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THE EFFECTS OF PROSTAGLANDINS E_2, $F_{2\alpha}$, PROSTACYCLIN, FLURBIPROFEN AND ASPIRIN ON ARRHYTHMIAS RESULTING FROM CORONARY ARTERY LIGATION IN ANAESTHETIZED RATS

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1 Various prostaglandins and inhibitors of prostaglandin synthesis were administered prior to acute coronary artery ligation in anaesthetized rats and their effects were assessed on the number and severity of the resulting early arrhythmias (ventricular ectopic activity; incidence and duration of ventricular tachycardia and of ventricular fibrillation).

2 Prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$ and prostacyclin all showed antiarrhythmic activity; in contrast flurbiprofen increased the incidence of ventricular fibrillation and mortality.

3 Both the number of ventricular ectopic beats and the incidence of ventricular fibrillation were reduced by aspirin.

4 The results suggest that the release of endogenous PGE_2, $PGF_{2\alpha}$ and prostacyclin could reduce early post-infarction ventricular arrhythmias whilst the protective effect of aspirin in this model adds further support for the hypothesis that thromboxane release is involved in the genesis of these arrhythmias.

Introduction

Since prostaglandin E_1 (PGE_1) was first shown to be antiarrhythmic by Zijlstra, Brunsting, Ten Hoor & Vergroesen in 1972, various other prostaglandins have been found to possess similar activity in a variety of animal models. For example, in rats $PGF_{2\alpha}$ has been shown to be effective against calcium chloride-induced arrhythmias (Förster, Mest & Mentz, 1973) and both PGE_2 and $PGF_{2\alpha}$ are effective against aconitine-induced arrhythmias (Förster, 1976). These prostaglandins also protect against ouabain-induced arrhythmias in cats (Somberg, Bounous, Cagin, Anagnostopoulos & Levitt, 1977) and barium chloride-induced arrhythmias in rabbits (Förster, 1976). Thus prostaglandins appear to be effective against arrhythmias induced chemically and this has led to the suggestion (Förster, 1976) that they may act as 'endogenous antiarrhythmic agents'.

However, there is some controversy regarding the effects of prostaglandins on arrhythmias resulting from coronary artery obstruction. In rats, exogenous PGE_2 reduces these arrhythmias whereas prostacyclin intensifies them (Au, Collins, Harvie & Walker, 1979). Similarly, in cats, prostacyclin exacerbates post-infarction arrhythmias (Dix, Kelliher, Jurkeiwicz & Lawrence, 1979) whereas in dogs it is apparently protective (Au *et al.*, 1979), as are PGE_2 and $PGF_{1\alpha}$ (Harvie, Collins, Miyagishima & Walker, 1978).

If prostaglandins have a functional role as endogenous antiarrhythmic agents then they should be effective against those arrhythmias that result from coronary artery ligation since this model probably bears a closer resemblance to the clinical situation (e.g. of infarction) than the other (chemical) models mentioned above. Furthermore, little is known about the effects of inhibitors of prostaglandin synthesis on these arrhythmias; if prostaglandins do indeed have a role as endogenous antiarrythmic agents then these drugs would be expected to exacerbate post-infarction arrhythmias. With this possibility in mind we have investigated the effects of PGE_2, $PGF_{2\alpha}$, prostacyclin, flurbiprofen and aspirin on the arrhythmias resulting from acute coronary artery ligation in anaesthetized rats.

Methods

Rats were prepared for coronary artery ligation according to the method of Clark, Foreman, Kane, McDonald & Parratt (1980). Male Sprague-Dawley rats (200–450 g) were anaesthetized with sodium pentobarbitone (60 mg/kg i.p.), additional doses of 3 mg/kg being administered intravenously if required. The electrocardiogram (ECG) was recorded from standard limb leads and a lead I ECG was continuously monitored and displayed on an oscilloscope (Racal Instruments Type 9383) along with arterial blood pressure, measured with an Elema-Schönander capacitance transducer (EMT 35,

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0–300 mm Hg) via a cannula inserted in the left common carotid artery. At appropriate times these parameters were recorded on an Elema-Schönander ink-jet recorder (Mingograf 81). A femoral vein was cannulated for drug administration.

A left thoracotomy was performed to expose the heart and the animals were respired with room air (2 ml/100 g) at a rate of 54 strokes/min. After pericardectomy a 6/0 braided silk suture attached to a 10 mm micro-point reverse cutting needle (Mersilk W812, Ethicon) was placed under the left coronary artery close to its origin and a 10 min recovery period allowed. If arrhythmias occurred at this stage the experiment was discontinued. Drugs, or the appropriate vehicles, were administered and 10 to 15 min later the left coronary artery was ligated and the resulting arrhythmias were quantified as previously described (Clark *et al.*, 1980).

Drugs

PGE_2 and $PGF_{2\alpha}$ (Upjohn Co.) were dissolved in ethanol to give stock solutions of 1 or 10 mg/ml and these were stored at 4°C. Immediately prior to administration they were diluted with 0.9% w/v NaCl solution and infused intravenously; the dilution was always sufficient to ensure that the final ethanol concentration was less than 10%.

Solid prostacyclin (generously provided by Upjohn, Kalamazoo, Michigan) was stored (desiccated) at 4°C. Each day a fresh stock solution in ethanol (1 mg/ml) was prepared. Immediately before use this was diluted with ethanol and infused into the animal along with a simultaneous saline infusion. The rate of saline infusion was at least 10 times that of the prostacyclin in ethanol and the two infusions were mixed (via a three-way stopcock) just before entering the animal. Thus the ethanol concentration was never more than 10% and the prostacyclin was only in aqueous solution for a short time (less than 1 min) before entering the circulation. Infusions were started 10 min before ligation and maintained until 30 min after ligation.

Flurbiprofen (Boots Co., Ltd) and aspirin (BDH), dissolved in saline or sodium acetate solution (0.15 M) respectively, were administered as an intravenous bolus 15 min before ligation.

Statistical analysis

All values quoted are the mean ± s.e.mean of n results. The statistical significance was determined using Student's t test for unpaired data. A Chi-squared test was used to compare the incidence of events. All results were considered to be significant at a probability level of $P < 0.05$.

Results

The electrocardiographic effects of coronary artery ligation in anaesthetized rats

Acute ligation of the left coronary artery in control rats resulted in the occurrence of arrhythmias which varied in nature from single ventricular ectopic beats to ventricular fibrillation and included periods of bigeminal rhythm and ventricular tachycardia. The onset of arrhythmias was generally 4 to 6 min post-ligation and these arrhythmias continued until 15 to 20 min after ligation. A unique feature of the rat coronary artery ligation model is that ventricular

Table 1 The effects of prostaglandins E_2 (PGE_2), $PGF_{2\alpha}$ and prostacyclin on the number and severity of the arrhythmias resulting from acute coronary artery ligation in the rat

	Ventricular ectopic beats	*Ventricular tachycardia*		*Ventricular fibrillation*		*Survival rate*
		duration (s)	*incidence* (%)	*duration* (s)	*incidence* (%)	
Control	1018 ± 148	43 ± 13	(100)	39 ± 19	(75)	7/8
PGE_2 1 $\mu g\,kg^{-1}\,min^{-1}$	1067 ± 332	82 ± 75	(88)	12 ± 9	(75)	6/8
Control	946 ± 143	37 ± 13	(100)	45 ± 13	(88)	6/8
PGE_2 10 $\mu g\,kg^{-1}\,min^{-1}$	320 ± 223*	14 ± 16	(50)	62 ± 69	(38)	7/8
Control	1051 ± 301	61 ± 26	(100)	49 ± 30	(62)	7/8
$PGF_{2\alpha}$ 10 $\mu g\,kg^{-1}\,min^{-1}$	1146 ± 562	129 ± 177	(75)	22	(25)	7/8
Control	992 ± 202	60 ± 19	(100)	9 ± 8	(25)	8/8
Prostacyclin 0.1 $\mu g\,kg^{-1}\,min^{-1}$	224 ± 69*	22 ± 10	(50)	—	(0)	8/8
Control	1006 ± 228	72 ± 23	(88)	14 ± 6	(38)	8/8
Prostacyclin 1 $\mu g\,kg^{-1}\,min^{-1}$	480 ± 201	45 ± 60	(75)	—	(0)	8/8

Mean ± s.e.mean, $n = 8$
*Statistically significant, $P < 0.05$, independent t test.

fibrillation is frequently not terminal, with spontaneous reversion to sinus rhythm occurring in many animals (Table 1). In each surviving animal the total number of ventricular ectopic beats, including those occurring as tachycardia, was counted in 1 min intervals for the first 30 min after ligation. The duration and incidence of ventricular tachycardia and ventricular fibrillation were also measured.

The effects of prostaglandins E_2 and $F_{2\alpha}$ on early post-ligation arrhythmias

An intravenous infusion of PGE_2 ($1\,\mu g\,kg^{-1}\,min^{-1}$) had no significant effect on the number of ventricular ectopic beats, the incidence of tachycardia or fibrillation or on the survival rate. However, when the concentration was increased to $10\,\mu g\,kg^{-1}\,min^{-1}$, a significant reduction in the number of ventricular ectopic beats and in the incidence of ventricular tachycardia and fibrillation resulted (Table 1). These findings indicate that in this model PGE_2 exerted an antiarrhythmic effect by reducing both the number and severity of post-infarction arrhythmias. $PGF_{2\alpha}$ ($10\,\mu g\,kg^{-1}\,min^{-1}$) had no significant effect on the total number of arrhythmias but did reduce the incidence of both ventricular tachycardia and ventricular fibrillation (Table 1). At this dose level therefore $PGF_{2\alpha}$ decreased the severity of the arrhythmias without altering the number of single ectopic beats. $PGF_{2\alpha}$ had no significant effect either on heart rate (479 ± 15 to 459 ± 13 beats/min) or on arterial blood pressure ($112 \pm 14/84 \pm 8$ mm Hg to $120 \pm 8/85 \pm 7$ mm Hg). At the higher infusion rate of $10\,\mu g\,kg^{-1}\,min^{-1}$, PGE_2 slightly decreased blood pressure, although not significantly, and reduced heart rate (from 435 ± 13 to 411 ± 13 beats/min: $P < 0.05$).

The effect of prostacyclin on early post-ligation arrhythmias

Intravenous infusions of prostacyclin reduced post-ligation arrhythmias and completely prevented ventricular fibrillation (Table 1). In contrast to the results obtained with PGE_2, there was a greater reduction in the number of ventricular ectopic beats with the lower dose of prostacyclin ($0.1\,\mu g\,kg^{-1}\,min^{-1}$); similarly the reduction in ventricular tachycardia was greater with this dose (Table 1). The higher dose of prostacyclin ($1\,\mu g\,kg^{-1}\,min^{-1}$) reduced arterial blood pressure (from $107 \pm 8/70 \pm 12$ to $89 \pm 8/57 \pm 9$ mm Hg) although there was considerable individual variation in the magnitude of the response. Heart rate was unchanged. It is possible that a reduction in arterial blood pressure, by decreasing perfusion within the acutely ischaemic myocardium, counteracts the antiarrhythmic effect of prostacyclin.

The effect of inhibitors of prostaglandin synthesis on early post-ligation arrhythmias

Fluribiprofen is a potent inhibitor of prostaglandin formation. Since it acts at the level of cyclo-oxygenase (Fitzpatrick & Wynalda, 1976) it prevents the synthesis of all the above-mentioned prostaglandins. When administered 15 min before ligation, flurbiprofen caused a dose-related exacerbation of the cardiac rhythm disturbances resulting from coronary artery ligation. At a dose of 0.1 mg/kg the number of ventricular ectopic beats was not significantly different from control and the survival rate was similar. However, increasing the dose to 0.3 mg/kg and 1 mg/kg caused a marked increase in the incidence of ventricular fibrillation to the extent that the survival rate decreased to 20% with the 0.3 mg/kg dose; none of the animals pretreated with 1 mg/kg flurbiprofen survived for more than 10 min after coronary artery ligation. All the deaths resulted from ventricular fibrillation (Table 2).

Flurbiprofen significantly reduced heart rate (e.g. from 486 ± 20 to 444 ± 14 beats/min with 0.1 mg/kg; $P < 0.01$) but had no effect on arterial blood pressure. The administration of this drug caused no alterations in the electrocardiogram and did not itself precipitate arrhythmias. Thus it appears that flurbiprofen alone is not arrhythmogenic but that it greatly increases the severity of the arrhythmias resulting from coronary artery ligation in this anaesthetized rat model.

Aspirin also inhibits the formation of prostaglandins but may have differential effects on prostacyclin and thromboxane formation. The administration of aspirin (30 and 100 mg/kg) resulted in a dose-related *decrease* in the number of ventricular ectopic beats (Table 2) and the incidence of ventricular fibrillation was also reduced. At the higher dose there was no ventricular fibrillation in any animal and all the animals survived. Apart from a decrease in heart rate with the higher dose (from 459 ± 19 to 434 ± 12 beats/min; $P < 0.05$) aspirin did not cause any significant changes either in heart rate or blood pressure.

Discussion

These results demonstrate that PGE_2, $PGF_{2\alpha}$ and prostacyclin possess antiarrhythmic activity in the very early stages of experimental myocardial infarction. Prostacyclin has been shown to be the major metabolite of arachidonic acid released from the rat isolated heart under conditions of ischaemia or hypoxia (De Deckere, Nugteren & Ten Hoor, 1977) and so this antiarrhythmic action is probably of more importance than that of either PGE_2 or $PGF_{2\alpha}$. The

Table 2 The effects of flurbiprofen and aspirin on the number and the severity of the arrhythmias resulting from acute coronary artery ligation in the rat

	Ventricular ectopic beats	*Ventricular tachycardia* duration (s)	incidence (%)	*Ventricular fibrillation* duration (s)	incidence (%)	*Survival rate*
Control	1070 ± 356	65 ± 30	(100)	23 ± 19	(62)	6/8
Flurbiprofen 0.1 mg/kg	1005 ± 175	51 ± 47	(89)	35 ± 30	(38)	8/9
0.3 mg/kg	1093	60	(100)		(100)	1/5
1 mg/kg			(100)		(100)	0/5†
Control	991 ± 320	59 ± 26	(100)	44 ± 42	(50)	7/8
Aspirin 30 mg/kg	670 ± 323	48 ± 77	(62)	28 ± 19	(38)	7/8
100 mg/kg	250 ± 50*	11 ± 3	(88)		(0)	8/8

Mean ± s.e.mean.
*Statistically significant, $P < 0.05$, independent t test; †statistically significant $P < 0.05$, chi-squared test.

results with prostacyclin are not entirely straightforward since a greater antiarrhythmic effect was seen with the lower infusion rate. This may be due to the fact that the higher concentration of prostacyclin had a more pronounced hypotensive effect. A reduction in blood pressure early in myocardial ischaemia might, by further reducing blood flow to the ischaemic region, induce ventricular arrhythmias.

Au *et al.* (1979) found that prostacyclin, in doses of 0.5 to 4.0 $\mu g\,kg^{-1}\,min^{-1}$, *increased* the number of arrhythmias resulting from coronary artery ligation in the rat and also increased the incidence of ventricular fibrillation. They did not, however, attribute these effects to prostacyclin-induced systemic hypotension. In the present study, concentrations greater than 1 $\mu g\,kg^{-1}\,min^{-1}$ could not be examined since in some animals they precipitated excessive decreases in mean arterial blood pressure, sometimes to below 40 mm Hg. Dix *et al.* (1979) have shown that in cats, prostacyclin can have both antiarrhythmic and arrhythmogenic activity, depending on dosage. In dogs prostacyclin in a dose of 0.5 $\mu g\,kg^{-1}\,min^{-1}$, has been shown to be antiarrhythmic (Au *et al.*, 1979).

In those studies in which prostacyclin has been shown to exacerbate early post-infarction arrhythmias, the concentrations have been at the upper end of the range. It is difficult to estimate the circulating levels of prostacyclin resulting from intravenous infusions, but at a rate of 1 $\mu g\,kg^{-1}\,min^{-1}$ levels as high as 10 to 50 ng/ml blood may be expected. These levels are certainly far in excess of those that can be predicted from our own measurements of plasma 6-keto $PGF_{1\alpha}$ (the major metabolite of prostacyclin) in greyhounds. We have found that control values in coronary venous blood ranged from 200 to 800 pg/ml and rose to 1 to 2 ng/ml after acute myocardial ischaemia (Coker, Ledingham, Parratt & Zeitlin, 1981a). Thus it is possible that the arrhythmogenic effect seen with high prostacyclin concentrations results from the administration of pharmacological concentrations of prostacyclin which exceed those that could result from endogenous synthesis.

The results obtained with the prostaglandin synthesis inhibitor, flurbiprofen, complement the results obtained with PGE_2, $PGF_{2\alpha}$ and prostacyclin and support the suggestion that these latter substances may perhaps act as endogenous antiarrhythmic agents. Flurbiprofen is a potent inhibitor of the cyclo-oxygenase enzyme which is responsible for the formation of the prostaglandin endoperoxides which are then converted either to the primary prostaglandins such as E_2 and $F_{2\alpha}$, or to prostacyclin, or to the thromboxanes. Since flurbiprofen thus inhibits the formation of all prostaglandins, the results obtained with this drug do not give any indication of the relative importance of the various prostaglandins as endogenous antiarrhythmic agents.

Aspirin has been shown to be antiarrhythmic in dogs subjected to non-thrombotic coronary occlusion (Moschos, Haider, De La Cruz, Lyons & Regan, 1978; Reiser, Gough & Anderson, 1980; Coker, Ledingham, Parratt & Zeitlin, 1981b). These results are in agreement with those described here. Since various prostaglandins have been shown to be antiarrhythmic and drugs such as flurbiprofen potentiate the arrhythmias resulting from coronary artery ligation, it appears that aspirin differs from other cyclo-oxygenase inhibitors. It has been shown that aspirin can selectively inhibit platelet cyclo-oxygenase (Smith & Willis, 1971; Korbut & Moncada, 1978) thus preventing the synthesis of thromboxane without inhibiting the production of the primary prostaglandins or of prostacyclin. The antiarrhythmic activity of aspirin may result from an increased production of the latter substances or could be taken as an indication that thomboxane A_2 may be involved in the genesis of arrhythmias. Moschos, Haider, Escobinas, Gandhi & Regan (1980) have recently found a similar effect when they compared aspirin

and indomethacin in dogs subject to non-thrombotic coronary artery occlusion. Aspirin markedly reduced mortality resulting from ventricular fibrillation whereas indomethacin had no effect.

The results presented in this paper support the theory that prostaglandins may be endogenous antiarrhythmic agents. PGE_2 and prostacyclin have been shown to be antiarrhythmic; inhibition of synthesis with flurbiprofen had the opposite effect. The results obtained with aspirin may also be taken as providing evidence for an antiarrhythmic role of prostaglandins if it is assumed that the doses used prevented the synthesis of thromboxanes without inhibiting the formation of the primary prostaglandins or prostacyclin.

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