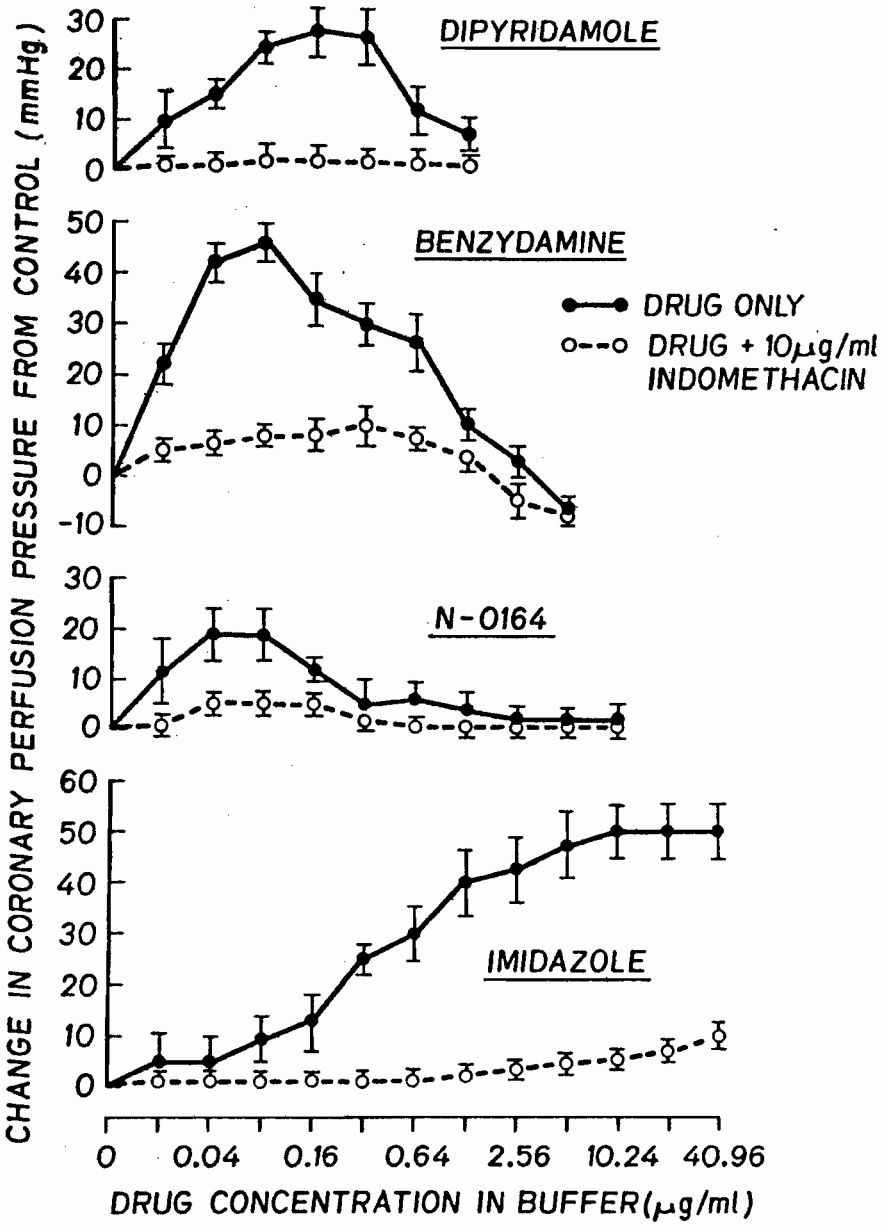

Figure 22. Effects of thromboxane A2 synthesis inhibitors on the coronary perfusion pressure in isolated rat hearts. Each drug concentration was tested either alone or in the presence of indomethacin (n=4)

(figure 22). Imidazole consistently increased the perfusion pressure until a plateau was reached. The significant ($P < 0.05$) concentrations of these agents having constricting effects were for dipyridamle 40 ng/ml (13.0 ± 1.0 mm Hg, 1.7 ± 0.1 kPa above control values), benzydamine 20 ng/ml (21.8 ± 2.0 mm Hg, 2.9 ± 0.3 kPa), N-0 164 40 ng/ml (18.4 ± 3.6 mm Hg, 2.4 ± 0.5 kPa) and imidazole 160 ng/ml (11.3 ± 2.2 mm Hg, 1.5 ± 0.3 kPa). The constricting actions of all these drugs were prevented by the presence of 10 µg/ml indomethacin.

Effects of Gonadal Steroids

Testosterone (1 to 32 ng/ml), estradiol (1 to 320 ng/ml) and progesterone (1 ng/ml to 1.24 µg/ml) had no significant effect on the perfusion pressure in normoxically perfused hearts. Also, as can be seen in table 10, estradiol or testosterone had no significant effect on the coronary artery response to hypoxia. When either of these steroids were present the average constricting response was higher than the response observed with drug-free buffer ($P > 0.05$). However, progesterone at 20 ng/ml significantly prevented the rise in perfusion pressure when already present in the buffer (figure 20, table 10, $P < 0.01$ at 60 minutes). In two experiments progesterone was not able to reverse significantly the constriction observed after beginning hypoxic perfusion. At 1 µg/ml only a slight reversal (about 4 mm Hg, 0.5 kPa) was seen after the addition of progesterone.

Table 12. Effects of gonadal steroids on the coronary artery response to hypoxia

TIME AFTER BEGINNING HYPOXIC PERFUSION (min)	BUFFER ONLY (n=12)	PROGESTERONE (20 ng/ml) (n=7)	ESTRADIOL (0.5 ng/ml) (n=5)	TESTOSTERONE (16 ng/ml) (n=5)
10	-24.2 ± 3.1	-27.3 ± 4.2	-26.5 ± 3.1	-20.4 ± 6.8
20	1.3 ± 2.6	-27.3 ± 4.1**	3.8 ± 0.6	4.7 ± 1.5
30	36.1 ± 9.3	-27.6 ± 4.7**	34.1 ± 8.7	38.9 ± 8.7
40	41.7 ± 9.2	-27.6 ± 4.9**	38.6 ± 7.0	43.6 ±10.4
50	39.8 ± 9.7	-27.8 ± 4.9**	40.4 ± 8.1	42.7 ± 9.5
60	40.6 ± 9.1	-27.6 ± 4.7**	43.6 ± 5.7	42.9 ± 8.7

Values in columns represent mean changes in coronary perfusion pressure (in mm Hg) ± SEM

** Indicates highly significant differences ($p < 0.01$) from mean values of drug-free buffer at the specific time interval

Drugs were present in the buffer before commencing hypoxic perfusion

DISCUSSION

The major results presented in this report will be discussed in general under various headings.

Prostaglandins and Cardiac Rhythm

There is extensive evidence available indicating that PGs can exert antiarrhythmic effects (see Literature Review). The studies reported here however also demonstrate that within critical concentrations PGs can induce rhythm instability in isolated hearts which disappear at high PG levels. There is much evidence, albeit indirect, that in fact PGs may be involved in arrhythmogenesis. Various drugs known to have antiarrhythmic properties have been shown to be able to antagonize the actions of PGs. These include chloroquine, quinidine, quinine and procaine (Manku and Horrobin 1976a) as well as diphenylhydantoin (Karmazyn *et al* 1977b). Also, the β blocker propranolol has been shown to antagonize the actions of PGE2. In unpublished work from this laboratory other agents having antiarrhythmic properties including disopyramide phosphate, ajmaline and mexiletine hydrochloride all behaved as PG antagonists in a rat vascular preparation. The mechanism of action of many of these agents are currently poorly understood and the possibility therefore exists that antagonism of PG action may be responsible for their antiarrhythmic properties. To further strengthen this hypothesis it was found that the ID50 of these drugs for their ability to antagonise PG action was well within their plasma therapeutic levels. It therefore seems possible that PG antagonism may be an important component of antiarrhythmic action although no proof at this time exists for such a role.

Other evidence may be found indicating that agents which antagonize PG action may exert cardio-protective effects. Rotenberg *et al* (1976) have presented an interesting relationship between coffee consumption and death from myocardial infarction suggesting that increased consumption of coffee may prevent death. In animal studies, they reported that rats given long-term caffeine administration had a reduced susceptibility to arhythmogenesis. Tachyarrhythmia threshold was twice as great in animals receiving oral caffeine as in control animals. It is well known that caffeine increases cAMP levels by phosphodiesterase inhibition. Such an action would be expected to contribute to arrhythmogenesis as will be discussed later. However it was recently shown that caffeine can act as a potent PG antagonist (Horrobin *et al* 1977b). It would therefore be attractive to speculate that this property may be responsible for its protective effect.

Various substances known to induce cardiac arrhythmias have been shown to stimulate PG production. One such agent is prolactin which causes arrhythmic behaviour in isolated rabbit hearts (Nassar *et al* 1974). There is now evidence that prolactin may stimulate PG biosynthesis (Horrobin *et al* 1974, Rillema 1975). Norepinephrine can cause a variety of electrical disturbances (Innes and Nickerson 1975) in the hearts. It is also a potent stimulator of cardiac PG synthesis (Pham-Huu-Chanh 1973). Acetylcholine which also stimulates PG synthesis (Junstad *et al* 1974) is known to produce or enhance the susceptibility to atrial fibrillation in experimental animals (Leveque 1956). Finally Meyer (1946) has shown that

myo-Inositol causes cardiac arrhythmias in rabbit hearts. Recently Karmazyn and coworkers (1977a) showed that this substance is a potent stimulator of PG synthesis in a rat vascular preparation.

In spite of the indirect evidence implicating PGs in cardiac arrhythmogenesis, the overwhelming evidence with direct PG studies indicate that PGs possess antiarrhythmic properties. In the present results PGE, PGF2 and PGI2 induced dysrhythmic behaviour at relatively low levels which gradually decreased while PG concentrations in the buffer increased. Perhaps more importantly, PG levels that did cause rhythm instability were within levels found in human coronary sinus plasma (Berger *et al* 1977). These results cast doubt on the concept that PGs are naturally occurring anti-arrhythmic agents although it does not dispute the fact that very high levels of PGs unlikely to be found *in vivo* may have this property. The ability of PGI2 to cause dysrhythmic behaviour was suprising in view of the fact that it is considered one of the "good" PGs which may play a role in the protection of the myocardium due to its coronary dilatating and anti-platelet actions (Gorman *et al* 1977, Dusting *et al* 1977). The ability of indomethacin to abolish spontaneous arrhythmias suggested that in this situation too, a PG may be responsible for the disturbances.

The reversal of the rhythm instability by chloroquine is compatible with the proposal that it is a PG antagonist (Manku and Horrobin 1976). Epidemiological studies have shown that low copper intake is associated with an increased risk of death from heart disease but possible mechanisms involved are not known (Klevay 1975). Copper was extremely potent in

stabilizing PG-induced rhythm instability suggesting a PG antagonistic action. Normal plasma copper concentrations range up to 2×10^{-5}M (Bearn 1972) and although much of this is bound the results presented here suggest that physiological copper concentrations could have effects on cardiac rhythm.

One of the possible explanations to the discrepancy between the results presented in this report and the well-known antiarrhythmic properties of PGs is the apparent property of many PGs to exert biphasic actions. This property has been demonstrated in many tissues. In a rat vascular preparation PGE1 and PGA1 first potentiate the pressor response to norepinephrine injection while higher PG levels have inhibitory actions (Manku *et al* 1977b). In the isolated frog sciatic nerve PGE1 up to 1 ng/ml increases the amplitude and velocity of the action potential but at higher concentrations these effects were reversed (Horrobin *et al* 1977). PGE2 increases lymphocyte growth at low concentrations while higher concentrations decreased growth (Karmali *et al* 1978). Similar biphasic actions of PGs were also observed in the heart as previously described (see Literature Review). Briefly, both PGE1 and PGE2 have been shown to have biphasic actions on coronary vascular resistance (Ogletree and Lefer 1977, Toda *et al* 1975). Also "bell-shaped" effects on various parameters of the cardiac action potential were observed with PGE2 administration (Kecskemati *et al* 1974, Scholkens and Kaiser 1977). It is therefore not suprising that low PG concentrations can have dysrhythmogenic actions at low levels while higher levels exhibit antiarrhythmic prpperties. Other possible reasons for variation in results will be discussed later.

The question that may be asked therefore is how do PGs exert their dysrhythmogenic actions. There are two main mechanisms for the generation of arrhythmias; abnormal pacemaker automaticity or an abnormality in the cardiac conduction system (re-entry phenomenon). Because various PGs have potent inotropic and chronotropic actions it is possible that they may make the heart more susceptible to arrhythmias when conditions are favourable such as stress (with increased catecholamine release) or myocardial ischemia. In the results described in this report PGE2 or PGF2α did not cause severe arrhythmias. Yet the main cardiac PG, prostacyclin did cause severe electrical disturbances in hearts from two animal species. This would greatly suggest that when PGs are released from the ischemic myocardium they may significantly reduce the arrhythmias threshold level. Because ionic imbalance may also induce arrhythmias, it may be of importance that an association between hypokalemia, PG administration and cardiac arrhythmias was recently demonstrated (Burt *et al* 1977). The phenomenon of re-entry is one of the most widely described electrical mechanisms suggested for arrhythmia generation. One of the main prerequisites for this phenomenon is prolonged conduction time through the conduction fibers. The effects of PGs on the action potential are difficult to interpret at this time since a myriad of actions have been observed. Yet a number of reports have demonstrated that PGE1 can prolong the duration of the action potential (Smejkal *et al* 1970, Mironneau and Grosset 1976). PGF2α (Mentz *et al* 1976b) as well as arachidonic acid (Szekeres *et al* *et al* 1976) can also prolong the duration of the cardiac action potential.

Although there has recently been intense progress in understanding the mechanism of arrhythmias at the electrophysiological level, the biochemical aspect of abnormal heart rhythm is not greatly understood. Calcium ions play an important role in the maintenance of cardiac function (Langer 1968). An acceleration of calcium influx into the heart would have a positive inotropic effect thus increasing oxygen demand. If however oxygen supply is limited, say due to coronary obstruction, cardiac arrhythmias would ensue (Lubbe *et al* 1974). A number of antiarrhythmic agents have been shown to inhibit calcium uptake by subcellular organelles. These include propranolol (Scales and McIntosh 1968, Dhalla and Lee 1976), quinidine (Harrow and Dhalla 1976) and verapamil (Entman *et al* 1972). In a variety of tissues there is evidence that calcium may play a crucial role in the actions of PGs (Ramwell and Shaw 1970, Shaw *et al* 1971, Manku and Horrobin 1976). In the heart PGs can increase calcium uptake and accumulation (Piccinini *et al* 1969, Sabatini-Smith 1969, 1971, Moura and Simpkins 1976). These results along with the calcium blocking/PG antagonist properties of various antiarrhythmic agents may offer one possible explanation for PG arrhythmogenic actions.

Another biochemical factor which has recently gathered much attention as a possible arrhythmogenic agent is cyclic adenosine monophosphate (cAMP). Rabinowitz *et al* (1974) have demonstrated a possible correlation between high plasma cAMP levels and poor prognosis after myocardial infarction in humans. In baboons with an experimental myocardial infarction, myocardial cAMP levels increase immediately preceeding ventricular fibrillation

(Podzuweit *et al* 1975). In the isolated rat heart dibutyryl cAMP lowers the ventricular fibrillation threshold and increases the duration of the vulnerable period (Lubbe *et al* 1976). Also, in ischemic rat hearts perfused without glucose, tissue levels of cAMP increase while at the same time the incidence of arrhythmias increase (Podzuweit *et al* 1976). Recently Corr and coworkers (1978) have demonstrated a possible relationship between increased tissue cAMP levels and cardiac arrhythmic activity in ischemic cat hearts.

Since cAMP is considered to be the second messenger of catecholamines, and since plasma catecholamine levels (McDonald *et al* 1969) and sympathetic nervous activity increase after myocardial infarction (Webb *et al* 1972) an important relationship may be suggested. The question then arises; where do the PGs "fit in"? PGs are known to inhibit neurotransmission in the heart by reducing the release of neurotransmitter substances (see Literature Review). Since PGs inhibit the synaptic release of norepinephrine in the heart a beneficial effect may be expected. However PGs have been shown by many workers to stimulate myocardial cAMP biosynthesis. The PGs so far shown to have this property include PGE1 (Klein and Levy 1971, Levy and Klein 1973, Moura and Simpkins1975, Sobel and Robison 1969), PGE2 (Klein and Levy 1971, Levy and Klein 1973), PGF1α (Sobel and Robison 1969) and PGF2α (Levy and Klein 1973). PGI2 has not yet been shown to stimulate cAMP production in the heart although it has this property in platelets (Gorman *et al* 1977). It is therefore possible that the rhythm instabilities observed in the present experiments may be due to increased cAMP production.

A hypothesis may therefore be suggested extending the results of the above studies to a role for PGs as well as cyclic nucleotides and calcium ions in arrhythmia generation. cAMP and calcium show close interaction in the heart (Robison *et al* 1965). Under hypoxic situations PG synthesis in the heart would be expected to rise as described in the Literature Review. This in turn would stimulate cAMP levels in the myocardium which may "trigger" arrhythmias through numerous actions. These include glycogenolysis (Dobson and Meyer 1973), increased lipolysis (Shug *et al* 1975), increased potassium loss (Lagslet 1970) or a direct effect increasing pacemaker activity in Purkinje fibers (Tsien *et al* 1972). Increased cAMP may also enhance calcium inflow into the myocardial cell (Robison *et al* 1965, Rich and Langer 1975) resulting in an increased oxygen demand. As previously described these calcium transport properties are also shared by many PGs. Since ischemically-perfused hearts do accumulate increased intracellular calcium (Henry *et al* 1977) resulting in undesirable effects and since PG synthesis in the heart is stimulated by ischemia, a direct relationship in this regard may be suggested.

This discussion has so far centered on calcium or cAMP as a second messenger of PG action on the heart. The possibility however, should not be ruled out, that PGs may be exerting some direct effect on the myocardium independant of any cyclic nucleotide or calcium second messenger.

Variation in the Cardiac Response to PGs

A phenomenon which has been referred to previously and deserves some discussion are the numerous contradicting results available in the literature regarding cardiac responses to the PGs. A number of factors can therefore be suggested as explanations for these variations. Differences in responses at different concentrations of the same PG have already been discussed.

There is also extensive evidence for species variation in the cardiac response to the PGs (see Literature Review). This was also clearly demonstrated in the present studies with PGI2. Although this substance increased the force of contraction and rate of the rat hearts, it had no effects on these parameters in the rabbit hearts.

Although species variation in the direct effects of PGs have been documented, no evidence is available regarding sexual variation in the cardiac response to the PGs. Uzunova *et al* (1977) did demonstrate sexual differences in arachidonate-induced arterial thrombosis in mice. The effects of various PGs on different parameters in isolated rat hearts from male and female normal and gonadectomized animals were therefore studied. The results indicate that the *in vitro* response to exogenous PGs can be dependant on sex or modified by gonadectomy. What role gonadal steroids play in this phenomenon as well as the effects of other PGs especially PGI2 remains to be studied.

Season may perhaps also influence the cardiac responses to PGs which may account for some of the discrepancies in the literature. However no such variation with PGs has yet been demonstrated. There is some evidence

however, that the cardiotonic effects of some agents may be dependant on the season. Weinstock and Shoham (1974) reported that the chronotropic effects of isoprenaline on the isolated guinea pig atrium were much more potent in the winter. Prolactin (which stimulates PGs) has also potent chronotropic and inotropic actions in the winter on isolated rabbit hearts (Nassar *et al* 1975). In that study very few effects were seen in the summer. While PG involvement in the myocardial response to these agents has not been demonstrated, it does perhaps suggest that PG action can follow a similar pattern.

Prostaglandins and the Coronary Circulation: A Possible Role in the Response to Hypoxia

Our current knowledge and understanding of the actions of PGs on the coronary circulation is fraught with confusion. Direct studies with PG administration indicate that they may constrict, dilate or have no effec

on the coronary arteries (table 4). Inhibition of PG synthesis reduces reactive hyperaemia after coronary artery occlusion (Kent *et al* 1973) yet various PGs have also been shown to have this effect (Bloor and Sobel 1970, 1973). Indomethacin and aspirin increased metabolically-induced coronary dilatation in rat hearts (Talesnik and Sunahara 1973) while PGE1 and PGE2 both prevented increases in blood flow (Sunahara and Talesnik 1974, Sen *et al* 1976).

In the results presented in this report, most of the PGs studied induced a coronary constriction as indicated by increased perfusion pressure. PGF2α and PGD2 constricted the coronary arteries at all concentrations studied. This is in agreement with most previous reports demonstrating coronary actions of these PGs (Kalsner 1975, Ogletree and Lefer 1977, Schror 1978). PGE2 on the other hand had a biphasic action, first constricting and then dilatating the arteries. A similar phenomenon was previously reported by Toda *et al* (1975). Most reports with isolated preparations have shown that PGE2 does contract coronary artery strips or perfused coronary vessels of the dog, cow and human (Strong and Bohr 1967, Kalsner 1975, Kulkarni *et al* 1976). Conversely PGE2 decreases coronary vascular resistance in the isolated guinea pig heart (Krebs and Schror 1975, 1976). This discrepancy may be due to species variation. PGF1α was found to be without coronary actions in isolated rat hearts (Vergroesen *et al* 1967). Similarly, very little effects were observed in the present study although a slight dilatating action was seen with very high PGF1α levels.

Probably the most unexpected results that were obtained were with PGI2, indicating that this substance is a potent coronary constrictor at low concentrations while dilating the vessels at high concentrations. This however should not be too surprising since, as referred to previously, many PGs have biphasic actions. PGE1 which is always considered to be a potent coronary dilatator has in fact also been demonstrated to constrict isolated canine coronary arteries at critical concentrations (Ogletree and Lefer 1977). Previous *in vitro* studies with PGI2 have been with concentrations much higher than those presented in this report. It therefore seems possible that PGI2 exerts similar biphasic effects on the coronary circulation in different animal species. The vasoconstrictor actions of PGI2 observed in this study as well as those recently reported on the porcine coronary arteries (Dusting *et al* 1977 b) indicate that this substance may not be a "pure" vasodilatator. The role of PGI2 in the control of the coronary circulation may therefore require reinterpretation.

The possible mechanism of action of PGs on the coronary vascular resistance is at present not understood. In the previous section it was mentioned that the arrhythmogenic actions of PGs may be due to the stimulation of cAMP production. In the coronary circulation a different mechanism may exist since increased cAMP levels would be expected to decrease coronary vascular resistance (Poch and Kukovetz 1972). The mechanism of action of PGs on the circulation has not been extensively

studied. Krebs and Schror (1975, 1976a) reported that the dilatating effects of PGE2 are not catecholamine-mediated since reserpine did not modify its actions. The adenosine antagonist aminophylline however did inhibit PGE1-induced coronary dilatation (Blass *et al* 1976). This may suggest that PGE1 may be working through the stimulation of adenosine formation. On the other hand, methyl xanthines can directly antagonize the actions of PGs on vascular smooth muscle (Horrobin *et al* 1977) which may explain the aminophylline effect in the coronaries.

Calcium probably plays an important role in the maintenance of coronary vascular tone (Fleckenstein-Grun and Fleckenstein 1975). It is therefore possible that PGs may modulate coronary changes by altering calcium transport. In a rat vascular preparation pressor responses to all vasconstrictor agents can be abolished by indomethacin and restored by PGs (Manku and Horrobin 1976). Since calcium availability to the actin-myosin complex is the final determinant for muscle contraction, it can be argued that PGs may influence this availability and that calcium transport may be dependant on the presence of PGs. It has in fact been suggested that calcium plays a crucial role in the action of PGs (Ramwell and Shaw 1970, Shaw *et al* 1971). This may be a simpple explanation for the constrictor actions of PGs on the coronary circulation. Horrobin (1977) has proposed that PGs are required for the activation of membrane calcium-binding sites. When the PG has a "bell-shaped" effect high PG levels will occupy all the PG receptors and at the same time react with the membrane in a way as to prevent calcium binding,resulting in an inhibitory effect (coronary dilatation). This may be the mechanism of action of PGE1, PGE2 and PGI2 on the coronary vasculature.

The question that should now be dealt with is what role do the PGs play in the maintenance of coronary function. Moretti *et al* (1976) demonstrated the need for PGs in the maintenance of coronary autoregulation during perfusion pressure changes in isolated rabbit hearts. Hintze and Kaley (1977) however have concluded that PGs play little or no role in the modulation of coronary blood flow in the dog. As described previously (see Literature Review) many workers have attempted to assign a role of PGs in the coronary artery response to hypoxia. It has been well established that the coronary arteries dilate as a result of myocardial oxygen insufficiency although the mechanism for this action is unknown. Berne and coworkers advocated that the hypoxia response is mediated via adenosine which is released from the myocardial cells (Berne 1964, Rubio and Berne 1975). In spite of this phenomenon of coronary dilatation due to hypoxia it has been suggested that coronary spasm may play an important role in both angina and myocardial infarction. This has generated much controversy as to whether coronary artery spasm can occur as a result of hypoxia or whether such spasm can actually cause a myocardial infarction (Hellstrom 1973). In Prinzmetal angina there seems to be little doubt that coronary constriction plays an important role (Oliva *et al* 1973). There is however much evidence that coronary spasm may play a crucial role in the genesis of many forms of coronary heart disease. A detailed description of such evidence is beyond the scope of this discussion. The evidence has however been summarized in three excellent reviews. Hellstrom (1973) has provided an extensive review suggesting

that coronary artery spasm might be important in ischemic heart disease. Similarly Boyd (1978) has comprehensively reviewed the evidence and concluded that actual spasm plays a major role in many patients with angina and with myocardial infarction. As a corollary he suggested that myocardial infarction is a potentially reversible event, possibly for several hours after the onset of symptoms. A recent review by Oliva (1978) also suggested an important role of vasospasm in the development of coronary heart disease. Oliva and Breckinridge (1977) have provided arteriographic evidence of coronary artery spasm in patients with acute myocardial infarction.

If coronary spasm can occur during myocardial ischemia, then the mechanism would be difficult to explain since myocardial metabolites released as a result of hypoxia are expected to contribute to a coronary dilatation. Most experimental evidence available has confirmed this view. On the other hand a recent report by Nayler and Seabra-Gomes (1976) using rat heart Langendorff preparations did demonstrate that perfusion with hypoxic medium for over 75 minutes leads to a coronary constriction after an initial dilatating period. It is possible that other workers may have failed to notice this effect because of the use of relatively short periods of hypoxia or of young animals.

In this report, unequivocal results are presented indicating that hypoxia can in fact lead to coronary artery spasm. The mechanism of this hypoxic constriction, although uncertain,does not seem to be mediated by endogenous release of catecholamines or angiotensin II since it was not influenced

by alpha-receptor blockade or by saralasin. PGs are suitable candidates for this phenomenon since they are released from the heart as a result of hypoxia and their is extensive evidence indicating that they cause coronary vasoconstriction. Both these aspects were revealed in the Literature Review. Furthermore, the finding that PGI2, the main cardiac PG causes coronary constriction also strengthens the theory for PG involvement. In the present experiment the PG outflow from the hearts were not measured but it was shown that two structurally different PG synthesis inhibitors, aspirin and indomethacin could prevent or reverse the hypoxic constriction. Moreover, chloroquine and procaine which have been shown to block some PG actions in blood vessels (Manku and Horrobin 1976a), nerves (Horrobin *et al* 1977a) and hearts (this report), and propranolol which blocks some PG actions on the rat stomach (Manku *et al* 1977a) also prevented the hypoxia-induced constriction.

It has been suggested that TXA2 when released from platelets may be important in coronary spasm (Ellis *et al* 1976). It was therefore expected that selective inhibition of TXA2 synthesis could prevent or reverse the hypoxic constriction. Imidazole, benzydamine and N-0164 which have been shown by others to inhibit TXA2 synthesis (Moncada *et al* 1977a, Needleman *et al* 1977a) and dipyridamole which has been found in this laboratory to have actions similar to TXA2 inhibition (Ally *et al* 1977) were used. All four agents had little effect in preventing the constrictor response which suggested that a product of PG synthesis other than TXA2 may be involved. More suprisingly, these agents when tested under normoxic

conditions all caused a coronary constriction at concentrations at which they have been reported to have selective effects on TXA2 synthesis. In all four cases the constriction could be prevented by indomethacin. Higher concentrations of some of these drugs have been reported to inhibit overall PG synthesis, possibly explaining the reversal of constriction as concentrations were increased.

These observations are consistent with the concept that the hypoxic constriction is mediated by a product of arachidonic acid metabolism other than TXA2. They do not prove this and confirmation will depend on accurate measurement of all the possible PGs and related substances. It was previously suggested that TXA2 exerts a negative feedback control over the arachidonic pathway and the removal of this control will lead to increased PG formation (Horrobin *et al* 1977b,1978). It is therefore possible that TXA2 could be indirectly involved in the hypoxic effect. The TXA2 synthetase system seems to have a binding site for imidazole and an imidazole group plays a critical role in the oxygenation and deoxygenation of heme-containing proteins (Chevion *et al* 1977). In preliminary experiments in this laboratory it was shown that oxy-hemoglobin but not carboxy-hemoglobin have actions consistent with the stimulation of TXA2 synthesis in a rat vascular preparation. Hemoglobin has been shown to stimulate the thromboxane synthetase system in platelets (Tai and Yuan 1977). If a TXA2 feedback system as previously described does exist then hypoxia could lead to increased PG formation secondary to a reduction in TXA2 synthesis. Whether such a mechanism actually exists is hypothetical. Inhibition of TXA2 synthesis has however been shown to

lead to increased production of other PGs particularly PGF2α (Nijkamp *et al* 1977) which is a potent coronary constrictor (Kulkarni *et al* 1976) and this report. It may be of significance therefore, that PGs of the F type are greatly elevated in the coronary sinus blood of patients suffering from angina (Berger *et al* 1977).

The lack of constriction observed in the presence of progesterone or in hearts from young animals is at present difficult to explain. The steroid action could possibly be due to selective antagonism of PG action although in preliminary experiments the author has failed to see such an effect with PGE2 or PGF2α . Possible interactions of progesterone with other PGs will be examined. The age effect observed in this study is also unclear. It may be due to increased PG availability in the myocardium in older animals resulting in coronary constriction. In man it has been observed that plasma PG levels increase with age (Siegler *et al* 1977).

A Possible Role of PGs in the Development of Coronary Heart Disease

In this report evidence was presented, using direct and indirect studies that PGs may have undesirable effects on the heart. Much of our current understanding of PGs is based on the actions of these agents unlikely to occur *in vivo* although results with low physiological concentrations have dissimilar effects. Coronary artery disease is associated with arrhythmias, pain and possibly coronary artery spasm. The latter point may be the predisposing factor to death from myocardial infarction since this can subsequently lead to lethal arrhythmias. If spasm in myocardial infarction does occur then it is possible that in the early phases acute

myocardial infarction may be a reversible event if the spasm can be prevented. This involves understanding the mechanism of the spasm.

From the data previously presented a mechanism for the generation of coronary artery disease can be suggested (figure 23). The process of infarct is initiated by physical narrowing of the coronary artery lumen possibly due to atherosclerotic plaque formation. The resulting hypoxia will initially lead to the release of vasoactive metabolites (eg. adenosine) resulting in coronary dilatation. If the blood supply reduction continues the dilatating process may pass over into the hypoxic constrictor phase. PG synthesis may be involved in this response since it can be prevented by either aspirin or indomethacin or by various PG antagonists. This increased PG production may be due to hypoxic reduction of TXA2 synthesis with a consequent removal of a feedback control as previously described. The subsubsequent constriction will lead to a vicious circle with further coronary constriction resulting in greater release of PGs. The PGs may also contribute to the generation of arrhythmias either by direct action or through cAMP/calcium interaction. As a result of their inotropic and chronotropic actions the PGs may also make the heart susceptible to arrhythmogenesis by other agents eg. catecholamines. The pain of angina and myocardial infarction may also be explained on the basis of increased PG synthesis.

These observations, particularly those related to hypoxic coronary artery constriction may explain a number of features of myocardial infarction. Hypoxia induced a coronary constriction only in mature rats which may partially explain age-related occurrence of coronary disease in humans. Smoking seems to be a major risk factor in myocardial infarction. In

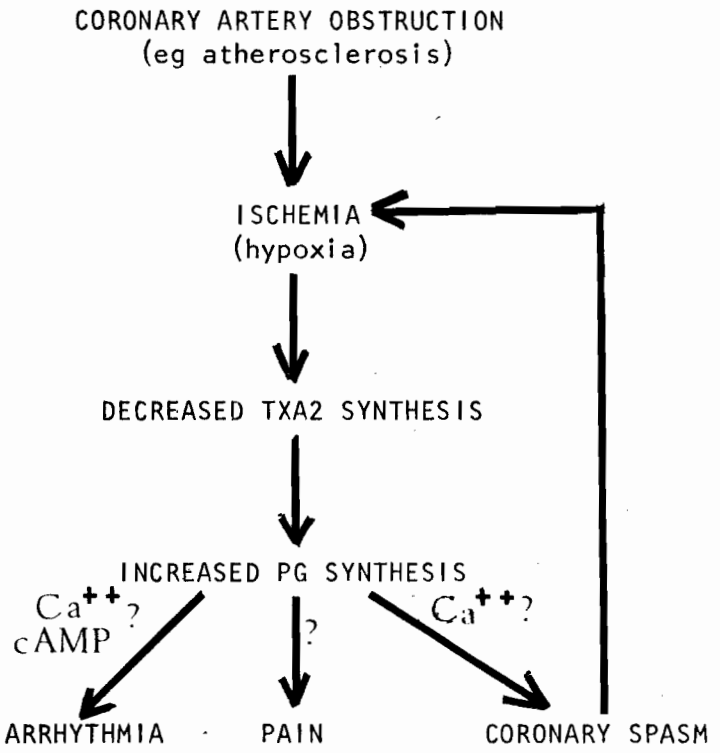

Fig 23. A POSSIBLE ROLE OF PROSTAGLANDINS IN THE DEVELOPMENT OF CORONARY ARTERY DISEASE

smokers up to 25% of hemoglobin may be in the form of carboxyhemoglobin (Smith *et al* 1978). This may result in a reduction of TXA2 synthesis and hence increased PG production leading to coronary spasm. It is also known that pre-menopausal women are relatively protected from coronary heart disease. Although much attention has been paid to the possibility that estrogens may be involved, there is extensive evidence that this hormone may actually increase the risk of coronary heart disease (Lancet 1977, Oliver 1977). The studies presented in this report suggest that progesterone may offer an explanation for this protection.

If a mechanism as described above does exist in humans, then these results could lead to a new approach in the therapy and prevention of coronary heart disease. For instance the combination of chloroquine and aspirin may effectively control platelet aggregation, coronary vasospasm and arrhythmia generation and may therefore prevent death from coronary artery disease. It may also hopefully lead to the development of new and more effective agents for the treatment of coronary artery disease.

SUMMARY AND CRITICAL EVALUATION

This study has presented diverse effects of PGs on various parameters of cardiac performance. These include dysrhythmic actions of various PGs at critically low concentrations. In addition the coronary and myocardial effects of various PGs at very low concentrations on the isolated rat heart is presented for the first time. A possible sexual variation in the cardiac response to PGs has also been introduced.

In the author's opinion one of the most interesting observations was that hypoxia, if prolonged can result in coronary spasm in the isolated rat heart after an initial dilatating response. The present results suggest that PGs or related substances may be involved in this phenomenon as various agents known to have effects on PG biosynthesis or action, reduced in varying degrees the coronary constrictor response to hypoxia. Indomethacin was the most effective in preventing or reversing the coronary spasm as well as preventing coronary constriction resulting from TXA2 synthesis inhibitors. It should however be stressed that indomethacin may have actions unrelated to PG biosynthesis (Northover 1967, 1977) and the possibility that the demonstrated effects are due to a non PG mechanism should not be ignored. In addition, aspirin, a known PG synthesis inhibitor was less potent in dilatating the coronary arteries or preventing the hypoxia-induced spasm again suggesting a lack of PG involvement. It should be noted however that the myocardial PG synthetase system is resistant to inhibition by aspirin (Limas and Cohn 1975) which may partially explain the weak aspirin effects. Also chloroquine, procaine and propranolol may also be influencing coronary resistance in a manner unrelated to PG action. Therefore these facts plus

the absence of any quantitative PG determinations may rule out the possibility of PG involvement in the coronary spasm process.

Another question that arises is, if molecular oxygen is required for the PG cyclo-oxygenase system how can hypoxia actually lead to an overproduction of PGs. Kalsner (1977) demonstrated that isolated bovine coronary arteries constrict under severe hypoxic conditions (pO2 9 mm Hg), a phenomenon associated with decreased PG production. One possible explanation for the differences between those results and the ones presented here is the different experimental design. The present thesis suggests that an increase in PG synthesis is due to TXA2 inhibition in the myocardium under hypoxia thus resulting in the overproduction of coronary constrictor PGs. Such a mechanism could not occur in isolated arteries since vascular smooth muscle is thought not to synthesize TXA2. Lands *et al* (1978) have demonstrated that the PG cyclo-oxygenase system in sheep vesicular glands is affected only at pO2's of less than 20 mm Hg. If this is true for the rat myocardium then the pO2 used in the present study (30-40 mm Hg) may not influence the cyclo-oxygenase system and thus may make the proposed TXA2 pathway feasible.

It may also be suggested that hypoxia-induced spasm may be due to oedema formation or tissue damage. This is difficult to ascertain from these experiments as histological studies were not performed and the heart weights before and after perfusion were not determined. However in the author's opinion these factors were not responsible for coronary changes since various drugs were able to reverse the effects. In addition it should be stated that isolated rat hearts perfused for up to 9 days under hypoxic conditions did not exhibit any significant histological alterations (Linask *et al* 1978).

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