Br. J. Pharmac. (1981), **74**, 553–562

STIMULATION OF PROSTACYCLIN RELEASE FROM THE EPICARDIUM OF ANAESTHETIZED DOGS

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1 The generation of prostanoids in the hearts of anaesthetized dogs was studied by irrigating *in situ* the epicardial surface with Krebs solution. Prostanoids were measured by direct bioassay on smooth muscles and by radioimmunoassay of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) and prostaglandin E_2 (PGE_2) in the epicardial irrigation fluid.

2 The epicardial irrigation fluid contained a prostacyclin-like substance, as indicated by the bioassay tissues, and immunoreactive 6-oxo-$PGF_{1\alpha}$; PGE_2-like materials were also detected. By both methods the output of the prostacyclin-like substance, which decreased with time of epicardial irrigation, was increased by manipulating the heart and by adding arachidonic acid (3 μg/ml), and decreased by adding indomethacin (1 μg/ml) to the irrigating fluid.

3 Bioassayed prostacyclin and immunoreactive 6-oxo-$PGF_{1\alpha}$ in the epicardial irrigation fluid increased by about 3–5 ng/ml during and after infusion of isoprenaline (0.1 $\mu g\,kg^{-1}\,min^{-1}$). The substance was not released by isoprenaline when indomethacin was added to the irrigating fluid, or when propranolol (0.5 mg/kg) was given intravenously.

4 Aortic constriction, bilateral carotid artery occlusion and intravenous angiotensin infusion all increased output of the prostacyclin-like substance into the epicardial irrigation fluid. The output was abolished by treating the heart with indomethacin (10 mg/kg intravenously or 1 μg/ml epicardially).

5 The prostacyclin-like substance was also released by all of the above stimuli after the parietal pericardium had been removed and replaced by a plastic sheet.

6 It is concluded that prostacyclin is continually released from tissues close to the epicardial surface and from the pericardium, and that prostacyclin generation increases when cardiac workload increases. Prostacyclin of epicardial or pericardial origin might therefore contribute to metabolic regulation of coronary blood flow.

Introduction

Changes in metabolic activity of the heart are normally accompanied by rapid adjustments of coronary blood flow. However, the factors which are responsible for maintaining the balance between changes in myocardial oxygen requirements and the volume of blood supplied to the myocardial cells are incompletely understood (Belloni, 1979). One concept of this mechanism is that a mediator or mediators generated locally in the myocardium acts as a dilator of arterioles, thereby increasing capillary blood flow. Prostacyclin is a potential candidate for this role since it is a potent coronary vasodilator (Dusting, Chapple, Hughes, Moncada & Vane, 1978a) and it is released from Langendorff-perfused hearts *in vitro* (De Deckere, Nugteren & Ten Hoor, 1977; Schror, Moncada, Ubatuba & Vane, 1978; Needleman, Bronson, Wyche, Sivakoff & Nicolaou, 1978).

Herman, Claeys, Moncada & Vane (1979) found that the pericardium and other serous membranes that are lined with mesothelium, spontaneously generate large amounts of prostacyclin *in vitro*, and also transform arachidonic acid into prostacyclin and lipoxygenase products. Moreover, prostacyclin elicits a rapid increase in coronary blood flow when applied to the epicardial surface of the dog's heart (Dusting *et al.*, 1978a). It therefore seemed possible that prostacyclin of pericardial origin might contribute to regulation of epicardial coronary blood flow. In this paper we show that prostacyclin is released from the epicardial surface of the heart, which includes a serous membrane covering of visceral pericardium. Significantly, biosynthesis of this substance increases in response to increased cardiac work.

0007-1188/81/110553-10 $01.00

Methods

Experimental animals

Mongrel dogs (15–35 kg) were anaesthetized with thiopentone (25–30 mg/kg intravenously) followed by α-chloralose (80–100 mg/kg intravenously) supplemented with further α-chloralose (20 mg/kg, intravenously) as required. Most dogs were artificially ventilated with air containing additional oxygen to maintain arterial blood pH and gases in the following ranges: pH 7.25 to 7.36, P_{O_2} 150 to 225, P_{CO_2} 25 to 37 mmHg. Three dogs, however, were initially ventilated without additional oxygen, and this maintained arterial P_{O_2} in the range 81 to 129 mmHg with pH and P_{CO_2} in the same ranges as ventilation with oxygen-enriched air. Arterial pressure was measured from a catheter in a femoral artery and heart rate was obtained from the pulse interval using a cardiotachometer coupler. All recordings were made on a Grass (Model 7) Polygraph. The chest was opened in the fifth intercostal space and the pericardium was opened into a cradle, care being taken to avoid leakage of blood into the pericardial sac. In three experiments the parietal pericardium was removed and the heart was supported in a plastic (poly-vinyl chloride) sheet. Drugs were infused into a femoral vein, or into the epicardial irrigating fluid (see below).

Epicardial irrigation and bioassay technique

The left ventricular epicardium was irrigated at 3 ml/min with warmed (37°C), oxygenated Krebs solution of the following (mM) composition: NaCl 118, KCl 4.6, $NaHCO_3$ 25, $CaCl_2$ 2.5, $MgSO_4$ 0.45, KH_2PO_4 1.0 and glucose 11.0. The fluid was immediately withdrawn from the pericardial cradle beneath the heart and used to superfuse a cascade of bioassay tissues (Figure 1). These tissues (a bovine coronary artery, a rat stomach strip and a rat colon) were continuously treated with a mixture of antagonist drugs (Gilmore, Vane & Wyllie, 1968) and in addition, (Sar[1]-Ile[8])-angiotensin II (0.03 μg/ml) and indomethacin (1 μg/ml) to increase the sensitivity and specificity of the tissues for detecting prostanoids (Dusting, Mullins & Nolan, 1981). Epicardial irrigation was continued throughout the experiments for up to 5 h.

Radioimmunoassay of prostanoids

Samples of the epicardial irrigation fluid were withdrawn from the pericardial cradle for direct radioimmunoassay of prostanoids. Assays were carried out, in duplicate, as described previously (Dusting *et al.*, 1981). Sensitivities of the assays, determined by the amount of standard per assay tube required to inhibit binding of the label to the antibody by 10%, were 5 pg for 6-oxo-$PGF_{1\alpha}$ and 25 pg for PGE_2. Cross reactivities with the PGE_2 antibody were: 6-oxo-$PGF_{1\alpha}$, 0.12%; $PGF_{2\alpha}$, 1%; TxB_2, 0.007% and arachidonic acid, 0.001%. Cross reactivities with the 6-oxo-$PGF_{1\alpha}$ antibody were: PGE_2, 0.5%; PGE_1, 0.25%; TxB_2, 0.0006%; $PGF_{2\alpha}$, 0.1%; arachidonic acid, 0.0002%. All samples were assayed by diluting the Krebs solution in Tris-gelatin buffer. Nonspecific effects of the Krebs buffer were ruled out by addition of standard prostanoid to Krebs buffer and dilution in Tris-gelatin buffer. The binding curve obtained was parallel to the curve with prostanoid in undiluted Tris-gelatin buffer. The differences in the concentrations of prostanoids determined by radioimmunoassays were evaluated for statistical significance by Student's *t* test.

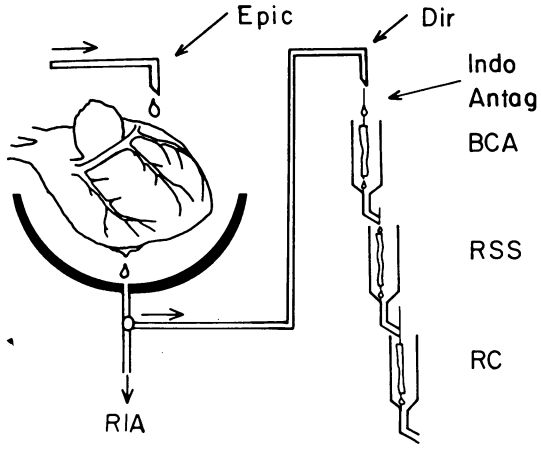

Figure 1 Diagrammatic representation of epicardial irrigation technique. The heart *in situ* was supported in a pericardial cradle, and Krebs solution which irrigated the epicardial surface was immediately withdrawn from a piece of soft tubing beneath the heart and passed over a cascade of bioassay tissues (bovine coronary artery, BCA; rat stomach strip, RSS; rat colon, RC) that were treated throughout with indomethacin (Indo, 1 μg/ml) and a mixture of antagonists (Antag, see text). The heart could be treated with substances added to the irrigating fluid epicardially (Epic) and substances could be given directly (Dir) over the tissues. Samples of irrigation fluid were also taken from time to time for subsequent radioimmunoassay (RIA).

Drugs

The following drugs were used: (Val[5])-angiotensin II (Hypertensin, Ciba), (Sar[1]-Ile[8])-angiotensin II (Bachem Inc.), arachidonic acid (Grade 1, Sigma), hyoscine hydrobromide (Buscopan, Boehringer-

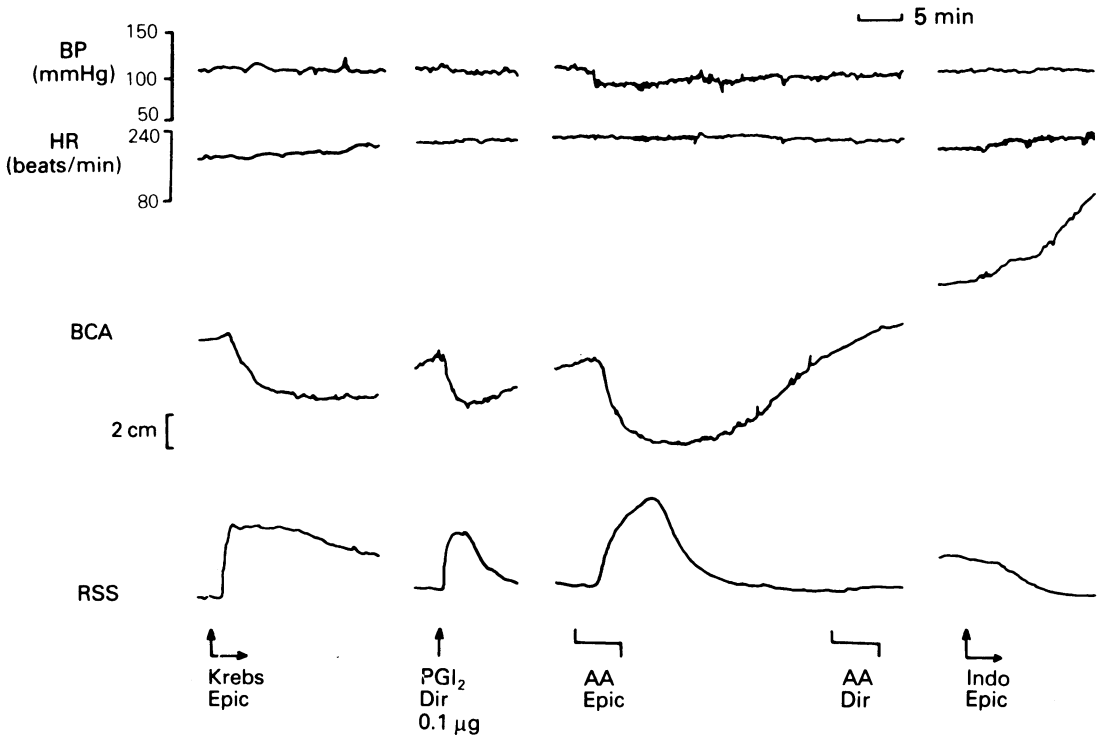

Figure 2 Effect of irrigating the heart with arachidonic acid (AA, 3 μg/ml) and indomethacin (Indo, 1 μg/ml), administered as indicated in Figure 1. Records, from the top down, are arterial blood pressure (BP), heart rate (HR) and tone of superfused strips of bovine coronary artery (BCA) and rat stomach (RSS). In the left hand panel the reactions of the bioassay tissues are shown when they were superfused with Krebs solution that had first irrigated the heart: superfusion with this solution was maintained for the rest of the experiment.

Ingelheim), indomethacin (Sigma), isoprenaline hydrochloride (Isuprel, Winthrop), mepyramine maleate (Anthisan, May and Baker), methysergide bimaleate (Sandoz), phenoxybenzamine hydrochloride (Dibenyline; Smith, Kline & French), propranolol hydrochloride (Sigma), PGE_2 (Upjohn), prostacyclin sodium salt (Upjohn), 6-oxo-$PGF_{1\alpha}$ (Upjohn). Solutions were prepared as previously described (Dusting, 1981; Dusting *et al.*, 1981).

Results

Initial epicardial irrigation with Krebs solution and manipulation of the heart

When the fluid superfusing the bioassay tissues was changed from fresh Krebs solution to one that had first irrigated the epicardium, the bioassay tissues responded in a manner consistent with the presence of prostacyclin in the superfusate, equivalent to 2–10 ng/ml (Figure 2). Epicardial irrigation with Krebs solution did not affect arterial pressure or heart rate. After 15–50 min of continuous epicardial irrigation the tone of the bioassay tissues superfused with this solution had returned to, or close to, their baselines, suggesting that the release of the prostacyclin-like substance from the epicardial surface had diminished. All these effects were observed whether the dogs were ventilated with or without additional oxygen. Handling the heart in the pericardium when the initial effects of irrigation had subsided caused a further release of the prostacyclin-like substance in amounts which depended on both the period of manipulation and the pressure applied to the epicardium through the pericardium.

Radioimmunoassays of diluted pericardial fluid revealed the presence of high concentrations of 6-oxo-$PGF_{1\alpha}$ with less PGE_2 (Table 1); the concentrations of both in the epicardial irrigation fluid decreased with time. After 20 min of irrigation, the concentration of 6-oxo-$PGF_{1\alpha}$ fell to a mean of 1.5 ng/ml (Table 1), but during the next 4–5 h the basal concentration declined more slowly to between 0.3 and 1.2 ng/ml in 8 dogs. These levels increased if the heart was handled between samples.

Table 1 Concentrations of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) and prostaglandin E_2 (PGE_2) in epicardial irrigation fluid as determined by direct radioimmunoassay

	Pericardial fluid	*During irrigation with Krebs solution* 5 min	10 min	20 min	n
6-oxo-$PGF_{1\alpha}$	7.2±2.6	5.6±2.1	3.4±1.0	1.5±0.3	6
PGE_2	4.0±3.7	0.6±0.4	0.3±0.2	0.2±0.1	4

Values are the mean±s.e. of concentrations (in ng/ml) determined in diluted pericardial fluid at various intervals after starting irrigation with Krebs solution. *n* is the number of dogs.

Epicardial superfusion with arachidonic acid and indomethacin

After the initial effects of epicardial irrigation had subsided, arachidonic acid (3 μg/ml) was added to the irrigation fluid for 5 min. In 3 out of 6 dogs this reduced arterial pressure by 5 to 18 mmHg and increased heart rate by 14 to 18 beats/min but in the other 3 dogs arterial pressure and heart rate did not change. In the 3 dogs which responded to arachidonic acid by hypotension and tachycardia, the bioassay tissues indicated, within 1–3 min, the output of a prostacyclin-like substance (Dusting, Moncada & Vane, 1978b; Dusting *et al.*, 1981), equivalent to more than 100 ng of standard prostacyclin (Figure 2). In the other 3 dogs, a prostacyclin-like substance was also released, which was equivalent to 100 ng or less of standard prostacyclin. However a higher concentration of arachidonic acid (10 μg/ml) produced more of the prostacyclin-like substance and greater cardiovascular effects in all dogs. The same concentration of arachidonic acid had no effect on the bioassay tissues when infused directly over them (Figure 2). When arachidonic acid (10 μg/ml) was added to the epicardial irrigating fluid in 2 dogs that had been previously treated with indomethacin (10 mg/kg, intravenously) there were no effects on blood pressure, heart rate, or on the bioassay tissues.

After 2–4 h of epicardial irrigation, indomethacin (1 μg/ml) was added to the irrigation fluid. In all

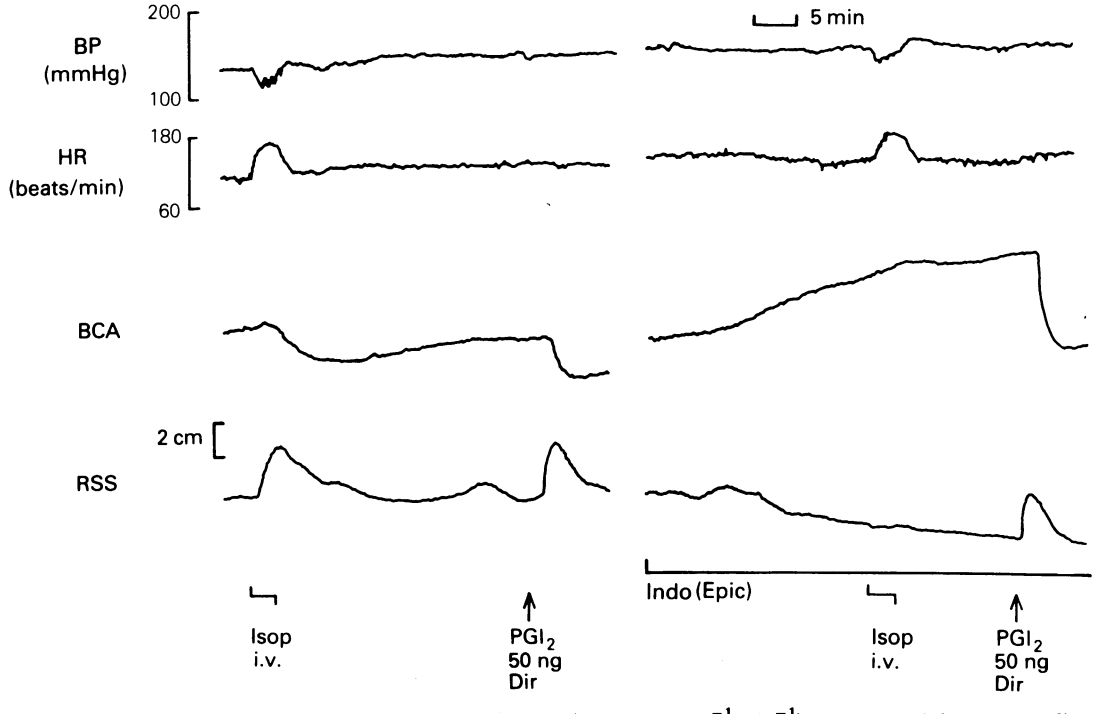

Figure 3 Effect of intravenous infusions of isoprenaline (Isop, 0.1 $\mu g\,kg^{-1}\,min^{-1}$) on output of the prostacyclin-like substance into the epicardial irrigation fluid. Records as in Figure 2. In the second panel indomethacin (Indo, 1 μg/ml) is superfused over the heart (Epic) instead of directly over the bioassay tissues.

Table 2 Effects of isoprenaline and angiotensin infusions, and bilateral carotid occlusion, on arterial pressure and heart rate

	Resting levels	*Changes induced by: Isoprenaline infusion*	*Angiotensin infusion*	*Carotid occlusion*
Mean arterial pressure (mmHg)	129±3 (22)	−18±2 (8)	+42±5 (8)	+49±4 (6)
Heart rate (beats/min)	138±7 (22)	+33±5 (7)	−18±4 (8)	+31±12 (6)

Values are mean ± s.e.; the number in parentheses is the number of dogs. All infusions were at the rate of 0.1 $\mu g\,kg^{-1}$ min^{-1}.

experiments this was followed within 5 to 10 min by an increase in tone of the bovine coronary artery and relaxation of the rat stomach strip (Figure 2) with little effect on the rat colon (not shown). Relaxation of the bovine artery induced by standard bolus doses of prostacyclin (25–100 ng) increased after treating the heart with indomethacin (Figure 3).

Effects of isoprenaline infusions

Bioassay In 8 dogs ventilated with oxygen-enriched air, infusion of isoprenaline (0.1 $\mu g\,kg^{-1}\,min^{-1}$ for 3–5 min) increased the heart rate by 33±5 beats/min and reduced the mean arterial pressure by 18±2 mmHg (Table 2). In all dogs this was followed within 2–3 min by release of a prostacyclin-like substance into the irrigating fluid, equivalent to 50–100 ng of prostacyclin (Figure 3) or steady-state concentrations of 2.5–5 ng/ml. In 5 dogs isoprenaline infusions were repeated, and in 4 of them the output of the prostacyclin-like substance increased to the same extent. In 3 of these dogs, the heart was subsequently irrigated with Krebs solution containing indomethacin (1 μg/ml). A third period of isoprenaline infusion, then had similar effects on arterial pressure and heart rate, but did not cause release of the prostacyclin-like substance (Figure 3). The fourth dog was treated with propranolol (0.5 mg/kg, intravenously), which did not change arterial pressure but reduced heart rate from 70 to 55 beats/min, and did not change the tone of the bioassay tissues. Subsequent infusion of isoprenaline in this dog had no cardiovascular effects and did not release the prostacyclin-like substance.

Radioimmunoassay Isoprenaline infusions (0.1 $\mu g\,kg^{-1}\,min^{-1}$ for 5 min) were repeated in a further 3 dogs, and samples of the epicardial irrigate were taken at 2 min intervals before and during the infusions for subsequent radioimmunoassay of 6-oxo-$PGF_{1\alpha}$ and PGE_2. The concentration of 6-oxo-$PGF_{1\alpha}$ in the irrigating fluid increased significantly ($P<0.05$) about 6 fold to 4 ng/ml, whereas the concentration of PGE_2 did not rise above 0.2 ng/ml during any period of infusion, and was not significant overall (Table 3).

Table 3 Radioimmunoassay of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) and prostaglandin E_2 (PGE_2) in epicardial irrigation fluid before and during intravenous infusions of isoprenaline

	Before	*During*	*Significance*
6-oxo-$PGF_{1\alpha}$	0.72±0.18	4.06±1.08	$P<0.05$
PGE_2	0.09±0.02	0.12±0.06	$P>0.05$

Values are the mean ± s.e. of concentrations (in ng/ml) determined during 5 periods of infusion in 3 dogs. Significance of the difference in mean is evaluated by Student's paired *t* test.

Effects of ventilation with oxygen-enriched air

Three dogs were initially ventilated with room air as described in Methods. Isoprenaline infusions (0.1 $\mu g\,kg^{-1}\,min^{-1}$ for 5 min) again caused reproducible cardiovascular effects and release of the prostacyclin-like substance into the epicardial irrigation fluid. Addition of oxygen to the ventilating air raised arterial P_{O_2} from 104±14 to 182±9 mmHg within 30 min, but did not change the tone of the bovine coronary artery or the rat stomach strip. Subsequent infusion of isoprenaline caused effects on blood pressure, heart rate and the bioassay tissues identical to the control responses, except in one of these dogs in which prostacyclin release during oxygen ventilation appeared to be of shorter duration than during air ventilation.

Prostacyclin release following increases of afterload

In dogs ventilated with oxygen-enriched air, afterload was increased by constricting the thoracic aorta with linen tape, by raising arterial pressure with intravenous infusions of angiotensin II, or increased

by eliciting the carotid sinus pressor reflex. Up to three of these procedures were carried out in each dog. The effects of the infusions and the pressor reflex on arterial pressure and heart rate are summarized in Table 2.

Aortic constriction In 3 dogs, constriction of the thoracic aorta to a degree which reduced femoral artery pulse pressure and reduced mean arterial pressure by up to 50 mmHg, caused release of the prostacyclin-like substance within 2 min, equivalent to more than 100 ng prostacyclin as indicated by bioassay. This did not occur following epicardial treatment of the heart with indomethacin (1 μg/ml).

Angiotensin infusion Intravenous infusion of angiotensin II, at 0.1 $\mu g\,kg^{-1}\,min^{-1}$ for 5 to 8 min, increased arterial pressure by 42 ± 5 mmHg (8 dogs) and caused bradycardia (Table 2). After 2–3 min of angiotensin infusion at 0.02–0.1 $\mu g\,kg^{-1}\,min^{-1}$ the bioassay tissues superfused with the epicardial irrigate indicated increased output of the prostacyclin-like substance, and the output still appeared to be increasing after 8 min of infusion. Infusion of angiotensin did not release the prostacyclin-like substance into the epicardial irrigation fluid in any of these dogs after treatment with indomethacin (10 mg/kg, intravenously or 1 μg/ml, epicardially).

In one further dog, prostacyclin was simultaneously assayed in arterial blood, as described by Dusting (1981). Intravenous infusion of angiotensin II (0.5 $\mu g\,kg^{-1}\,min^{-1}$) released a prostacyclin-like substance both into arterial blood and into the epicardial irrigating fluid (Figure 4). Indomethacin (1 μg/ml) was added to the epicardial irrigating fluid, and subsequent angiotensin infusion caused a similar relaxation of the coronary artery bathed in arterial blood, but had no effect on the tissues superfused with the epicardial irrigate.

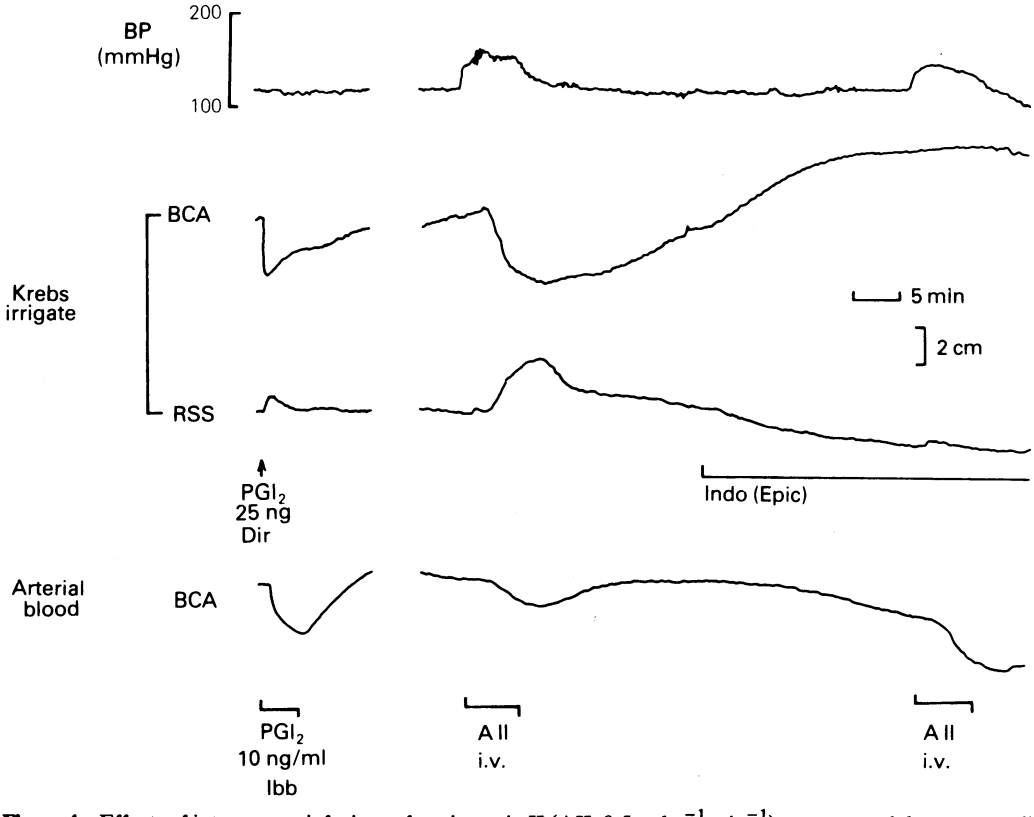

Figure 4 Effects of intravenous infusions of angiotensin II (AII, 0.5 $\mu g\,kg^{-1}\,min^{-1}$) on output of the prostacyclin-like substance into the epicardial irrigation fluid (Krebs irrigate) and into arterial blood. The upper three records are as in Figure 2, and the last is a bovine coronary artery (BCA) bathed in arterial blood from the heparinized dog (Dusting, 1981). Prostacyclin (PGI_2) is administered directly over the tissues (Dir and Ibb) as indicated beneath the records. Epicardial irrigation with indomethacin (Indo) abolishes output of the prostacyclin-like substance from the heart, but does not reduce the release of the substance into arterial blood.

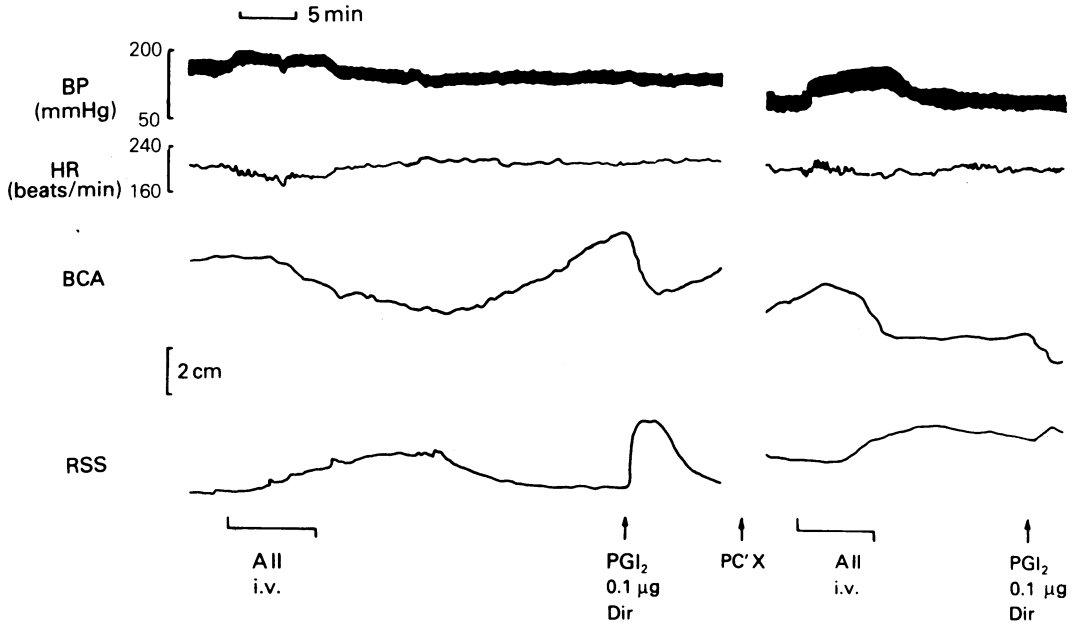

Figure 5 Release of the prostacyclin-like substance by angiotensin II (AII, 0.1 $\mu g\,kg^{-1}\,min^{-1}$) after removing the parietal pericardium during the interval marked PC'X. Records as in Figure 2. Note that the baselines of the bioassay tissues are altered by pericardiectomy.

Carotid occlusion When arterial pressure and heart rate were raised reflexly in 6 dogs (Table 2) by occlusion of both common carotid arteries for 5 min, this was also followed by increased output of the prostacyclin-like substance into the epicardial irrigation fluid, equivalent to 50–100 ng of standard prostacyclin.

Effects of pericardiectomy

After 2–3 h of epicardial irrigation in 3 dogs, the parietal pericardium was removed and the heart was supported in a sheet of plastic sutured to the thoracic walls, and irrigation was resumed after about 10 min. When this epicardial irrigation fluid replaced the fresh Krebs solution which had superfused the bioassay tissues during the interval, there was an immediate relaxation of the bovine coronary artery and contraction of the rat stomach strip and the rat colon. These effects were much greater and more prolonged than the reactions of the bioassay tissues which occurred initially on epicardial irrigation. The bioassay tissues had reached relatively stable baselines after a further 50–60 min, but had not reached control levels (Figure 5). Epicardial irrigation with arachidonic acid (3 $\mu g/ml$), intravenous infusion of isoprenaline (0.1 $\mu g\,kg^{-1}\,min^{-1}$), aortic constriction, bilateral carotid occlusion, and infusion of angiotensin (0.1 $\mu g\,kg^{-1}\,min^{-1}$) all caused increased output of the prostacyclin-like substance from the epicardial surface. The increase in output of the prostacyclin-like substance with angiotensin was greater after parietal pericardiectomy than before in one dog (Figure 5) but in two others the output in response to angiotensin, isoprenaline, and carotid occlusion was less than occurred in response to these stimuli when the parietal pericardium was present.

Release of prostacyclin from the vascular compartment into the epicardial irrigation fluid

Prostacyclin, infused intravenously at 30, 100 and 300 $ng\,kg^{-1}\,min^{-1}$, decreased arterial pressure by up to 60 mmHg and increased heart rate up to 80 beats/min as observed previously (Dusting *et al.*, 1978a). However, even at the highest infusion rate, prostacyclin could just be detected in the epicardial irrigation fluid by the bioassay tissues, in amounts equivalent to less than 1–2 ng/ml.

Discussion

It has been known for some years that the heart has the capacity to produce prostaglandins (see review by Needleman & Kaley, 1978). Prostaglandin generation has been measured in a microsomal fraction of dog heart (Limas & Cohn, 1973), in coronary sinus

blood of dogs' hearts, and in the effluent of Langendorff-perfused hearts of the rabbit, guinea-pig and rat (Needleman & Kaley, 1978). Needleman and his colleagues concluded that the site of cardiac prostaglandin biosynthesis is the coronary vasculature (Sivakoff, Pure, Hsueh & Needleman, 1979), in which prostacyclin is the predominant metabolite of arachidonic acid (Dusting, Moncada & Vane, 1977; DeDeckere *et al.*, 1977; Schror *et al.*, 1978; Needleman *et al.*, 1978). However, it has recently been shown that isolated cardiac myocytes and mesenchymal cells (probably fibroblasts) may also generate prostanoids (Vahouny, Bolton, Chanderbhan, Bryant, Bailey & Weglicki, 1979; Ahumada, Sobel & Needleman, 1980). We have now demonstrated that prostacyclin is released in large amounts from the epicardial surface of the heart *in situ*, and that arachidonic acid is rapidly converted into prostacyclin when it is superfused over this surface.

The parietal pericardium *in vitro* is a very active producer of prostacyclin (Herman *et al.*, 1979), a property it has in common with other mesothelium-rich loose connective tissues such as pleura and omentum. Prostacyclin measured in the epicardial irrigation fluid in the present experiments is derived at least partly from the epicardial surface, for release continued after the parietal pericardium had been removed. It is likely that the visceral membrane of the pericardium, which is also lined with mesothelium, is a major source of this substance because the myocardium is a poor producer of prostanoids (Sivakoff *et al.*, 1979; our unpublished observations). Furthermore, very large concentrations of prostacyclin in the coronary vasculature, which had marked effects on coronary vascular resistance and arterial pressure (Dusting *et al.*, 1978a), were necessary to achieve release of 1–2 ng/ml into the epicardial irrigation fluid. Higher concentrations of endogenous prostacyclin were reached in the epicardial fluid with moderate elevations of cardiac work. Therefore, prostacyclin measured here appears unlikely to arise from the coronary blood vessels, although these *in vivo* experiments cannot entirely rule out a contribution from extra-membranous sources in the epicardium.

Prostacyclin is also released from the epicardial surface when heart rate and force of contraction are stimulated by isoprenaline, and when afterload is increased. Although not assessed in these experiments, all of these stimuli for prostacyclin release might be expected to result in increased cardiac work and oxygen consumption. The effect of hypoxia on prostacyclin generation has not been studied in these experiments, but elevation of arterial P_{O_2} to around 180 mmHg did not greatly alter the increase in prostacyclin output induced by increasing cardiac work. An increase in cardiac work by all of the methods used is associated with increased stretching of the epicardium and pericardium. These mechanical disturbances may be the common stimulus for increased prostacyclin output, for manipulation of the heart in the pericardial sac and the disturbance caused by suspending the heart in a plastic sheet also increased the release of prostacyclin. Certainly, stretching of isolated blood vessels and cutting vessel rings increases the output of prostacyclin from these tissues (Moncada & Vane, 1977; Ten Hoor & Quadt, 1979) and the manipulation of the heart involved in setting up these experiments is probably the cause of the high initial output of prostacyclin which diminishes as the experiment progresses.

Since prostacyclin induces coronary vasodilatation when applied to the epicardial surface (Dusting *et al.*, 1978a) it is possible that prostacyclin of epicardial or pericardial origin might contribute to the regulation of coronary blood flow. However, on the whole, the evidence is against prostaglandins making a major contribution to coronary autoregulation following changes in perfusion pressure, to hypoxic coronary vasodilatation, or to reactive hyperaemia (with the possible exception of the increases in blood flow following long periods of coronary occlusion) (Needleman & Kaley, 1978; Belloni, 1979). The concentrations of prostacyclin achieved in the epicardial fluid in the present experiments, high as they are, may not be sufficient to influence total coronary vascular resistance (Dusting *et al.*, 1978a).

Prostacyclin released from the epicardium or pericardium might have other roles. Aiken, Gorman & Shebuski (1979) have shown that prostacyclin, applied epicardially to the dog's heart, can prevent or reverse platelet aggregation at sites of obstruction in the major coronary arteries. The endothelial vascular surface of the coronary circulation may not always be a sufficient source of prostacyclin to protect against thrombosis, particularly when the output of this substance is diminished by atherosclerotic vascular disease (Dembinska-Kiec, Gryglewska, Zmuda & Gryglewski, 1977; Sinzinger, Feigel, Silberbauer, 1979). During sympatho-adrenal activation, circulating adrenaline may activate platelets and make them more prone to aggregating stimuli (Johnson, Rao & White, 1980). Under these conditions increased release of prostacyclin from pericardial tissues might be an important supplement to that reaching the coronary circulation from other sources. However, the proposal of such a role for pericardial prostacyclin first requires demonstration of an antithrombotic effect during increased cardiac work.

It is likely that a functional role of pericardial prostacyclin is to prevent platelet aggregation and clotting of blood (Vermylen, 1978) in the pericardial cavity should haemorrhage occur (Herman *et al.*, 1979). Similarly, prostacyclin of pleural origin is

probably responsible for the well known observation that extravasated blood in the thorax does not defibrinate or clot.

Finally, prostaglandins have effects on cardiac reflexes. Staszewska-Barczak, Ferreira & Vane (1976) originally proposed that prostaglandins which are released from the heart during ischaemia (Needleman & Kaley, 1978) have a role in eliciting nociceptive chemo-reflexes after myocardial infarction. Bradykinin, a potent algesic substance, is also formed in the ischaemic myocardium (Kimura, Hashimoto, Furukawa & Hayakawa, 1973) and this substance, acting in concert with prostaglandins, may be the natural stimulus for excitation of chemosensitive nerve endings in the left ventricle and pericardium which signal the pain of myocardial ischaemia (Baker, Coleridge, Coleridge & Nerdrum, 1980). The chemoreflex elicited by bradykinin has both afferent and efferent arcs within the sympathetic division of the autonomic nervous system, and is characterized by a rise in blood pressure and heart rate (Staszewska-Barczak *et al.*, 1976; Staszewska-Barczak & Dusting, 1977). The reflex is potentiated by prostacyclin applied epicardially (Staszewska-Barczak & Dusting, 1981), and prostacyclin also causes short-lasting hyperalgesia (Ferreira, Nakamura & De Abreu Castro, 1978). Therefore, increased generation of prostacyclin by pericardial tissues during exercise may contribute to the pain and reflex events associated with the onset of angina pectoris. In contrast, prostacyclin reaching the myocardium from the general circulation or coronary blood vessels may act to reduce cardiac work and oxygen consumption, for circulating prostacyclin, in low concentrations, sensitizes vagal nerve endings which subserve depressor chemoreflexes (Staszewska-Barczak & Dusting, 1981). Moreover, bradykinin injected into the coronary circulation also elicits a vagally-mediated depressor chemoreflex (Neto, Brasil & Antonio, 1974; Staszewska-Barczak *et al.*, 1976). Therefore, whenever the oxygen demand of the myocardium exceeds the capacity of the coronary circulation to supply it, the resulting formation of bradykinin and prostacyclin together might simultaneously initiate two events. The first is to signal the pain of the ensuing anginal attack as a warning that the inappropriate stimulus to the heart should be withdrawn. The second consequence of bradykinin and prostacyclin formation in the vicinity of the vagal chemosensitive nerve endings would be to lower reflexly the arterial pressure and heart rate, thereby reducing oxygen demand. This may be the basis of the ill-defined 'protective' effects of prostacyclin demonstrated in acute myocardial ischaemia in cats (Ogletree, Lefer, Smith & Nicolaou, 1979). However, further studies are needed to clarify the significance of endogenous prostacyclin in myocardial ischaemia.

G.J.D. is a Senior Research Fellow of the National Heart Foundation (NHF) of Australia. The work was supported by grants from the NHF and NH and MRC of Australia. We are grateful to Wendy Davies and Tracey Drysdale for skilful technical assistance. Prostacyclin was a gift from J. Pike (Upjohn, Kalamazoo), and the antibodies for PGE_2 and 6-oxo-$PGF_{1\alpha}$ radioimmunoassays were generously donated to T.J. Martin by L. Levine and L. Best, respectively.

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(Received February 20, 1981.
Revised May 26, 1981.)