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Effects of aspirin and prostacyclin on arrhythmias resulting from coronary artery ligation and on infarct size

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1 The effects of pretreatment with aspirin, and of prostacyclin (PGI_2) infusions, on responses to myocardial ischaemia and infarction produced by ligation of a coronary artery were investigated in conscious rats.

2 Surgical preparation, under halothane anaesthesia, consisted of implanting exteriorized aortic and jugular cannulae, ECG leads and a polypropylene/polyethylene occluder for the left anterior descending coronary artery. Ligation of the coronary artery was performed six to nine days after surgery.

3 Aspirin pretreatment consisted of 100 mg/kg given intravenously 1 or 36 h before ligation. PGI_2 infusions (10–400 ng kg^{-1} min^{-1}, i.v.) were begun 2 min before ligation and continued for 4 h afterwards.

4 ECG, blood pressure, heart rate and arrhythmias were recorded starting 30 min before, and continuing for 4 h after, ligation. Twenty-four hours after ligation, in surviving animals, the heart was removed for estimation of occluded and infarcted zones.

5 Some treatments provided antiarrhythmic and other protection in the first 30 min post-ligation. By 4 and 24 h post-ligation, protective effects were lost.

6 Both aspirin pretreatment and low doses of prostacyclin reduced arrhythmias occurring within 30 min of ligation. The highest dose of prostacylin (400 ng kg^{-1} min^{-1}) was arrhythmogenic.

7 None of the treatments influenced the amount of cardiac tissue occluded or infarcted by ligation.

8 The conclusions from this study in conscious rats were that acute aspirin pretreatment and low doses of infused prostacyclin have limited beneficial actions which are mainly confined to the earliest post-ligation period.

Introduction

Many workers, including ourselves (Harvie, Collins, Miyagishima & Walker, 1978), have shown some prostaglandins to be antiarrhythmic against arrhythmias produced by ligation of a coronary artery. It was therefore natural that, once discovered, prostacyclin should also be tested for its antiarrhythmic action. Prostacyclin infusions have been reported, in various species, to have beneficial effects in myocardial ischaemia (Lefer, Ogletree, Smith, Silver, Nicolaou, Barnette & Gasic, 1978; Ribeiro, Brandon, Hopkins, Reduto, Taylor & Miller, 1981). In the dog, we found prostacyclin to be antiarrhythmic in a manner similar to other prostaglandins, whereas in the rat prostacyclin was arrhythmogenic (Au, Collins, Harvie & Walker, 1979; 1980). However, in the same rat preparation, prostaglandin (PGE_2) was antiarrhythmic.

Using anaesthetized rats, Coker & Parratt (1981a) also found PGE_2 to be antiarrhythmic as were infusions of prostacyclin at doses of 100 and 1000 ng kg^{-1} min^{-1}.

In most of the above studies, observations on arrhythmias were apparently confined to the first hour of ligation, and since the interpretation of results from many, but not all (see Ribiero *et al.*, 1981), studies are complicated by short post-ligation observation periods, and by the use of anaesthesia for acute surgical preparation, we have developed a chronic conscious rat model. In this model, the effects of treatment following ligation of a coronary artery can be assessed over 4 h in terms of arrhythmias, blood pressure, heart rate, ECG changes ('S-T' segment and Q-wave appearance), and over 24 h for mortality and occluded zone and infarct zone sizes

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(Johnston, MacLeod & Walker, 1981; MacLeod, Augereau & Walker, 1983). This preparation gives an overall comprehensive picture of various responses to ligation and so we have used it to investigate the effects of aspirin pretreatment and of low dose prostacyclin infusions, continued for 4 h, on responses to ligation.

We have also investigated the effects of aspirin pretreatment. Aspirin regimens have previously been used to manipulate endogenous prostacyclin levels preferentially (Livio, Villa & de Gaetano, 1978; Korbut & Moncada, 1979; Masotti, Poggesi, Galanti, Abbate & Neri Sereni, 1979; Ellis, Wright, Jones, Richardson & Ellis, 1980) and aspirin has also been shown to be antiarrhythmic in the rat (Coker & Parratt, 1981a; Lepran, Koltai & Szekeres, 1981). However, the salicylate ion alone is also antiarrhythmic in the dog (Regan, Moore, Bakth, Moschos & Oldewurtal, 1980).

In this study in conscious rats, treatment consisted of prostacyclin infusion for 4 h and aspirin pretreatment. A preliminary account of some of this work has been given (Au, Harvie, Johnston, MacLeod & Walker, 1981).

Methods

Coronary ligation techniques in conscious rats

Our conscious rat preparation (Johnston *et al.*, 1980; MacLeod *et al.*, 1982) is an extension of methods used in anaesthetized rats by ourselves (Au *et al.*, 1979) and others (Clark, Foreman, Kane, McDonald & Parratt, 1980). Six to nine days before ligation rats were anaesthetized with halothane and the chest opened under positive-pressure respiration. A polypropylene suture (5–0) was passed through the myocardium surrounding the left anterior descending coronary artery and exteriorized in a polythene guide (PE 50) in the mid-scapula region of the neck. Permanent stainless steel ECG leads were placed subcutaneously in each limb (using a long subdermal trocar) and exteriorised near the occluder. A chest lead was also used and this was placed in the pectoralis muscle overlying the chest incision (4-5th intercostal space). In most animals permanent aortic and jugular cannulae were implanted, at the same operation, by the technique of Weeks (1979). In some rats permanent external venous jugular and arterial cannulae were implanted under halothane anaesthesia a day or two before ligation. In this limited number of animals the arterial cannula consisted of a teflon catheter (Jelco No. 22) inserted into a ventral tail artery and attached to polythene tubing (PE 90) exteriorised at the neck.

Experimental procedure

On the day of ligation, cannulae and leads were appropriately connected to a Grass Polygraph, and infusion pump, and the animal left in its cage for 1 h before ligation. To obtain ligation, sufficient traction was exerted between the polypropylene suture and the polythene guide to close the ligature. Ligation was easily completed within seconds in the unrestrained animal. The blood pressure, ECG and behaviour of the animal was continuously monitored for 30 min before ligation and for 4 h after.

Ligation was performed during daylight hours on rats in their home cages and animals tended to sleep during the various procedures. Rats responded to ligation with few apparent outward signs of discomfort (restlessness and mild pilo-erection). Generally, no other marked changes in behaviour were seen and many animals resumed a sleeping position shortly after ligation.

Four hours post-ligation, cannulae were closed by heat-sealing, leads were disconnected, and animals returned to the animal house. After 24 h of ligation, blood pressure and ECG were re-recorded for 30 min before the animal was killed and its heart rapidly removed. The heart was perfused (Langendorff technique) with Krebs solution at 37°C and 100 mmHg pressure for 5 min to remove all blood. A bolus of 2.0 ml of cardio-green dye (Indocyanine Green; Hynson, Westcott and Dunning Inc., Baltimore, MD 21201 U.S.A.; 1.0 mg/ml in Krebs solution) was then used to differentiate perfused (green) from underperfused, or occluded, tissue (pink). The underperfused region was immediately cut out and weighed to give an occluded zone as percentage of total ventricular weight. Immediately thereafter, the heart tissue was sliced longitudinally into 1.0 mm thick sections and incubated in tetrazolium dye (10 mg 2-, 3-, 5-triphenyltetrazolium chloride/ml of 70 mM sodium phosphate buffer pH 8.5) at 37°C for 30–45 min. At the end of the incubation period all sections were placed in 10% formaldehyde (in normal saline) for 2 days before the undyed (white) infarcted tissue was dissected from viable tissue (purple). Infarcted tissue was expressed as a percentage by weight of total ventricular tissue (infarct zone).

Measured variables

Heart rate, blood pressure and ECG were recorded continuously for 30 min before, and for 4 h after, ligation with a final reading 24 h post-ligation in surviving animals. From the ECG record, the time to Q-wave (25μV or more negative deflection preceding R wave) appearance was recorded and the height of the 'S-T' segment above the iso-electric line measured. The latter was measured 20 ms after the initial

R-wave and normalised for signal size (RS height) according to the formula: 'S-T' corrected = ('S-T' – 'S-T' control) × (RS control/RS).

Arrhythmias were detected from ECG and blood pressure traces as premature ventricular contractions (PVC), ventricular tachycardia-flutter (VT) and ventricular fibrillation (VF). Four or more PVC occurring consecutively were considered to be VT. Ventricular tachycardia, in its purest form (which occurred infrequently), had a rate of at least 500 beats/min with an ECG trace which showed absence of sinus rhythm and clearly defined R waves. With tachycardia the blood pressure fell only slightly, if at all. In ventricular flutter the ECG was typically cyclical in appearance with no sharp R wave and blood pressure fell to 30–60 mmHg with a very small pulse pressure wave. In ventricular fibrillation either a *torsade de pointes*, or true fibrillation, pattern was seen on the ECG and this occured with a precipitous fall in blood pressure to 0 mmHg. Since these three ventricular arrhythmias could not always be distinguished from each other, and tachycardia often degenerated to flutter and flutter to fibrillation, all such major arrythmias were recorded as either ventricular tachycardia (VT) or ventricular fibrillation (VF). If VF did not spontaneously revert within 10 s, attempts were made to obtain reversal by standardized precordial taps and chest massage. If 5 min of such resuscitation failed to revive a rat its heart was excised for estimation of occluded zone as described previously.

All arrhythmia histories were scored on a 0–8 arrhythmia scoring scale for 0–30 min or 0–4 h post-ligation observation periods. The value 0 was given for 0–50 PVC with no VT or VF over the observation period; 1, for 50–500 PVC only; 2, for > 500 PVC, or one episode of spontaneously reversible VT or VF; 3, for more than one episode of spontaneously reversible VT and/or VF or, one or more, episodes of non-spontaneously reversible VT and/or VF lasting less than 60 s; 4, for reversible VT and/or VF episodes lasting 60–120 s; 5, for VT and/or VF episodes lasting more than 120 s; 6, for irreversible VF causing death within 15–240 min of ligation; 7, for fatal VF within 4–15 min; 8, for fatal VF within 4 min. This scale is an extension of previous scales (Au *et al.*, 1979) and was chosen so as to give a normally distributed (Gaussian) curve for 64 untreated ligated animals. An 'F-test' was routinely used to check that variances of control and test groups were not significantly different. In its present form, the scoring scale has been used to demonstrate antiarrhythmic effects of drugs in this preparation (MacLeod *et al.*, 1983). Arrhythmias were recorded as the incidence of VT and/or VF in a group, incidence of VF (all types) and incidence of non-spontaneously reversible VF (i.e. VF reversed by pre-cordial taps) as well as arrhythmia score and $\log_{10}$ PVC. This total system of arrhythmia scoring was designed to quantify the total arrhythmia history by means of normally distributed variables suitable for parametric statistical testing. Total arrhythmic histories have been recorded for over 250 rats (64 untreated controls) in our laboratory and those pooled data subjected to analysis by standard computer packages (UBC Computer Centre) designed to test for various types of distributions (normal Gaussian, log normal, Poisson, etc.). Such analysis showed arrhythmia score and $\log_{10}$ PVC (but not PVC) fitted normal Gaussian distribution curves. However, variables such as the number of episodes of VT and/or VF per rat or duration of VT or VF episodes did not fit such distribution curves and therefore were not considered suitable for parametric testing. However, exclusion of zero times and expressing durations as $\log_{10}$ duration gave distributions closer to normal. This latter measure was not used since the arrhythmia score incorporates the time element on a log-type scale.

Occluded zone size, infarct size and mortality at 4 h and 24 h post-ligation were also recorded.

Drug treatment

Groups ($n = 5–9$) of animals were treated in a random and blind fashion with aspirin pretreatment and/or prostacyclin infusions together with appropriate controls. Aspirin pretreatment was 100 mg/kg i.v. given 1 or 36 h before ligation as a 100 mg/ml in a bicarbonate-saline solution (pH 7.0) prepared just before administration. An aspirin control group received bicarbonate-saline solution alone. Prostacyclin infusions, at 0°C, were dilute solutions of a stock solution of prostacyclin in a saline-bicarbonate buffer at pH 10 (Weeks, 1979). Control infusions consisted of buffer without prostacyclin. Infusions of prostacyclin were begun 2 min pre-ligation and continued for 4 h post-ligation. The infusion volume was always below 1 ml kg^{-1} h^{-1}. In one group of rats, treated with 100 ng kg^{-1} min^{-1} prostacyclin, infusions were begun 2 min post-ligation.

Estimates of occluded and infarcted zones, and all record analysis, was performed 'blind'. All data, kept on computer files, were subjected to analysis using computer packages for analysis of variance by an Anova programme with differences between means tested by various mean tests (Duncan's, Tukey's and Newman-Keul's) according to the programmes of Gregg & Osterlin (1977) and using the general statistical guideline of Wallenstein, Zucker & Fliess (1980). Duncan's mean range test was usually taken as standard. Control values were accumulated after it was determined that no statistically significant differences existed between different control groups.

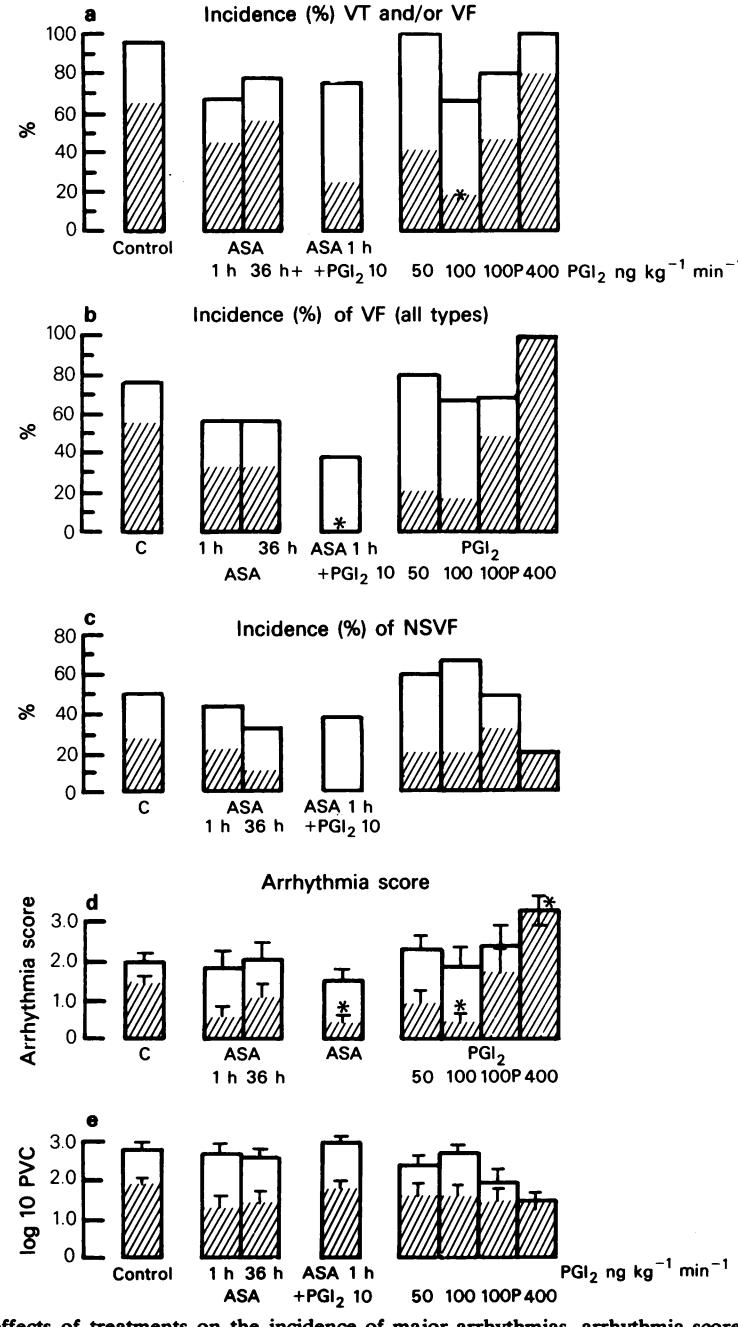

Figure 1 The effects of treatments on the incidence of major arrhythmias, arrhythmia score, and premature ventricular contraction (PVC) occurrence following ligation of a coronary artery in conscious rats. The effect of aspirin (ASA) and prostacyclin treatment on the incidence, as percentage of animals in group, of: (a) ventricular tachycardia and/or ventricular fibrillation (VT and/or VF); (b) ventricular fibrillation (VF) (of all types); and (c) non-spontaneous reversible ventricular fibrillation (NSVF); (d) Shows the effect of treatments on mean arrhythmia score ($\bar{x} \pm$ s.e.mean) while (e) shows the effect on PVC as $\bar{x} \pm$ s.e.mean of $\log_{10}$ PVC. Treatments were ASA (aspirin) 100 mg/kg i.v. 1 or 36 h (1 and 36) before ligation with, or without, prostacyclin (PGI_2) infusions of 50, 100, and 400 ng kg^{-1} min^{-1} begun 2 min pre-ligation and continued for 4 h. 'P' indicates infusion begun 2 min post-ligation. For exact details of measurements see Methods. The open columns are for the 0–4 h post-ligation period while the hatched columns are for the 0–30 min post-ligation period. * indicates a statistically significant difference from control at $P < 0.05$ by ANOVA.

Results

Figure 1 summarizes the effects of treatments on arrhythmias as measured by the incidence of VT and/or VF, VF (total) or non-spontaneously reversible VF, arrhythmia score and $\log_{10}$ of total PVC. As indicated in the Methods section, the arrhythmia score included weighting for duration and frequency of VT and/or VF. All variables were recorded for the periods 0–30 min and 0–4 h post-ligation. The division of measurement periods into 0–30 min and 0–4 h post-ligation was adopted because of the temporal aspects of arrhythmia appearance. Most arrhythmias, whether PVC, VT or VF, appeared in the periods 2–25 min and 1.5–4 h post-ligation. A quiescent period with few arrhythmias occurred between 25 and 90 min post-ligation.

The most interesting finding shown in Figure 1 is the reduction in the incidence of severe arrhythmias in various treatment groups during the first 30 min post-ligation. In this period, most treatments, except for the highest infusion level of prostacyclin (400 ng kg^{-1} min^{-1}), lowered the incidence of VT and VF from the control incidence. Statistically significant reductions occurred with aspirin plus 10 ng kg^{-1} min^{-1} prostacyclin and 100 ng kg^{-1} min^{-1} prostacyclin. Both treatments lowered all types of VF incidence. The highest prostacyclin dose level was arrhythmogenic.

The above findings applied particularly to the early 0–30 min post-ligation period since comparison of the 0–30 min with the 0–4 h period showed that antiarrhythmic effects of treatments were most marked in this early period. By 4 h post-ligation, the incidence figures for VT and/or VF, VF and non-spontaneously reversible VF were the same for all treatments except for 400 ng kg^{-1} min^{-1} prostacyclin.

In the early arrhythmia period other indices were also reduced by all treatments except for the highest dose level of prostacyclin. Mean arrhythmia scores were statistically significantly lower for the aspirin plus 10 ng kg^{-1} min^{-1} prostacyclin group and for the 100 ng kg^{-1} min^{-1} prostacyclin group. These differences were lost by 4 h post-ligation where no differences between means were seen for any of the groups except for the highest dose prostacyclin group, where the mean score was statistically higher.

Overall the reduction in arrhythmias during the early period (0–30 min) seen with the various treatments was almost balanced by extra arrhythmias in the period 30–240 min post-ligation. This is most striking for the most effective treatment (aspirin plus prostacyclin) which reduced the mean arrhythmia score from a control of 4.0 to 3.0 (a 25% fall) in the 4 h post-ligation period whereas the fall by 30 min was 68% (2.8 to 0.9).

Log_{10} PVC values reflected the findings with other measures of arrhythmias for most treatments resulted in lower $\log_{10}$ PVC scores by 30 min, but not by 4 h, post-ligation. However, where death from irreversible VF occurred early after ligation, only a limited number of PVC could occur. This was the reason for the low PVC counts seen with the highest dose of prostacyclin.

While all treatments, except the high dose of prostacyclin, tended to be antiarrhythmic, by most measures, it is interesting to note that 100 ng kg^{-1} min^{-1},

Table 1 Mortality and cardiac tissue loss following ligation of a coronary artery in conscious rats treated with aspirin (ASA) and/or prostacyclin

Treatment	n	*Mortality in group (as %) by* 4 h Post-ligation	24 h Post-ligation	*Tissue loss (% ventricular weight)* Occluded zone	Infarcted zone
Control	25	20	24	29 ± 1	24 ± 2
ASA 1 h	9	33	33	31 ± 3	23 ± 3
ASA 36 h	9	44	44	25 ± 3	24 ± 2
ASA 1 h + PGI_2 10 ng kg^{-1} min^{-1}	8	0	13	27 ± 3	25 ± 5
PGI_2 ng kg^{-1} min^{-1} 50	5	50	40	32 ± 3	26 –
PGI_2 ng kg^{-1} min^{-1} 100	6	17	17	27 ± 2	17 ± 7
PGI_2 ng kg^{-1} min^{-1} 100P	5	40	67	33 ± 3	29 –
PGI_2 ng kg^{-1} min^{-1} 400	5	80°L	80°L	32 ± 5	24 –

All animals dying within 4 h, except one each in control and ASA 1 h groups, died of irreversible VF.
Treatment consisted of ASA (1 or 36 h pre-ligation), aspirin 1 h pre-ligation with 10 ng kg^{-1} min^{-1} prostacyclin, 50, 100, 100P (began 2 min post (P) ligation) or 400 ng kg^{-1} min^{-1} prostacyclin begun 2 min pre-ligation and continued for 4 h. Occluded and infarcted zones were estimated as indicated in the Methods section. Values are percentages or mean ± s.e. mean. S.e. mean values were excluded when insufficient animals were left to give a meaningful s.e.mean.
° Indicates significantly different from low values $P < 0.05$ with L indicating difference from low values and H from high values in Table.

begun 2 min after ligation, was always less antiarrhythmic than the same infusion given before ligation.

Mortality, infarct zone, occluded zone and ECG findings in the various groups are summarised in Table 1 and 2. Mortality by 4 h and 24 h post-ligation was not statistically significantly reduced by any of the treatments (Table 1) and was actually increased by the highest dose of prostacyclin. In the 4 h post-ligation period, 90% of deaths were due to irreversible ventricular fibrillation and chance observations of deaths occurring between 4 and 24 h suggested these were often due to ventricular fibrillation. A lower incidence of irreversible VF accounted for the lower mortality with the aspirin plus prostacyclin and with the 100 ng kg^{-1} min^{-1} prostacyclin treatments. The increase in mortality with 400 ng kg^{-1} min^{-1} prostacyclin resulted from an increased incidence of irreversible ventricular fibrillation.

None of the treatments appeared to change occluded zone size whose group mean values varied from 27% to 33% with an accumulated control value of 28.7 ± 1.2($\pm$s.e.mean). The findings for infarct zone reflected those for occluded zone and again no statistically significant differences were found between any of the groups. Infarct zones were, on average, 20% lower than occluded zones but expressing infarct zone values as a percentage of occluded zone did not materially effect findings. Thus none of the treatments appeared to 'salvage' cardiac tissue.

ECG findings were somewhat erratic (Table 2). This was especially so for Q-wave alterations where apparently large changes were not statistically significant. Where a Q-wave appeared in the ECG before the animal was killed, and after 4 h post-ligation, a value at 240 min was given. The absence or presence of this correction factor revealed no difference in the time to Q-wave appearance in any of the groups. As ligation produced a rapid (within 1 min) increase in R-S wave size, values for 'S-T' segment changes were corrected for this increase. Such a correction decreased the variance between observations. All aspirin treatments appeared to reduce 'S-T' segment changes, while the effects of prostacyclin were inconsistent. The importance of such findings, which did not readily relate to occluded or infarct zone sizes, is difficult to determine.

Table 3a and b summarizes cardiovascular changes with treatment and ligation. Mean pre-ligation (−1 min) heart rates varied significantly from group to group, being lower in the aspirin-treated groups and higher for the prostacyclin-treated animals. Ligation did not markedly change rates either in control or treated groups.

Pre-ligation blood pressures (−1 min), in the various groups varied from 102 mmHg systolic/78 mmHg diastolic to 123 mmHg/89 mmHg. As expected, prostacyclin infusions (begun 2 min pre-ligation) were associated with lower pressures. Aspirin pretreatments were associated with higher pressures. Ligation produced falls in systolic and diastolic pressure which persisted throughout the 4 h post-ligation period. The ligation-induced falls in pressure were statistically greater in the prostacyclin-treated animals and were greatest with 400 ng kg^{-1} min^{-1}. The only other statistically significant finding was for the aspirin 36 h group where blood pressure values were higher than the low pressures encountered in the prostacyclin-treated groups.

Discussion

In the acutely-prepared anaesthetized rat we have

Table 2 ECG changes following ligation of a coronary artery in conscious rats treated with aspirin (ASA) or prostacyclin

Treatment	*% of group showing Q-wave by 24 h*	*Appearance time of Q-wave when seen* (min)	*'S-T' corrected 15 min post-lig.* (mV)
Group controls	92	72 ± 12	0.38 ± 0.07
ASA, 1 h	67	54 ± 13	0.30 ± 0.06*
ASA, 36 h	56	66 ± 22	0.24 ± 0.05*
ASA, 1 h + PGI_2 10 ng kg^{-1} min^{-1}	75	53 ± 27	0.11 ± 0.04*
PGI_2 ng kg^{-1} min^{-1} 50	100	81 ± 15	0.50 ± 0.12
PGI_2 ng kg^{-1} min^{-1} 100	50	65 −	0.64 ± 0.20
PGI_2 ng kg^{-1} min^{-1} 100P	33	45 −	0.28 ± 0.08*
PGI_2 ng kg^{-1} min^{-1} 400	(excessive early deaths)		0.66 −

The effects of ASA pretreatment (100 mg/kg i.v.) 1 or 36 h before ligation, with or without prostacyclin (PGI_2) infusions on ECG responses to ligation are summarised above. ECG measurements are explained in the Methods section. Other than percentage values, all values are the mean ± s.e.mean for groups of from 5–26 (grouped controls) animals. '−' indicates insufficient number of animals left in group to give a meaningful s.e.mean.

* Indicates a value statistically significantly different from control with $P < 0.05$ by ANOVA.

Table 3 The effect of ligation, with and without aspirin (ASA) or prostacyclin treatment, on (a) heart rate and (b) blood pressure of conscious rats

Treatment	*1 min Pre-ligation*	*+10 min*	*+1 h*	*+4 h*
		(Post-ligation)		
	(a) Heart rate (beats/min)			
Controls	360± 6	360± 9	370± 8	380±13
ASA, 1 h	340±15	370± 9	340±16	350±11
ASA, 36 h	350± 6	360±11	350± 7	350±15
ASA, 1 h + PGI_2 10 ng kg^{-1} min^{-1}	340± 8	350±12	370±20	370±18
PGI_2 ng kg^{-1} min^{-1} 50	460±15*	440±17*	420 –	452 –
PGI_2 ng kg^{-1} min^{-1} 100	460±19*	420±27*	420±13*	420±17*
PGI_2 ng kg^{-1} min^{-1} 400	450±12*	460 –	440 –	350 –
	(b) Blood pressure (systolic, mmHg/diastolic, mmHg)			
Controls	118±4/88±2	113±3/87±3	106±3/85±2	101±4/80±3
ASA 1h	124±7/87±4	120±6/88±2	109±6/84±	108±5/82±4
ASA 36 h	123±4/89±3	115±3/92±3	116±6/91±3*L	115±3/92±2
ASA 1 h+ 10 ng kg^{-1} min^{-1} PGI_2	110±5/89±4	106±6/89±6	107±6/89±5	103±9/81±7
PGI_2 ng kg^{-1} min^{-1} 50	110±3/92±7	105±3/87±3	98±3/88±2	115 –/90 –
PGI_2 ng kg^{-1} min^{-1} 100	108±7/80±4	93±5/68±2	102±7/77±5	93±13/73±4*H
PGI_2 ng kg^{-1} min^{-1} 400	102±6/78±7	80 –/55 –	80 –/60 –	60 –/40

Blood pressure and heart rate were continuously recorded from conscious rats from 30 min before, to 4 h after, ligation of LAD coronary artery. All values are the mean for the group ± s.e.mean values. Pre-ligation refers to values obtained 1 min before ligation.

* indicates a statistically significantly different mean with $P<0.05$ (with H and L as in Table 1) by ANOVA. Treatments and *n* values as in previous Tables.

previously shown that infusions of some prostaglandins, begun before ligation, reduce arrhythmic responses to ligation of a coronary artery (Au *et al.*, 1979; 1980), as have other workers (Coker & Parratt, 1981a; Martinez & Crampton, 1981). Treatment with aspirin, or other prostaglandin synthetase inhibitors, has also been reported to have various influences on arrhythmia and other outcomes of myocardial ischaemia in rats (Martinez & Crampton, 1980; 1981; Coker & Parratt, 1981a; Lepran *et al.*, 1981) and dogs (Moschos, Haider, De La Cruz, Lyons & Regan, 1978; Jugdutt, Becker, Bulkley & Hutchins, 1978).

We found that prostacyclin infused at 500 ng kg^{-1} min^{-1}, and above, in acutely prepared anaesthetized rats, produced adverse effects; arrhythmias were worse and more animals died with treatment (Au *et al.*, 1979). Coker & Parratt (1981a) found that 100 and 1,000 ng kg^{-1} min prostacyclin in a similar preparation reduced arrhythmias although, the degree of effectiveness was not obviously dose-related. In both above studies the arrhythmic observation period occupied only the first 30 min post-ligation.

None of the prostacyclin treatments we tested in the conscious rat reduced infarct size, although in other species possible infarct reducing actions have been reported (Moschos *et al.*, 1978; Ogletree, Lefer, Smith & Nicolaou, 1979; Coker & Parratt, 1981b).

In anaesthetized dogs, using various indices, beneficial actions (including antiarrhythmic ones) have been claimed for prostacyclin in myocardial ischaemia by Au *et al.*, 1979; Starnes, Primm, Woosley, Oates & Hammon, 1980; Coker & Parratt, 1981b; Ribeiro *et al.*, 1981. Studies in cats also showed beneficial effects (Ogletree *et al.*, 1979), although these were dose-related with adverse effects on arrhythmias and infarct size occurring at higher doses (Dix, Kelliher, Jurkiewicz & Lawrence, 1979). Our best treatment was where low levels of exogenous prostacyclin were added after aspirin pretreatment for 1 h.

In most, but not all, studies aimed at demonstrating a beneficial action for prostacyclin in myocardial ischaemia and infarction, a limited number of variables were measured and the total cardiovascular and mortality responses not recorded. We monitored effects on arrhythmias, blood pressure, heart rate and occluded and infarcted areas. In addition we also recorded mortality as the adverse outcome. Thus, the total beneficial, or adverse, effect of treatment was assessed. If only one measure of potential beneficial effect is taken, then it is relatively easy to distort the overall picture. Figure 1 illustrates that animals observed only for 30 min post-ligation would show antiarrhythmic benefit, whereas fuller temporal analysis showed that, despite continued treatment, beneficial effects were lost. However, although such limitations may apply to many studies, Ribiero *et al.* (1981) have shown reduced mortality with prostacyclin given to dogs for 6 h.

Overall analysis also showed that while prostacyclin infusion regimens had few maintained beneficial effects the highest infusion level of 400 ng kg^{-1} min^{-1} was detrimental. This latter finding is in keeping with our previous studies (Au *et al.*, 1979; 1980).

In conclusion, our study, appears to indicate that, at least for the rat, exogenous prostacyclin has no maintained major benefical actions in reducing adverse responses to ligation of the left anterior descending coronary artery.

Aspirin has been tested by a number of workers for its ability to reduce the effects of myocardial ischaemia and infarction. In anaesthetized rats, it has been reported that aspirin treatment (30 mg/kg, i.v.) does not significantly reduce arrhythmias induced by ligation of a coronary artery (Martinez & Crampton, 1981) while in conscious rats, oral aspirin treatment (200 mg/kg) 1 h before ligation had a small, but significant, antiarrhythmic effect against ligation-induced arrhythmias (Lepran *et al.*, 1981). Coker & Parratt (1981a) also showed an antiarrhythmic action for aspirin (100 mg/kg) given intravenously 15 min pre-ligation; a lower dose had no effect. Beneficial (Moschos *et al.*, 1978) and adverse (Jugdutt *et al.*, 1978) actions for aspirin have also been reported in other species, while, in the dog, Regan *et al.* (1980) found the salicylate ion to be antiarrhythmic, thus arguing for aspirin having antiarrhythmic actions independent of cyclo-oxgenase inhibition.

Our aspirin treatment schedules may have abolished all prostaglandin production (1 h pretreatment) or favoured formation of prostacyclin (36 h treatment) (Livio *et al.*, 1978; Korbut & Moncada, 1979; Masotti *et al.*, 1979; Ellis *et al.*, 1980), but regardless of whether such differences in prostaglandin production occurred, treatments had no major effect on responses to ligation of a coronary artery. Arrhythmias were reduced (in accord with other workers findings), but not to a statistically significant degree, with both aspirin treatments, 'S-T' segment changes were also reduced after aspirin although there were no changes in either occluded or infarct zone sizes. The only other possible beneficial effect of aspirin pretreatment (36 h before ligation) was the higher blood pressure; the significance of this finding is not known.

From this study it would appear that manipulation of endogenous or exogenous prostacyclin levels does not have a major maintained influence on the various adverse responses that occur when a major coronary artery is ligated in the conscious rat.

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