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Effects of zaprinast and rolipram on platelet aggregation and arrhythmias following myocardial ischaemia and reperfusion in anaesthetized rabbits

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1 This study was designed to compare the effects of two selective inhibitors of certain phosphodiesterase (PDE) isoenzymes on arrhythmias induced by coronary artery occlusion and reperfusion. The drugs used were zaprinast which inhibits guanosine 3':5'-cyclic monophosphate (cyclic GMP)-specific PDE (PDE V) and rolipram which inhibits cyclic GMP-insensitive, adenosine 3':5'-cyclic monophosphate (cyclic AMP)-specific PDE (PDE IV).

2 Pretreatment of anaesthetized rabbits with zaprinast ($300\,\mu g\,kg^{-1}$ plus $30\,\mu g\,kg^{-1}\,min^{-1}$) had no significant effect on ischaemia- or reperfusion-induced ST-segment changes, or arrhythmias. In contrast, rolipram ($30\,\mu g\,kg^{-1}$ plus $3\,\mu g\,kg^{-1}\,min^{-1}$) and ($100\,\mu g\,kg^{-1}$ plus $10\,\mu g\,kg^{-1}\,min^{-1}$) increased the severity of arrhythmias. With the higher dose of rolipram, ST-segment changes were increased in magnitude and mortality due to ventricular fibrillation during ischaemia or reperfusion was increased to 80% compared with 30% in controls ($n = 10$ per group).

3 Zaprinast caused small but significant increases in heart rate and arterial blood pressure whereas rolipram decreased diastolic arterial pressure, increased left ventricular (LV) dP/dt_{max} and substantially increased heart rate.

4 At the end of each experiment platelet aggregation was measured *ex vivo*. Pretreatment of rabbits with either dose of rolipram had no significant effect on platelet aggregation induced by adenosine diphosphate (ADP), collagen, arachidonic acid or thrombin or on isoprenaline- or prostacyclin-induced inhibition of aggregation. Aggregatory responses to ADP and collagen were increased in platelets obtained from rabbits which had received zaprinast.

5 These results indicate that in the dose used here, the PDE V inhibitor zaprinast had no significant effect on arrhythmias. The effects of the PDE IV inhibitor rolipram on haemodynamics, combined with its lack of antiplatelet activity, may have contributed to the exacerbation of arrhythmias observed during myocardial ischaemia and reperfusion.

Keywords: Arrhythmias; myocardial ischaemia; reperfusion; zaprinast; M&B 22,948; rolipram; phosphodiesterase inhibitors; platelet aggregation

Introduction

We have demonstrated previously that the non-selective phosphodiesterase (PDE) inhibitor isobutylmethylxanthine (IBMX) had antiarrhythmic activity in anaesthetized rabbits subject to acute coronary artery occlusion, whereas the selective PDE III inhibitor, milrinone had no significant antiarrhythmic effects (Holbrook & Coker, 1989). Although the mechanism(s) responsible for the antiarrhythmic action of IBMX could not be determined from this previous study, alterations in platelet aggregation did not seem to be directly relevant since milrinone had greater anti-platelet activity than IBMX. One possible explanation for the antiarrhythmic effect of IBMX was that it may be related to the ability of this compound to increase guanosine 3':5'-cyclic monophosphate (cyclic GMP) rather than adenosine 3':5'-cyclic monophosphate (cyclic AMP).

In the last decade several different PDE isoenzymes have been identified and it has been suggested recently that there may be at least five distinct, but related, gene families coding for cyclic nucleotide PDEs (see Beavo & Reifsnyder, 1990, for descriptions and nomenclature). IBMX inhibits all PDEs whereas milrinone is selective for the cyclic GMP-inhibited, cyclic AMP-specific PDE (PDE III). Selective increases in cyclic AMP alone, can also be achieved by inhibition of the cyclic GMP-insensitive, cyclic AMP-specific PDE (PDE IV) with a drug such as rolipram. Conversely, zaprinast will increase cyclic GMP by inhibiting the cyclic GMP-specific PDE (PDE V, previously termed PDE I, e.g. see Weishaar, 1987).

Both cyclic AMP and cyclic GMP can mediate vasodilator responses and impair platelet aggregation (Packham & Mustard, 1980; Waldman & Murad, 1987; Lincoln, 1989). During acute myocardial ischaemia, alterations in vascular tone or platelet aggregation in the coronary circulation may be important determinants of the severity of subsequent arrhythmias. Thus the aim of the present study was to extend our knowledge of the consequences of selective PDE inhibition by examining the effects of rolipram and zaprinast in our anaesthetized rabbit model of acute myocardial ischaemia and reperfusion.

Methods

Animal preparation

Experiments were performed in male New Zealand White rabbits (2.1 to 3.2 kg) purchased from Hylyne, Cheshire. Anaesthesia was induced by i.m. injection of diazepam $2.5\,mg\,kg^{-1}$ followed by Hypnorm $0.4\,ml\,kg^{-1}$. Rabbits were then prepared for coronary artery occlusion as described previously (Coker, 1989). Briefly, a Lead II ECG was recorded along with arterial blood pressure, left ventricular pressure and its first derivative with time. Drugs were administered into the vena cava via a right femoral venous catheter. After cannulation of the trachea, sodium pentobarbitone was administered i.v. (24 to $48\,mg\,kg^{-1}$) to maintain anaesthesia.

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A left thoracotomy was performed at the fourth intercostal space and the rabbits were ventilated with room air at 38 strokes per min, 12 to 18 ml per stroke, with a positive end expiratory pressure of 1 to 2 cmH_2O. Arterial blood gases and pH were measured (Corning 158 Analyzer) and stroke volume was adjusted to maintain P_{CO_2} within normal limits. The pericardium was incised and a fine silk ligature was placed around the major anterolateral branch of the left circumflex coronary artery.

Experimental protocol

A stabilization period of at least 15 min was allowed after the completion of the surgical procedures. A drug or vehicle was then administered i.v. as an initial bolus dose followed immediately by a continuous infusion. Zaprinast was given at a dose of 300 $\mu g\,kg^{-1}$ plus 30 $\mu g\,kg^{-1}\,min^{-1}$ whereas two doses of rolipram were studied; 30 $\mu g\,kg^{-1}$ plus 3 $\mu g\,kg^{-1}\,min^{-1}$ and 100 $\mu g\,kg^{-1}$ plus 10 $\mu g\,kg^{-1}\,min^{-1}$. Control rabbits received equivalent volumes of the vehicle (25% v/v polyethylene glycol 300 in water). There were 10 rabbits in each group. The coronary artery was occluded 10 min after commencing drug administration and myocardial ischaemia was maintained for 20 min, after which time the ligature around the coronary artery was released to allow reperfusion. At the end of each experiment, i.e. after 10 min of reperfusion or after 3 min of continuous ventricular fibrillation (if this occurred), blood was removed from the right ventricle via a 21G needle to permit the study of platelet aggregation *ex vivo*. The heart was then removed, the aorta cannulated and after retying the ligature around the coronary artery, a 1% w/v solution of Coomassie Blue was injected retrogradely into the aorta to stain the non-ischaemic myocardium. After dissecting the unstained tissue from the rest of the ventricles, both ischaemic and normal regions were weighed. The 'area at risk' was calculated as a percentage of the total ventricular mass. Changes in the ST-segment of the ECG and ischaemia- and reperfusion-induced arrhythmias were analyzed as described previously (Coker, 1989).

Exclusion criteria

Animals were excluded from further study or final analysis if any of the following occurred: (a) arrhythmias prior to coronary artery occlusion; (b) ST-segment changes prior to coronary artery occlusion; (c) area at risk less than 30% of total ventricular mass; (d) area at risk greater than 60% of total ventricular mass.

Platelet aggregation

Blood samples were placed in plastic tubes containing 3.8% w/v trisodium citrate solution (1 ml to 9 ml blood) and centrifuged at 220 *g* for 10 min. The supernatant, platelet rich plasma (PRP) was removed and the remnants recentrifuged at 2000 *g* to give platelet poor plasma (PPP). The number of platelets in the PRP was counted and then the PRP was diluted with PPP to give a final platelet count of 2.5 to 3.0 $\times 10^5\,\mu l^{-1}$. Aliquots of 100 μl of PRP were placed in cuvettes in a Payton dual channel aggregometer and stirred at 900 r.p.m. at 37°C. After an equilibration period of 3 min, aggregating agents were added and platelet aggregation was measured as the change in light transmission with the aggregometer set so that light transmission was 0% with PRP and 100% with PPP. In experiments where inhibition of platelet aggregation was studied, the inhibitory agent (isoprenaline or prostacyclin) was added 1 min before the aggregating agent (ADP).

Drugs

Zaprinast (M&B 22,948) and rolipram were gifts from Rhone-Poulenc, Dagenham and Schering AG, Berlin respectively. Due to its limited solubility, rolipram was dissolved in 25% v/v polyethylene glycol 300 in water with the aid of sonication. This vehicle was also used for zaprinast and was given in appropriate volumes to the control rabbits. Adenosine diphosphate (ADP), arachidonic acid, collagen, isoprenaline, prostacyclin and thrombin were purchased from Sigma, Poole; trisodium citrate from BDH, Poole; diazepam injection from the Royal Liverpool Hospital Pharmacy; Hypnorm (which contains 0.315 mg fentanyl citrate and 10 mg fluanisone per ml) from Janssen, Wantage and sodium pentobarbitone (Sagatal) from Rhone-Poulenc, Dagenham.

Statistics

Where appropriate, values have been expressed as the mean $\pm$ s.e.mean of n experiments. Comparisons between groups were made with an unpaired t test and within groups with a paired t test. The incidence of events was compared by Fisher's exact test.

Results

In total, 44 rabbits were used in this study. Of these, 4 were excluded; 2 which had ventricular premature beats (VPBs) prior to coronary artery occlusion and 2 in which the area of ventricle at risk of ischaemia was below the acceptable minimum (<30%). This left 10 rabbits in each group, all of which had similar sized areas at risk of ischaemia. The values were 42 $\pm$ 3% of total ventricular mass in controls; 39 $\pm$ 4% in the zaprinist group; 38 $\pm$ 3% in the low dose rolipram group and 41 $\pm$ 3% in the high dose rolipram group.

Arrhythmias and ST-segment changes

During the 20 min period of myocardial ischaemia VPBs were observed in all rabbits except one in the control group, whereas following reperfusion, ectopic activity occurred in all rabbits. Pretreatment with zaprinast did not significantly alter the incidence of ventricular tachycardia, ventricular fibrillation or the mortality resulting from acute myocardial ischaemia or reperfusion (Figure 1). In contrast the severity of arrhythmias was increased in the rabbits which received rolipram, resulting in an increase in mortality (due to terminal ventricular fibrillation) following the combined insult of ischaemia plus reperfusion (Figure 1).

Normally, in this model, ischaemia-induced ventricular tachycardia is a rare event and in this particular study none of the rabbits in the control group or the zaprinast-treated group had ventricular tachycardia during myocardial ischaemia. In the group of rabbits which received the lower dose of rolipram, however, 70% had ventricular tachycardia during the ischaemic period ($P = 0.003$ compared with control, Fisher's exact test). Although only 20% of the rabbits pretreated with the higher dose of rolipram had ventricular tachycardia, this was because most of the animals in this group went straight into ventricular fibrillation without a preceding episode of ventricular tachycardia.

Ventricular fibrillation occurred during ischaemia in 5 out of the 10 control rabbits but in 2 of these animals it was self-terminating (see Figure 1). In all other rabbits in which ventricular fibrillation occurred it was sustained, resulting in death. Most ventricular fibrillation occurred during ischaemia but 3 of the remaining rolipram-treated rabbits fibrillated following reperfusion (Figure 1). As well as increasing the severity of arrhythmias, ventricular fibrillation also appeared to occur earlier in the rabbits which received rolipram. In controls, ventricular fibrillation is most frequently observed between 10 and 15 min after coronary artery occlusion. In 1 rabbit which received the higher dose of rolipram, ventricular fibrillation occurred during the 3rd min of myocardial ischaemia and 4 rabbits had fibrillated by 10 min post-occlusion (see Figure 2, upper panel).

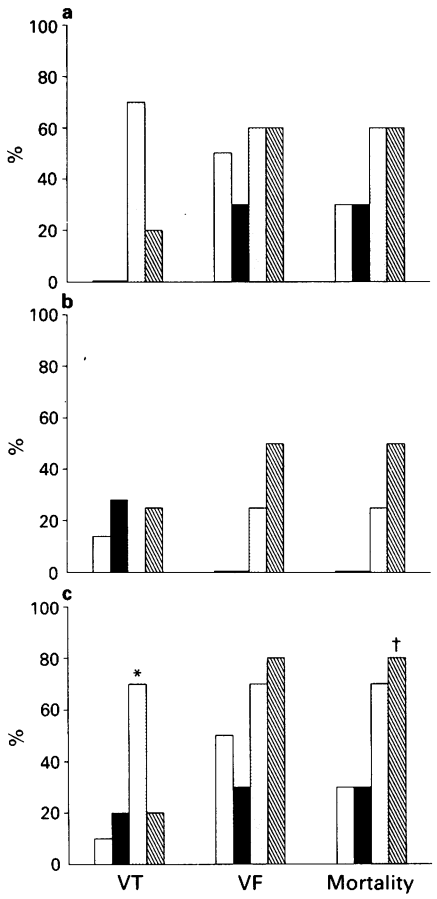

Figure 1 The incidence of ventricular tachycardia (VT), ventricular fibrillation (VF) and the mortality (a) during myocardial ischaemia, (b) following reperfusion in survivors and (c) the total values after ischaemia plus reperfusion, in controls (open columns) and in rabbits pretreated with zaprinast (solid columns), low dose rolipram (stippled columns) or high dose rolipram (hatched columns), $n = 10$ per group. * $P = 0.019$; † $P = 0.070$, compared with corresponding value in controls, Fisher's exact test.

Significantly greater changes in the ST-segment of the ECG were observed in the rabbits which received the higher dose of rolipram. Although the mean values for ST-segment change during ischaemia (Figure 2) also appear to be greater in the zaprinast-pretreated rabbits the variation in values within this group was larger, and thus the differences from the control group were not statistically significant. To avoid the risk of generating false positives by performing repetitive t tests, comparisons were made only at two time points; 7 min post-occlusion, which is before arrhythmias start in most controls, and at the end of the ischaemic period (i.e. 20 min post-occlusion).

Haemodynamics and blood gases

Within 1 min of starting drug administration, significant haemodynamic changes were observed. With zaprinast, heart rate and arterial blood pressure were increased and remained elevated after 10 min of drug infusion (Table 1). The lower dose of rolipram caused sustained increases in heart rate and left ventricular dP/dt_{max} but only a transient increase in systolic blood pressure. After 10 min of infusion of this lower dose of rolipram a reduction in diastolic blood pressure was evident. The same pattern of responses was observed with the higher dose of rolipram although the magnitude of some changes was greater (Table 1).

In control rabbits coronary artery occlusion caused immediate reductions in heart rate, systolic blood pressure and left ventricular dP/dt_{max} which gradually reversed with time. By the end of the ischaemic period the only significant haemodynamic change was an increase in left ventricular end-diastolic pressure (Table 1). Similar ischaemia-induced alterations in haemodynamics were observed in the drug-treated rabbits, with the exception that no reduction in heart rate was seen in either group that had received rolipram.

Arterial blood gases and pH were measured before drug administration and again just before coronary artery occlusion, i.e. after 10 min of drug administration. The values are detailed in Table 2. There were no differences between the baseline values in any of the groups and none of the drugs altered arterial Po_2, Pco_2 or pH.

Platelet aggregation

Platelets obtained from all of the rabbits aggregated in response to ADP and arachidonic acid. The magnitude of the responses to arachidonic acid (10^{-3} M) was similar in all the groups: 51 ± 3% in controls, 52 ± 3% zaprinast, 50 ± 3% low dose rolipram and 53 ± 3% high dose rolipram. In contrast, although ADP produced concentration-dependent platelet aggregation in all the groups, some of the responses were greater in platelets from rabbits that had received zaprinast (Figure 3). The effects of ADP on the aggregation of platelets obtained from rabbits that had received rolipram

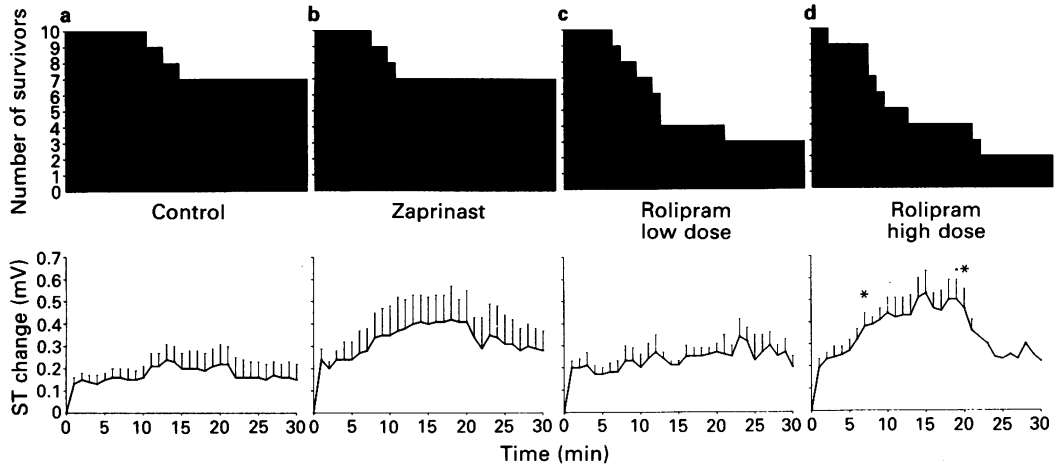

Figure 2 The number of survivors and the ST-segment change (mean with vertical bars indicating the s.e.mean) following coronary artery occlusion at time 0 and reperfusion at 20 min in controls (a) and in rabbits pretreated with zaprinast (b), low dose rolipram (c) or high dose rolipram (d).
* $P < 0.05$ compared with corresponding time point in controls, independent t test.

Table 1 The effects of drug (or vehicle) administration at −10 min, coronary artery occlusion at time 0 and reperfusion at 20 min on heart rate (HR), systolic and diastolic blood pressures (SBP and DBP), left ventricular end-diastolic pressure (LVEDP) and left ventricular (LV) dP/dt_{max}

Time (min)	n	*HR* (beats min^{-1})	*SBP* (mmHg)	*DBP* (mmHg)	*LVEDP* (mmHg)	*LV*dP/dt$_{max}$ (mmHg s^{-1})
Control						
−11	10	293 ± 10	76 ± 4	45 ± 3	3.5 ± 0.5	3640 ± 130
−9	10	294 ± 10	80 ± 3	48 ± 2		3690 ± 130
−1	10	292 ± 10	80 ± 3	47 ± 3	3.2 ± 0.4	3720 ± 120
1	10	287 ± 8†	72 ± 4††	45 ± 4		3330 ± 130†††
19	7	279 ± 13	77 ± 2	48 ± 2	8.4 ± 2.0†	3290 ± 130
30	7	288 ± 13	79 ± 4	50 ± 3	3.3 ± 0.5	3440 ± 190
Zaprinast (300 μg kg^{-1} plus 30 μg kg^{-1} min^{-1})						
−11	10	291 ± 10	84 ± 3	50 ± 3	3.8 ± 0.4	3680 ± 200
−9	10	299 ± 10*	99 ± 3***	61 ± 4**		3940 ± 290
−1	10	301 ± 9*	98 ± 3***	58 ± 5	3.8 ± 0.7	3970 ± 270
1	10	291 ± 10††	88 ± 6†††	51 ± 9†		3430 ± 240††
19	7	281 ± 12	89 ± 5	47 ± 4	8.4 ± 2.0†	3290 ± 250
30	7	289 ± 13	92 ± 5	48 ± 4	5.3 ± 1.4	3500 ± 170
Rolipram (30 μg kg^{-1} plus 3 μg kg^{-1} min^{-1})						
−11	10	283 ± 10	83 ± 3	52 ± 5	3.1 ± 0.4	3500 ± 70
−9	10	313 ± 10***	89 ± 3**	53 ± 4		3690 ± 90**
−1	10	317 ± 8***	81 ± 3	46 ± 4*	3.6 ± 0.7	3690 ± 70***
1	10	312 ± 9	73 ± 5††	40 ± 5		3110 ± 170††
19	4	308 ± 16	79 ± 6	51 ± 9	6.0 ± 1.1†	3440 ± 120
30	3	317 ± 17	80 ± 10	54 ± 10	2.8 ± 1.5	3290 ± 110
Rolipram (100 μg kg^{-1} plus 10 μg kg^{-1} min^{-1})						
−11	10	273 ± 8	78 ± 4	45 ± 4	2.7 ± 0.3	3360 ± 100
−9	10	310 ± 10***	84 ± 4***	46 ± 4		4030 ± 180***
−1	10	316 ± 12***	77 ± 5	38 ± 2*	3.3 ± 0.6	3840 ± 130***
1	10	316 ± 13	68 ± 4†	40 ± 4		3090 ± 230††
19	4	289 ± 20	71 ± 3	37 ± 5	5.6 ± 0.8††	3410 ± 80
30	2	328 ± 15	68 ± 8	41 ± 6	6.8 ± 3.3	3190 ± 60

Each value is the mean ± s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with pre-drug (−11 min) value; † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ compared with pre-occlusion (−1 min) value, paired t test.

Table 2 Arterial blood gases and pH measured before drug administration and after 10 min of drug infusion

		pH (units)	Pco_2 (mmHg)	Po_2 (mmHg)
Control	Pre-drug	7.45 ± 0.02	36.8 ± 0.7	94.6 ± 3.2
	Post-drug	7.44 ± 0.01	37.0 ± 0.8	95.0 ± 3.8
Zaprinast	Pre-drug	7.44 ± 0.03	37.2 ± 0.3	98.8 ± 4.6
	Post-drug	7.45 ± 0.02	36.3 ± 0.4	94.7 ± 3.7
Rolipram low dose	Pre-drug	7.43 ± 0.03	36.1 ± 0.1	89.6 ± 3.0
	Post-drug	7.44 ± 0.03	36.8 ± 0.3	92.1 ± 2.9
Rolipram high dose	Pre-drug	7.44 ± 0.02	37.0 ± 0.1	99.9 ± 2.7
	Post-drug	7.44 ± 0.02	36.8 ± 0.3	100.5 ± 3.1

Each value is the mean ± s.e.mean. $n = 10$ per group.

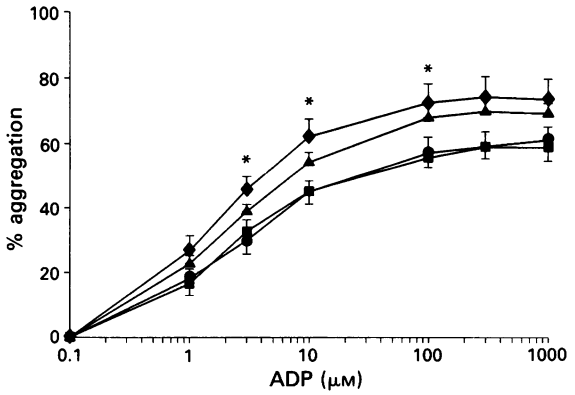

Figure 3 ADP-induced aggregation of platelets from control rabbits (●) and from rabbits that had received zaprinast (◆), low dose rolipram (▲) or high dose rolipram (■). Each value is the mean with vertical bars indicating the s.e.mean, $n = 10$ per group. * $P < 0.05$ compared with corresponding value in controls, independent t test.

were not significantly different from control. Thrombin (2 units ml^{-1}) caused the aggregation of platelets from 70 to 90% of the rabbits in each group. Although there were no significant differences in the number of rabbits in each group whose platelets responded to collagen, the magnitude of the response was significantly enhanced in platelets from rabbits pretreated with zaprinast (Table 3).

The addition of isoprenaline (10^{-8} to 10^{-5} M) to platelets prior to ADP (10^{-5} M) caused concentration-dependent inhibition of the ADP-induced platelet aggregation (Figure 4). At higher concentrations of isoprenaline (10^{-4} and 10^{-3} M) this effect started to reverse, presumably due to additional stimulation of α_2-adrenoceptors by isoprenaline. In platelets from rabbits that had received either zaprinast or rolipram, the effects of isoprenaline on ADP-induced aggregation were not different from those in controls (Figure 4). Prostacyclin also caused concentration-dependent inhibition of ADP-induced platelet aggregation, with no differences observed between the responses in platelets from control rabbits or those that had received either phosphodiesterase inhibitor (Figure 5).

Table 3 The number of rabbits whose platelets responded and the magnitude of the response to collagen ($0.2\,mg\,ml^{-1}$)

	Number responding	*% aggregation*
Control	6/10	54 ± 8
Zaprinast	8/10	86 ± 6*
Rolipram low dose	7/10	71 ± 7
Rolipram high dose	8/10	65 ± 4

Values are the mean ± s.e.mean. * $P < 0.01$ compared with control, unpaired t test.

Discussion

The results of this study indicate that zaprinast (in the dose used here, $300\,\mu g\,kg^{-1}$ plus $30\,\mu g\,kg^{-1}\,min^{-1}$) had no significant effects on ischaemia- or reperfusion-induced arrhythmias. In contrast, rolipram exacerbated arrhythmias during acute myocardial ischaemia and reperfusion, to the extent that mortality due to sustained ventricular fibrillation was significantly increased in the group that received the higher dose of rolipram. This latter result suggests that increasing cyclic AMP, by inhibition of PDE IV, in the setting of acute myocardial ischaemia has detrimental effects.

In some respects this is hardly surprising since a large amount of evidence has accumulated which implicates local increases in myocardial cyclic AMP in the genesis of ischaemia-induced arrhythmias (see Podzuweit, 1982). Both clinical and experimental studies have suggested that PDE III inhibitors such as amrinone and milrinone can have arrhythmogenic effects during myocardial ischaemia or reperfusion (Collucci *et al.*, 1986; Lukas & Ferrier, 1988; Lynch *et al.*, 1989). Although we did not see any significant arrhythmogenic activity with milrinone in our previous study in rabbits (Holbrook & Coker, 1989) it is possible that a higher dose may have exacerbated arrhythmias.

The doses of rolipram used in the present study were chosen on the basis of its reported relative potency as an inhibitor of certain PDE isoenzymes and from preliminary experiments on its effects on heart rate, arterial blood pressure and $LVdP/dt_{max}$ in anaesthetized rabbits in our laboratory. Although rolipram is slightly less potent as an inhibitor of PDE IV than milrinone is as an inhibitor of PDE III (Beavo, 1988), we decided to use rolipram in the same doses that we had used milrinone previously (Holbrook & Coker, 1989). A major reason for this decision was that we wanted to avoid causing large reductions in arterial pressure with rolipram because we had evidence that the rabbits which received milrinone and subsequently fibrillated during myocardial ischaemia were those in which milrinone caused greater decreases in blood pressure (see Holbrook & Coker, 1989). The reduction in diastolic blood pressure observed 10 min after starting administration of rolipram was almost identical to that seen previously with milrinone but in the present study there were no differences in pressures between the rolipram-treated rabbits that died and those that survived. Thus it is unlikely that the detrimental effects of rolipram on arrhythmias were due to reductions in diastolic blood pressure.

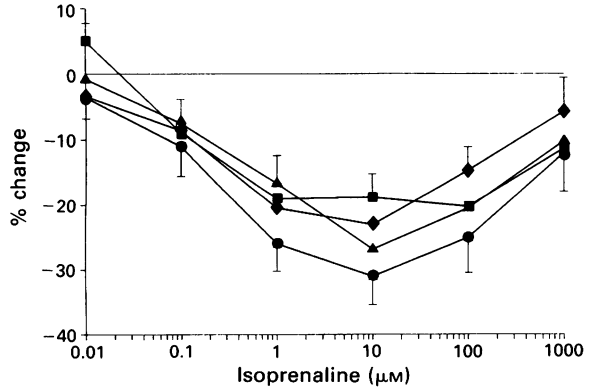

Figure 4 The effect of isoprenaline on 10^{-5} M ADP-induced aggregation of platelets from control rabbits (●) and from rabbits that had received zaprinast (◆), low dose rolipram (▲) or high dose rolipram (■). Each value is the mean with vertical bars indicating the s.e.mean, $n = 10$ per group. Some error bars have been omitted for clarity.

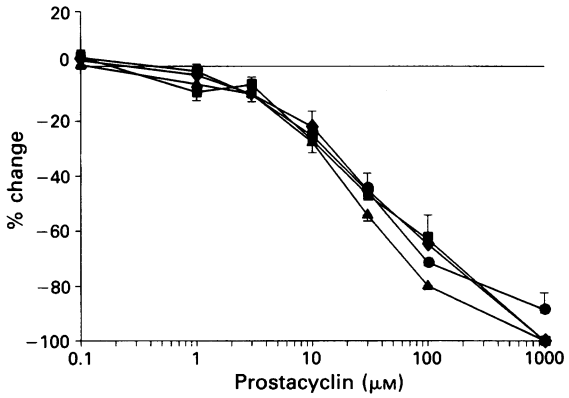

Figure 5 The effect of prostacyclin on 10^{-5} M ADP-induced aggregation of platelets from control rabbits (●) and from those that had received zaprinast (◆), low dose rolipram (▲) or high dose rolipram (■). Each value is the mean with vertical bars indicating the s.e.mean, $n = 10$ per group. Some error bars have been omitted for clarity.

In the present study, the haemodynamic effects of rolipram were different in some ways from those of milrinone, since systolic blood pressure was maintained and $LVdP/dt_{max}$ was increased by rolipram. Although PDE IV has been reported to be present in ventricular tissue in the rabbit (Kithas *et al.*, 1988; Shahid & Nicholson, 1990) and other species (Weishaar *et al.*, 1987) it has been observed that rolipram alone appears to be devoid of inotropic activity *in vitro* (Weishaar *et al.*, 1987; Muller *et al.*, 1990; Shahid & Nicholson, 1990). However, when cyclic AMP is increased either by stimulation of adenylate cyclase or by inhibition of PDE III, rolipram can have positive inotropic actions (Muller *et al.*, 1990; Shahid & Nicholson, 1990). Thus the increased myocardial contractility observed here *in vivo* with rolipram suggests that cyclic AMP may already have been elevated. This could simply be a reflection of the level of sympathetic tone in these anaesthetized rabbits or it may be related to the ability of rolipram to increase noradrenaline turnover (Wachtel, 1983). Elevated catecholamine concentrations would increase cyclic AMP further, thus providing an additional mechanism for the exacerbation of ischaemia-induced arrhythmias by rolipram.

The increases in heart rate resulting from the administration of rolipram may also have contributed to the greater incidence of ventricular fibrillation. Positive correlations between heart rate and the occurrence of ischaemia-induced and reperfusion-induced ventricular fibrillation have been reported (Coker & Parratt, 1985; Bolli *et al.*, 1986). In the control rabbits (and those which received zaprinast), 1 min after coronary artery occlusion heart rate was significantly reduced suggesting increased vagal activity. It has been proposed that the balance between sympathetic and parasympathetic tone may be an important factor in arrhythmogenesis during ischaemia (Zaza & Schwartz, 1985), with increased vagal activity being associated with reductions in ventricular fibrillation (Collins & Billman, 1989; Billman, 1990). No ischaemia-induced reductions in heart rate were observed in the rabbits that had received rolipram which again suggests that rolipram may have elevated sympathetic tone.

It is possible that increased oxygen demand (due to increased heart rate and myocardial contractility) may have contributed to the increased severity of arrhythmias observed

in rabbits which received rolipram. However, calculation of the rate-pressure product, an estimate of oxygen demand, revealed that zaprinast increased this parameter as much as either dose of rolipram (see Table 1 for mean rates and pressures). Since zaprinast did not exacerbate arrhythmias it seems unlikely that increased oxygen demand could account for the effect of rolipram.

Another possible explanation for the difference between the effects of rolipram and milrinone on arrhythmias involves platelets. In our previous study, milrinone did not alter arrhythmias but had significant antiplatelet activity (Holbrook & Coker, 1989). Under identical conditions, pretreatment of rabbits with rolipram had no effect on platelet aggregation measured *ex vivo*. The lack of activity of rolipram is probably due to the absence of PDE IV in platelets (Simpson *et al.*, 1988; Shahid *et al.*, 1990). We have been unable to demonstrate the presence of a rolipram-sensitive, cyclic GMP-insensitive, cyclic AMP-specific PDE (PDE IV) in rabbit platelets (Holbrook *et al.*, 1990). Thus rolipram, unlike milrinone, may exacerbate ischaemia-induced arrhythmias because the arrhythmogenic actions of increasing myocardial cyclic AMP cannot be offset by the antiarrhythmic consequences of reducing platelet aggregation (via increased platelet cyclic AMP).

The enhancement of ADP- and collagen-induced aggregation of platelets obtained from the rabbits that had received zaprinast was surprising since increases in cyclic GMP (e.g. induced by nitrovasodilators or endothelium-derived relaxing factor (EDRF)) have been associated with reduced platelet aggregation (Hogan *et al.*, 1988; Lidbury *et al.*, 1989; Willis *et al.*, 1989). Similarly the haemodynamic effects of zaprinast were unexpected. Zaprinast has been shown to decrease arterial blood pressure in anaesthetized and conscious rats (Banerjee *et al.*, 1989; McMahon *et al.*, 1989; Dundore *et al.*, 1990) albeit at much higher doses than that used here. The dose of zaprinast was chosen on the basis of its reported activity against PDE V relative to its ability to inhibit other PDE isoenzymes (Beavo, 1988). We decided not to use a higher dose of zaprinast since we wanted to use a dose which would only increase cyclic GMP by inhibition of PDE V. It is possible, however, that the dose used ($300\,\mu g\,kg^{-1}$ plus $30\,\mu g\,kg^{-1}\,min^{-1}$) was insufficient to increase cyclic GMP *in vivo*. The data from the rat studies quoted above, which have been published since we started our experiments, do suggest that *in vivo*, high doses of zaprinast are required to produce significant decreases in blood pressure. It is interesting to note, however, that Dundore *et al.* (1990) reported a small but significant increase in arterial pressure with $3\,mg\,kg^{-1}$ zaprinast, which was similar to the increase in blood pressure that we observed here in rabbits, but they did not suggest any explanation for this effect of zaprinast. It is possible that the small increases in heart rate, arterial blood pressure and platelet aggregation observed in the present study were due to increased sympathetic tone, but whether this is a direct or reflex effect of zaprinast remains to be determined. Alternatively, the apparently enhanced aggregation of platelets from the rabbits that received zaprinast may simply be due to variability between groups.

In summary, therefore, we cannot draw any firm conclusions about the actions of zaprinast since only one dose was examined and no significant effects on arrhythmias were observed. Rolipram, however, was devoid of anti-platelet activity, increased heart rate and $LVdP/dt_{max}$ and increased the severity of arrhythmias induced by acute myocardial ischaemia and reperfusion in anaesthetized rabbits.

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