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Comparison of the effects of isobutylmethylxanthine and milrinone on ischaemia-induced arrhythmias and platelet aggregation in anaesthetized rabbits

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1 The aim of this study was to compare the effects of the non-selective phosphodiesterase (PDE) inhibitor, isobutylmethylxanthine (IBMX) and the selective PDE III inhibitor, milrinone, in a rabbit model of acute myocardial ischaemia.

2 Coronary artery occlusion caused changes in the ST-segment of the ECG and ectopic activity in all control rabbits. Ventricular fibrillation occurred in 10 out of 14 (71%) of these animals. Pretreatment with IBMX $100\,\mu g\,kg^{-1}$ plus $10\,\mu g\,kg^{-1}\,min^{-1}$, starting 10 min before coronary artery occlusion, reduced ischaemia-induced ST-segment changes and ventricular fibrillation occurred in only 10% of this group ($n = 10$). A similar dose of milrinone had no antiarrhythmic activity, whereas with a lower dose of milrinone, $30\,\mu g\,kg^{-1}$ plus $3\,\mu g\,kg^{-1}\,min^{-1}$ ($n = 10$), only 30% of rabbits fibrillated and ST-segment changes were attenuated.

3 Acute administration of both IBMX and milrinone reduced arterial blood pressure. With the higher dose of milrinone a significant effect was still present after 10 min of drug infusion. A greater hypotensive response to the higher dose of milrinone was observed in the rabbits which subsequently fibrillated during ischaemia. A marked tachycardia was also observed after administration of the higher dose of milrinone.

4 At the end of the experiment platelet aggregation was studied *ex vivo*. ADP-induced aggregation was reduced by pretreatment of the rabbits with milrinone but not IBMX. Both PDE inhibitors enhanced the ability of isoprenaline to inhibit ADP-induced platelet aggregation but milrinone was more effective, particularly at the higher dose.

5 The results demonstrate that IBMX was antiarrhythmic but that this activity was not directly related to inhibition of platelet aggregation. Adverse haemodynamic effects may explain the failure of milrinone to have similar activity during myocardial ischaemia.

Introduction

Several novel phosphodiesterase (PDE) inhibitors are being developed currently for use in the treatment of heart failure. Drugs such as milrinone (Alousi *et al.*, 1983) have both positive inotropic and vasodilator activity which may contribute to their therapeutic effects. However, patients with heart failure may also have or be at risk of developing myocardial ischaemia. It is possible that because of their mechanism of action, PDE inhibitors may have a significant influence on the development of myocardial ischaemia. For example, increasing adenosine 3′:5′-cyclic monophosphate (cyclic AMP) in the myocardium may precipitate arrhythmias (Podzuweit, 1982) thus exacerbating ischaemia. In contrast, elevated cyclic AMP in platelets would inhibit their aggregation (Packham & Mustard, 1980) and this may protect against ischaemia since inappropriate platelet aggregation has been implicated in the genesis of sudden cardiac death (Haerem, 1972).

Most of the novel PDE inhibitors have selective activity on PDE III, an isoenzyme found in cardiac muscle which hydrolyses only cyclic AMP (Weishaar *et al.*, 1986). Isobutylmethylxanthine (IBMX) is a non-selective PDE inhibitor which will prevent the breakdown of cyclic GMP as well as cyclic AMP (Mushlin *et al.*, 1981). Inhibition of the hydrolysis of cyclic GMP may have additional effects during acute myocardial ischaemia. For example, the activity of endothelium-derived relaxing factor (EDRF) will be

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prolonged thus enhancing vasodilatation (Martin *et al.*, 1986) and inhibiting platelet aggregation (Hogan *et al.*, 1988). Thus the aim of the present study was to compare the effects of selective and non-selective PDE inhibitors on arrhythmogenesis and platelet aggregation in anaesthetized rabbits subject to acute myocardial ischaemia.

Methods

Animal preparation

Male New Zealand White rabbits (1.9 to 3.1 kg) purchased from Hylyne, Cheshire, were prepared for coronary artery occlusion by techniques that have been described in detail previously (Coker, 1989). Anaesthesia was induced by i.m. injection of diazepam 2.5 mg kg^{-1} followed by Hypnorm 0.4 ml kg^{-1}. A Lead II ECG was recorded along with arterial blood pressure, left ventricular pressure and its first derivative with time. A catheter was also placed in the vena cava (via the right femoral vein) for drug administration. After cannulation of the trachea, sodium pentobarbitone was administered i.v. (24 to 48 mg kg^{-1}) to maintain anaesthesia. 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 were measured with a Corning 158 blood gas/pH analyser 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 which supplies the majority of the free left ventricular wall in the rabbit.

Experimental protocol

After completion of the surgical preparation a stabilisation period of at least 15 min was allowed. Any rabbits which had arrhythmias or ST-segment changes at this time were excluded from the study (4 out of a total of 48). Drugs were then administered i.v. as an initial bolus dose followed immediately by a continuous infusion. IBMX ($n = 10$) was given at a dose of 100 $\mu g\,kg^{-1}$ plus 10 $\mu g\,kg^{-1}\,min^{-1}$ for the duration of the experiment. Milrinone ($n = 10$) was also administered at this dose with a further group ($n = 10$) receiving a lower dose of 30 $\mu g\,kg^{-1}$ plus 3 $\mu g\,kg^{-1}\,min^{-1}$. Rabbits in the control group ($n = 14$) received equivalent volumes of 0.9% w/v NaCl solution. Ten min after starting drug administration the coronary artery was occluded. After 20 min of myocardial ischaemia 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, blood was removed from the right ventricle via a 21G needle so that platelet aggregation could be studied *ex vivo*. The heart was also 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. The unstained tissue was dissected from the rest of the ventricles and both ischaemic and normal regions were weighed. The 'area at risk' was calculated as a percentage of the total ventricular mass. Ischaemia- and reperfusion-induced arrhythmias were analysed as described previously (Coker, 1989).

Platelet aggregation

Blood samples were placed in plastic tubes containing 3.8% w/v sodium 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 were centrifuged at 2000 *g* to give platelet poor plasma (PPP). After performing a platelet count, PRP was diluted with PPP to give a final platelet count of 2.5 to 3.0 $\times$ 10^5 μl^{-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.

Drugs

IBMX and milrinone were gifts from Organon Laboratories Ltd., Newhouse and were made up fresh in saline each day. Adenosine diphosphate (ADP), isoprenaline, collagen, arachidonic acid and thrombin were purchased from Sigma, Poole; sodium pentobarbitone (Sagatal) from May and Baker, Dagenham, diazepam from the Royal Liverpool Hospital Pharmacy and Hypnorm (which contains 0.315 mg fentanyl citrate and 10 mg fluanisone per ml) from Janssen, Wantage.

Statistics

Where appropriate, values have been expressed as the mean $\pm$ s.e.mean of n experiments. Comparisons within groups were made with a paired t test and between groups with an unpaired t test. A probability of $P < 0.05$ was considered to be significant. The incidence of events was compared by Fisher's exact test.

Results

Arrhythmias and ST-segment changes

Ischaemia-induced ventricular premature beats (VPBs) occurred in all of the rabbits in the control and high dose milrinone groups, 80% of the IBMX group and only 60% of the group that received the lower dose of milrinone. In this model ventricular tachycardia (VT, defined as 4 or more consecutive VPBs) is a rare event. It was observed in only 2 control rabbits and in 2 rabbits that had been pretreated with the higher dose of milrinone. In contrast, ventricular fibrillation occurred in more than half of the control animals, usually with little or no warning and commonly between 8 and 12 min post-occlusion. Spontaneous reversal of ventricular fibrillation to normal sinus rhythm was observed in 6 rabbits (4 controls, 1 IBMX and 1 low dose milrinone). Figure 1 illustrates clearly that pretreatment of rabbits with IBMX before coronary artery occlusion reduced ventricular fibrillation and mortality during the first 20 min of myocardial ischaemia. Milrinone given at the same dose, $100\,\mu g\,kg^{-1}$ plus $10\,\mu g\,kg^{-1}\,min^{-1}$ was devoid of antiarrhythmic activity, however, ventricular fibrillation and mortality were observed less frequently in the group that received the lower dose of milrinone, $30\,\mu g\,kg^{-1}$ plus $3\,\mu g\,kg^{-1}\,min^{-1}$ (Figure 1).

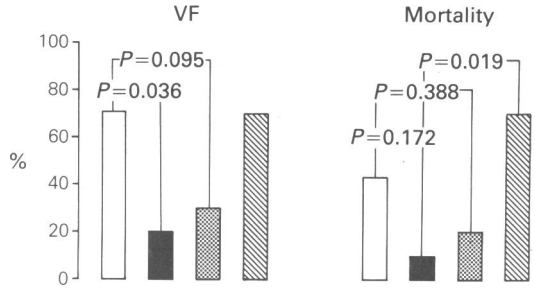

Figure 1 The incidence of ventricular fibrillation (VF) and mortality resulting from coronary artery occlusion in control rabbits (open columns, $n = 14$) and in those which received isobutylmethylxanthine (IBMX) $100\,\mu g\,kg^{-1}$ plus $10\,\mu g\,kg^{-1}\,min^{-1}$ (solid columns, $n = 10$), milrinone $30\,\mu g\,kg^{-1}$ plus $3\,\mu g\,kg^{-1}\,min^{-1}$ (stippled columns, $n = 10$) or milrinone $100\,\mu g\,kg^{-1}$ plus $10\,\mu g\,kg^{-1}\,min^{-1}$ (hatched columns, $n = 10$). *P* values were determined by Fisher's exact test.

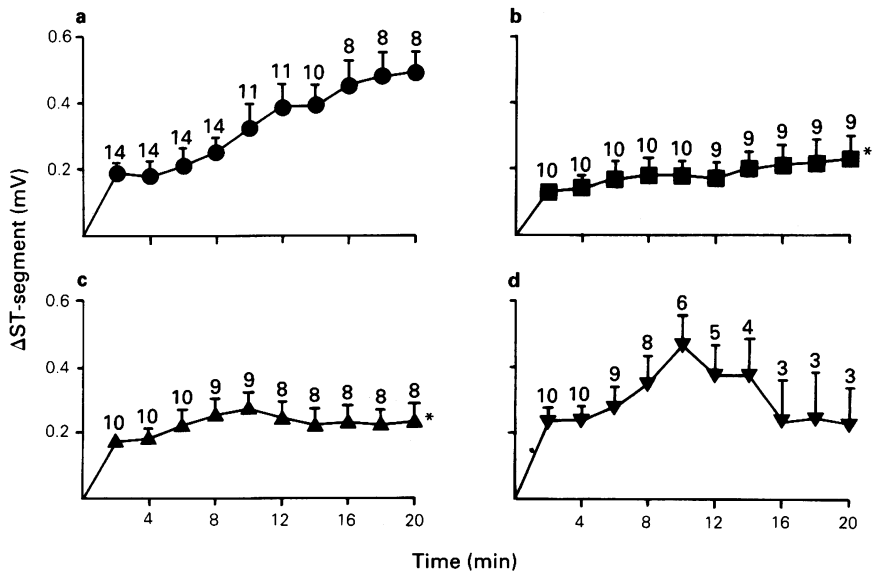

Figure 2 ST-segment changes during the first 20 min of coronary artery occlusion in control rabbits (a; ●) and in those which received isobutylmethylxanthine (b; ■), low dose milrinone (c; ▲) or high dose milrinone (d; ▼). Each value is the mean with vertical bars indicating the s.e.mean. Numbers above each point indicate the number of survivors at that time.
*$P < 0.05$ compared with the control value at 20 min. Comparisons were also made at the 8 min time point, which is before the onset of arrhythmias in the majority of rabbits, but no significant differences were found, 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	14	277 ± 9	75 ± 4	45 ± 3	2.2 ± 0.2	3290 ± 160
−1	14	278 ± 10	75 ± 3	45 ± 3	2.3 ± 0.3	3250 ± 160
1	14	272 ± 9	68 ± 3†††	40 ± 3†		2730 ± 180††
19	8	282 ± 15	78 ± 4	48 ± 4	7.8 ± 2.0†	3280 ± 210
30	8	289 ± 15	79 ± 3	48 ± 3	3.9 ± 0.7	3310 ± 180
IBMX ($100\,\mu g\,kg^{-1}$ plus $10\,\mu g\,kg^{-1}\,min^{-1}$)						
−11	10	280 ± 14	78 ± 5	45 ± 3	3.4 ± 0.4	3300 ± 240
−1	10	290 ± 15**	76 ± 3	42 ± 2	3.3 ± 0.5	3310 ± 230
1	10	285 ± 14	67 ± 4††	38 ± 3†		2790 ± 240††
19	9	300 ± 10	74 ± 4	44 ± 3	5.7 ± 1.8	2940 ± 280
30	9	311 ± 11	73 ± 5	41 ± 3	4.7 ± 1.8	2860 ± 310
Milrinone ($30\,\mu g\,kg^{-1}$ plus $3\,\mu g\,kg^{-1}\,min^{-1}$)						
−11	10	276 ± 6	76 ± 4	48 ± 3	2.3 ± 0.3	3480 ± 100
−1	10	290 ± 6***	72 ± 4*	46 ± 3	2.0 ± 0.3	3530 ± 70
1	10	283 ± 5	65 ± 3	42 ± 4		3230 ± 110††
19	8	283 ± 6	69 ± 3	47 ± 3	3.6 ± 0.4†	3360 ± 140
30	8	283 ± 7	66 ± 4	45 ± 4	2.3 ± 0.3	3410 ± 150
Milrinone ($100\,\mu g\,kg^{-1}$ plus $10\,\mu g\,kg^{-1}\,min^{-1}$)						
−11	10	279 ± 12	79 ± 6	50 ± 6	3.2 ± 0.7	3140 ± 180
−1	10	322 ± 13***	63 ± 6***	41 ± 5*	3.4 ± 0.5	3240 ± 210
1	10	316 ± 12	54 ± 7††	38 ± 6		2700 ± 200††
19	3	338 ± 33	71 ± 14	49 ± 10	2.8 ± 0.6	2670 ± 580
30	3	335 ± 33	68 ± 16	48 ± 9	2.0 ± 0.9	2960 ± 570

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.

Coronary artery occlusion caused changes in the ST-segment of the ECG in all rabbits, except one that had been pretreated with IBMX. The magnitude of the ischaemia induced ST-segment changes was reduced by IBMX and the lower dose of milrinone but not by the higher dose of milrinone (Figure 2). The reduction in the magnitude of the ST-segment changes was confined to the latter half of the occlusion period.

These effects were not due to variations in the size of the occluded zone. The areas at risk were 39 ± 3% of total ventricular mass in the controls; 37 ± 1% in the IBMX group; 43 ± 3% in the low dose milrinone group and 41 ± 2% in the high dose milrinone group.

In all surviving rabbits release of the ligature around the coronary artery after 20 min of ischaemia resulted in immediate reduction, but not complete reversal, of the ischaemia-induced ST-segment changes. This was accompanied by reperfusion-induced VPBs in 88% of the control group ($n = 8$), 55% of the IBMX group ($n = 9$), 50% of the low dose milrinone group ($n = 8$) and 100% of the high dose milrinone group ($n = 3$). Only one rabbit (which had received the lower dose of milrinone) had VT following reperfusion. The reperfusion-induced arrhythmias were rapid in onset and of short duration, normally terminating within 2 to 3 min. In this study none of the rabbits fibrillated after reperfusion.

Haemodynamics and blood gases

Administration of the initial bolus dose of the PDE inhibitors reduced arterial blood pressure. As the subsequent infusion of drug continued, arterial blood pressure returned toward pre-drug values and the heart rate gradually increased. After 10 min of administration of the higher dose of milrinone there was still a significant reduction in both systolic and diastolic pressure (Table 1). In addition, subdivision of this group into those which fibrillated during coronary artery occlusion and those which survived revealed that those which fibrillated had lower arterial pressures, after drug administration but before coronary artery occlusion, than the survivors; 52 ± 6/36 ± 5 mmHg, $n = 7$ and 79 ± 13/56 ± 6 mmHg, $n = 3$, respectively. No such difference was observed in the control group where those that survived ($n = 8$) had pre-occlusion arterial pressures of 76 ± 3/45 ± 3 mmHg and the values in those that

died ($n = 6$) were 74 ± 6/45 ± 4 mmHg. The higher dose of milrinone also caused a marked tachycardia which was sustained for the duration of the experiment. In the control group, coronary artery occlusion reduced arterial pressure and left ventricular *dP/dt max* transiently and caused a sustained increase in left ventricular end-diastolic pressure (LVEDP). Neither IBMX nor milrinone prevented the initial reduction in blood pressure but both drugs attenuated the ischaemia-induced increase in LVEDP (Table 1).

In control rabbits, before administration of saline the arterial blood gas values were; P_{O_2} 88 ± 3 mmHg; P_{CO_2} 38 ± 1 mmHg; pH 7.44 ± 0.03 units ($n = 14$). Similar values were observed in the other groups. Administration of IBMX or either dose of milrinone did not alter blood gases or pH.

Platelet aggregation

Platelets taken from all of the rabbits aggregated in response to ADP and to arachidonic acid (10^{-3} M). Thrombin (2 units ml^{-1}) caused platelet aggregation in 70 to 80% of the samples in each group whereas the number of rabbits whose platelets responded to collagen (0.2 mg ml^{-1}) varied; 64% in controls, 40% IBMX, 60% low dose milrinone and 30% of the group that received the higher dose of milrinone. Although all platelets responded to ADP the magnitude of this response was reduced significantly by the higher dose of milrinone whereas IBMX had no effect (Figure 3).

Addition of isoprenaline to the platelets prior to ADP (10^{-5} M) did not reduce the response of platelets from the control rabbits and with high concentrations of isoprenaline, enhancement of ADP-induced aggregation was observed presumably due to stimulation of α-adrenoceptors by isoprenaline. In the platelets from the rabbits that had received a PDE inhibitor, however, isoprenaline reduced ADP-induced platelet aggregation (Figure 4). Milrinone was more effective than IBMX in this situation.

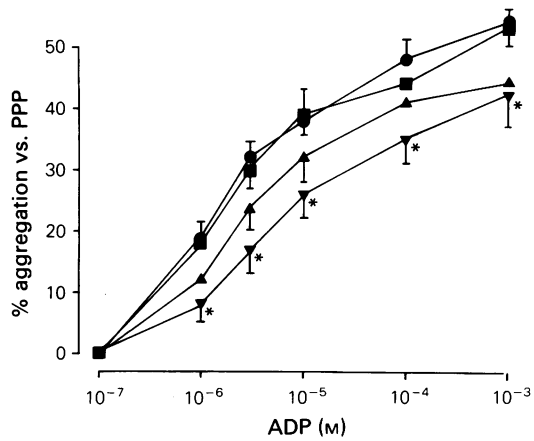

Figure 3 ADP-induced aggregation of platelets obtained from control rabbits (●) and from those which had received isobutylmethylxanthine (■), low dose milrinone (▲) or high dose milrinone (▼). Each value is the mean with vertical bars indicating the s.e.mean. Some error bars have been omitted for clarity. *$P < 0.05$ compared with the corresponding control value, independent t test.

Discussion

The results of this study indicate that the non-selective PDE inhibitor IBMX had significant anti-arrhythmic activity in anaesthetized rabbits subject to acute myocardial ischaemia. In contrast, a dose of the PDE III inhibitor milrinone, with similar haemodynamic actions appeared to be less effective and a higher dose of milrinone was completely devoid of benefit during ischaemia. A different profile of activity was observed, however, when platelet aggregation was studied *ex vivo*. In these experiments milrinone reduced platelet aggregation induced by ADP and revealed the β-adrenoceptor-mediated ability of isoprenaline to inhibit platelet aggregation. The higher dose of milrinone had greater activity than the lower dose, whereas IBMX had little effect. Consideration of these facts suggests that the antiarrhythmic activity of IBMX is not related to the ability of PDE inhibitors to reduce platelet aggregation. However, in view of the limited range of drugs and doses studied, firm conclusions cannot be drawn at this stage.

It is also possible that other adverse effects of milrinone may have counteracted any benefit resulting from its antiplatelet activity. The higher dose of milrinone produced a marked tachycardia and it has been shown that there is a positive correlation between heart rate and the prevalence of ischaemia-induced ventricular fibrillation (Bolli *et al.*, 1986). A significant reduction in arterial blood pressure was also observed after administration of the higher dose of milrinone and the rabbits which subsequently fibrillated had lower pressures than those which survived. Systemic vasodilatation would have reduced arterial driving pressure and thus reduced blood flow to the ischaemic myocardium (Coker & Parratt, 1981). If milrinone also caused direct dilatation of coronary blood vessels supplying the normal region of the myocardium this could result in a 'coronary steal' effect (Parratt *et al.*, 1980) and further exacerbation of ischaemia. A related compound, amrinone, has been reported to exacerbate ischaemia in dogs

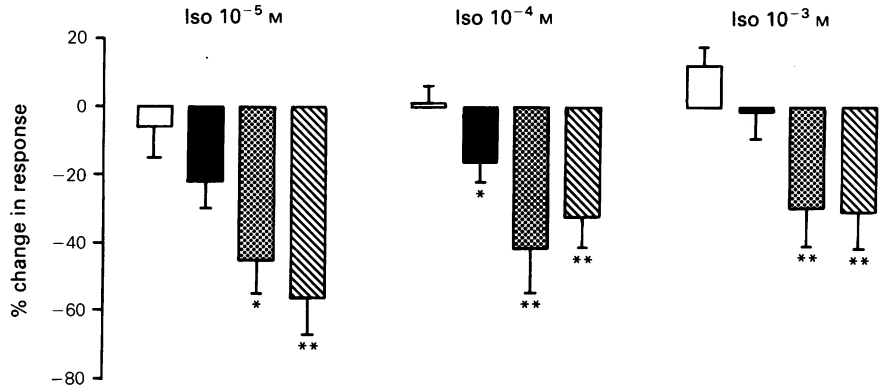

Figure 4 The effect of isoprenaline (Iso) on platelet aggregation induced by ADP (10^{-5} M) in platelet-rich plasma (PRP) from control rabbits (open columns, $n = 14$) and those which were pretreated with isobutylmethylxanthine (solid columns, $n = 10$), low dose milrinone (stippled columns, $n = 10$) or high dose milrinone (hatched columns, $n = 10$).
Each value is the mean with vertical bars indicating the s.e.mean.
$*P < 0.05$, $**P < 0.01$ compared with control, independent t test.

subject to acute coronary artery occlusion (Rude *et al.*, 1980). These arguments may therefore explain why the higher dose of milrinone did not protect against ischaemia-induced ST-segment changes and ventricular fibrillation. The lower dose of milrinone, however, had very similar haemodynamic effects to IBMX, greater antiplatelet activity but less antiarrhythmic effect. This suggests that other factors may need to be considered to explain the different effects of these two drugs observed in the present study.

Electrophysiological studies have suggested that milrinone and amrinone sensitize isolated cardiac muscle to the arrhythmogenic effects of reperfusion following ischaemia (Lukas & Ferrier, 1988). A review of clinical studies also indicates that milrinone and amrinone exacerbate ventricular arrhythmias (Colucci *et al.*, 1986). Since considerable experimental evidence exists to support the theory that cyclic AMP precipitates arrhythmias (Podzuweit, 1982) it is possible that the arrhythmogenic activity of PDE III inhibitors is related to their ability to increase cyclic AMP. However, non-selective PDE inhibitors such as IBMX will also increase cyclic AMP and yet antiarrhythmic activity was observed in the present study. IBMX has been reported to exacerbate ischaemia-induced arrhythmias in anaesthetized rats (Kane *et al.*, 1985) but the dose of IBMX which had this effect was much higher (approximately 10 fold) than that used in the present study and it caused a marked tachycardia. Kane *et al.* (1985) also showed that pretreatment with IBMX increased cyclic AMP in the ischaemic myocardium by approximately 33% whereas cyclic GMP concentrations were increased by more than 50%. Thus it is possible that the dose of IBMX used in the present study was insufficient to increase cyclic AMP to arrhythmogenic concentrations. Alternatively, a concomitant increase in cyclic GMP may have had some influence.

Although IBMX did not cause marked systemic vasodilatation it may have had a beneficial effect on blood flow to the ischaemic myocardium via its ability to increase cyclic GMP. A recent study (Nichols *et al.*, 1988) has demonstrated that coronary vasodilator reserve is impaired in regions of the myocardium that had previously been subject to ischaemia for a period of 1 hour. It was concluded that this effect may be related to loss of EDRF. If this EDRF was released during the ischaemic period, IBMX would potentiate its vasodilator effect (Martin *et al.*, 1986). Thus, unlike milrinone, IBMX could preferentially increase blood flow to the ischaemic myocardium rather than causing marked systemic vasodilatation and possible coronary steal.

The effects of cyclic GMP on cardiac muscle have received far less attention than those of cyclic AMP. The results of Kane *et al.* (1985) show that dibutyryl cyclic GMP increased arrhythmias in rats subject to coronary artery occlusion but dibutyryl cyclic AMP had greater effects. In contrast, cyclic GMP and cyclic AMP have been claimed to have opposite effects on the slow inward calcium current in single heart cells (Hartzell & Fischmeister, 1986). Obviously further work is required to determine whether increases in cyclic GMP are an important factor in the antiarrhythmic activity of IBMX.

Recently, two subclasses of PDE III have been identified in cardiac muscle, only one of which is inhibited by selective PDE inhibitors like milrinone

(Weishaar *et al.*, 1987). It has been shown in other tissues that IBMX inhibits both of these subclasses of PDE III (Elks & Manganiello, 1984). Thus it is possible that the beneficial effects of IBMX during myocardial ischaemia may be related to its effect on the milrinone-insensitive subclass of PDE III. Another important point that has emerged from the work on subclasses of PDE III in cardiac muscle is that there are significant species differences (Weishaar *et al.*, 1987). In view of this it would be sensible to exercise caution when comparing the present study with results obtained in other species.

One final point that should be considered is that the antiarrhythmic activity of IBMX may be completely independent of its ability to inhibit the breakdown of cyclic nucleotides. Although the precise mechanism(s) underlying the beneficial effects of IBMX remain to be clarified the results of the present study suggest that inhibition of platelet aggregation alone is not a major contributory factor.

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References

ALOUSI, A.A., CANTER, J.M., MONTENARO, M.J., FORT, D.J. & FERRARI, R.A. (1983). Cardiotonic activity of milrinone, a new potent cardiac bypyridine, on normal and failing heart of experimental animals. *J. Cardiovasc. Pharmacol.*, **5**, 792–803.

BOLLI, R., FISHER, D.J. & ENTMAN, M.L. (1986). Factors that determine the occurrence of arrhythmias during acute myocardial ischemia. *Am. Heart J.*, **111**, 261–270.

COKER, S.J. (1989). Anesthetized rabbit as a model for ischemia and reperfusion induced arrhythmias: effects of quinidine and bretylium. *J. Pharmacol. Methods*, (in press).

COKER, S.J. & PARRATT, J.R. (1981). Prostacyclin-induced changes in coronary blood flow and oxygen handling in the normal and acutely ischaemic canine myocardium. *Basic Res. Cardiol.*, **76**, 457–462.

COLUCCI, W.S., WRIGHT, R.F. & BRAUNWALD, E. (1986). New positive inotropic agents in the treatment of congestive heart failure. Mechanisms of action and recent clinical developments. *New Engl. J. Med.*, **314**, 349–358.

ELKS, M.L. & MANGANIELLO, V.C. (1984). Selective effect of phosphodiesterase inhibitors on different phosphodiesterases, adenosine 3',5' monophosphate metabolism and lipolysis in 3T3-L1 adipocytes. *Endocrinology*, **115**, 1262–1268.

HAEREM, J.W. (1972). Platelet aggregates in intramyocardial vessels of patients dying suddenly and unexpectedly of coronary artery disease. *Atherosclerosis*, **15**, 199–213.

HARTZELL, H.C. & FISCHMEISTER, R. (1986). Opposite effects of cyclic GMP and cyclic AMP on Ca^{2+} current in single heart cells. *Nature*, **323**, 273–275.

HOGAN, J.C., LEWIS, M.J. & HENDERSON, A.H. (1988). In vivo EDRF activity influences platelet function. *Br. J. Pharmacol.*, **94**, 1020–1022.

KANE, K.A., MORCILLO-SANCHEZ, E.J., PARRATT, J.R., RODGER, I.W. & SHAHID, M. (1985). The relationship between coronary artery occlusion-induced arrhythmias and myocardial cyclic nucleotide levels in the anaesthetized rat. *Br. J. Pharmacol.*, **84**, 139–145.

LUKAS, A. & FERRIER, G.R. (1988). Electrophysiological effects of amrinone and milrinone in an isolated canine cardiac tissue model of ischemia and reperfusion. *J. Pharmacol. Exp. Ther.*, **244**, 348–354.

MARTIN, W., FURCHGOTT, R.F., VILLANI, G.M. & JOTHIANANDAN, D. (1986). Phosphodiesterase inhibitors induce endothelium-dependent relaxation of rat and rabbit aorta by potentiating the effects of spontaneously released endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther.*, **237**, 539–547.

MUSHLIN, P., BOERTH, R.C. & WELLS, J.N. (1981). Xanthine derivatives that selectively inhibit cyclic GMP hydrolysis potentiate cardiac contractile effects of isoproterenol but not those of bethanecol. *Mol. Pharmacol.*, **20**, 190–194.

NICHOLS, W.W., MEHTA, J.L., DONNELLY, W.H., LAWSON, D., THOMPSON, L. & TER REIT, M. (1988). Reduction in coronary vasodilator reserve following coronary occlusion and reperfusion in anesthetized dog: role of endothelium-derived relaxing factor, myocardial neutrophil infiltration and prostaglandins. *J. Mol. Cell. Cardiol.*, **20**, 943–954.

PACKHAM, M.A. & MUSTARD, J.F. (1980). Pharmacology of platelet-affecting drugs. *Circulation*, **62**, suppl. V, 26–41.

PARRATT, J.R., MARSHALL, R.J. & LEDINGHAM, I. McA. (1980). Interventions for improving blood flow, oxygen availability and the balance between oxygen supply and demand in the acutely ischaemic myocardium. *J. Physiol. (Paris)*, **76**, 791–803.

PODZUWEIT, T. (1982). Early arrhythmias resulting from acute myocardial ischaemia; possible role of cyclic AMP. In *Early Arrhythmias resulting from Myocardial Ischaemia. Mechanisms and Prevention by Drugs.* ed. Parratt, J.R. pp. 171–198. London: Macmillan.

RUDE, R.E., KLONER, R.A., MAROKO, P.R., KHURI, S., KARAFFA, S., DE BOER, L.W.V. & BRAUNWALD, E. (1980). Effects of amrinone on experimental acute myocardial ischaemic injury. *Cardiovasc. Res.*, **14**, 419–427.

WEISHAAR, R.E., BURROWS, S.D., KOBYLARZ, D.C., QUADE, M.M. & EVANS, D.B. (1986). Multiple molecular forms of cyclic nucleotide phosphodiesterase in cardiac and smooth muscle and in platelets. Isolation, characterization and effects of various reference phosphodiesterase inhibitors and cardiotonic agents. *Biochem. Pharmacol.*, **35**, 787–800.

WEISHAAR, R.E., KOBYLARZ-SINGER, D.C., STEFFEN, R.P. & KAPLAN, H.R. (1987). Subclasses of cyclic AMP-specific phosphodiesterase in left ventricular muscle and their involvement in regulating cardiac contractility. *Circ. Res.*, **61**, 539–547.

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