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The relationship between coronary artery occlusion-induced arrhythmias and myocardial cyclic nucleotide levels in the anaesthetized rat

K.A. Kane[1], E.J. Morcillo-Sanchez*, J.R. Parratt, I.W. Rodger & M. Shahid**

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, Department of Pharmacology,* University of Alcala de Henares, Spain and Organon Laboratories**, Newhouse, Lanarkshire

1 The aims of this study were to determine whether a relationship exists between the occurrence of coronary artery occlusion-induced arrhythmias in the anaesthetized rat and the levels of cyclic AMP and cyclic GMP in both normal and ischaemic myocardium, and to assess whether such arrhythmias were modified by pretreatment with the phosphodiesterase inhibitors, quazodine and isobutyl methylxanthine (IBMX), or with the butyryl derivatives of cyclic AMP and cyclic GMP.

2 At 5 min after coronary artery ligation (when only a few arrhythmias had occurred) both cyclic AMP and cyclic GMP levels were elevated in normal myocardium whereas in ischaemic tissue only cyclic AMP was raised.

3 At the peak of the arrhythmic activity and after cessation of the arrhythmias, i.e. at 10 and 30 min post-ligation respectively, levels of both nucleotides had fallen in ischaemic although not in normal tissue.

4 The severity of these occlusion-induced arrhythmias was exacerbated by pretreatment intravenously with quazodine, IBMX, dibutyryl cyclic AMP and dibutyryl cyclic GMP.

5 Pretreatment with IBMX was also shown to elevate significantly both cyclic AMP and cyclic GMP content of left ventricular tissue before occlusion.

6 None of the drug pretreatments markedly affected mean arterial blood pressure but heart rate was significantly increased following quazodine and IBMX administration.

7 We conclude that in the pentobarbitone-anaesthetized rat the occurrence of occlusion-induced arrhythmias was not accompanied by a rise in cyclic nucleotide content of the ischaemic myocardium but agents which may elevate either myocardial cyclic AMP or cyclic GMP levels exacerbate such arrhythmias.

Introduction

Since the observation by Wollenberger *et al.* (1969) that a rapid stimulation of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) production occurs in dog myocardium subsequent to the arrest of blood flow, the relationship between myocardial ischaemia and the level of cyclic AMP in the heart has been extensively studied. For instance, in the dog (Krause *et al.*, 1978), the cat (Corr *et al.*, 1978) and the baboon (Podzuweit *et al.*, 1978) coronary artery ligation results in a rise in myocardial cyclic AMP levels, the time course and magnitude of which is species dependent. These results together with other findings on the arrhythmogenic activity of phosphodiesterase inhibition (Ueda & Okumura, 1971; Sugiura *et al.*, 1979) have led to the suggestion that the rise in myocardial cyclic AMP levels may be causally related to the occurrence of ventricular fibrillation (Podzuweit *et al.*, 1976) and other ventricular arrhythmias (Opie & Lubbe, 1979).

Both ventricular fibrillation and marked extrasystolic activity occur in the anaesthetized rat, following occlusion of the left main coronary artery (Clark *et al.*, 1980). One of the aims of this study was to determine, in this species, the relationship, if any, between the occurrence of these arrhythmias and the levels of cyclic nucleotides in normal and ischaemic myocardium. Cyclic GMP (guanosine 3′,5′-cyclic monophosphate) as well as cyclic AMP levels were

[1]Correspondence.

measured since it has been shown that transient rises in myocardial cyclic GMP levels also occur following ischaemia (Nesher *et al.*, 1977; Busuttil *et al.*, 1978). The other aim of this study was to investigate whether or not the arrhythmias observed in this experimental model were affected by the administration of the phosphodiesterase inhibitors quazodine (Amer & Browder, 1971) and isobutyl methylxanthine (Mushlin *et al.*, 1981a) or by the butyryl derivatives of cyclic AMP and cyclic GMP.

Methods

Coronary artery ligation and evaluation of arrhythmias

Male Sprague-Dawley rats (250–350 g body weight) were anaesthetized with pentobarbitone sodium, 60 mg kg^{-1} intraperitoneally, with small additional amounts administered intravenously if necessary. Catheters were placed in the left common carotid artery and in a jugular vein for measurement of systemic arterial blood pressure and drug administration, respectively. The trachea was cannulated to allow artificial ventilation. Arterial blood pressure and a standard lead I or II electrocardiogram (ECG) were monitored continuously on an oscilloscope and recorded using a mingograph 81 ink-jet recorder (Elema-Schönander, Stockholm). Rectal temperature was maintained at approximately 38°C. The chest was opened by left thoracotomy at the fifth intercostal space and the fifth and fourth ribs were sectioned approximately 2 mm from the left margin of the sternum. Immediately after opening the chest, the animals were ventilated with room air using a stroke volume of 2 ml 100 g^{-1} and a rate of 54 strokes min^{-1}. These ventilation parameters maintain arterial P_{O_2}, P_{CO_2} and pH within the normal range. After opening the pericardium the heart was exteriorized by gentle pressure on the chest walls and a 6/0 braided silk suture attached to a 10 mm micropoint reverse cutting needle (Mersilk W812, Ethicon) was placed under the left coronary artery as described by Selye *et al.* (1960). The heart was repositioned in the thoracic cavity and any animal in which this procedure itself produced arrhythmias or a sustained fall in mean arterial blood pressure (MABP) to less than 70 mmHg was discarded from the study at this point. After an equilibration period of 15 min the ligature was tied in control animals and the blood pressure and ECG recorded for 30 min.

During the initial 30 min post-ligation period, all animals exhibited arrhythmic activity which commenced between 1 and 7 min and continued for about 25 min. The severity of the arrhythmias during this period was assessed by noting the mortality, the incidence and duration of ventricular fibrillation (VF; which in this species can spontaneously revert to sinus rhythm) and of ventricular tachycardia (VT; defined as a run of seven or more consecutive ventricular extrasystoles) and by counting the total number of ventricular extrasystoles (VEB).

Drug studies

All drugs were dissolved in saline and administered intravenously after the end of the 15 min equilibration period. Quazodine (Mead Johnson) and isobutylmethlxanthine (IBMX, Aldrich) were given as a bolus injection followed by a 15 min infusion before ligation. The doses of quazodine were 1 mg kg^{-1} and 1 mg kg^{-1} min^{-1} and of IBMX were 0.1 mg kg^{-1} and 0.1 mg kg^{-1} min^{-1}. Dibutyryl cyclic AMP and dibutyryl cyclic GMP (Sigma) were administered as an infusion at a rate of 10 μg kg^{-1} min^{-1} for 30 min before and 5 min following coronary artery ligation and subsequently at a rate of 2.5 μg kg^{-1} min^{-1} until the end of the ligation period. All drugs were diluted such that a total volume not exceeding 1 ml was administered.

Control studies were performed alongside each drug study such that on each day both control and drug pretreated animals were used. This was to eliminate any possible variation in response due to the time of day, temperature, laboratory conditions or seasonal and hormonal variations.

Cyclic nucleotide measurements

Myocardial cyclic AMP and cyclic GMP levels were measured in a separate series of rats subjected to one of the following experimental procedures: sham operation (including positioning of the ligature and a 15 min stabilization period), coronary artery ligation for a period of 5, 10 or 30 min or pretreatment with IBMX as described for the arrhythmia experiments. In these studies, the systemic arterial blood pressure and ECG were also monitored.

At the conclusion of the appropriate periods the heart was rapidly removed from the animal, the right and left ventricular free walls excised, blotted dry on absorbent tissue paper and frozen in liquid nitrogen. The mean freezing times for the left (ischaemic) and right (non-ischaemic) ventricular free wall samples were 18.7 ± 1.8 s and 25.2 ± 1.2 s, respectively. This protocol was used in the light of pilot experiments which established that it was not technically feasible to freeze clamp the heart *in situ* with the correct orientation such that it was then possible to separate reliably the ischaemic from non-ischaemic myocardium, even using a dye indicator.

The frozen samples of myocardium were weighed, pulverized under liquid nitrogen and then transferred

Table 1 The effects of pretreatment with quazodine, isobutyl methylxanthine (IBMX), dibutyryl cyclic AMP and dibutyryl cyclic GMP on mortality and on the incidence and duration of ventricular arrhythmias occurring in the first 30 min after coronary artery ligation in anaesthetized rats

	n	*Mortality* (%)	*Incidence of VF* (%)	*Duration(s) of* (min) *VT*	*VF*
Control	36	22	58	100.9 ± 23.4	20.4 ± 4.8
Quazodine	8	62.5*	100*	184.3 ± 35.8	113.7 ± 33.8
IBMX	11	82**	100*	187, 415	119, 136
db cyclic AMP	8	50	100*	258.8 ± 46.2**	89.2 ± 32.3*
db cyclic GMP	9	33	100*	176.7 ± 35.2*	64.5 ± 35.4

Abbreviations: VT, ventricular tachycardia; VF, ventricular fibrillation; *n*, the number of animals subjected to coronary artery occlusion.
The values for the durations of VT and VF are calculated in those animals surviving the initial 30 min period of coronary artery occlusion. Individual values are quoted when the number of survivors is less than 3. For the statistical analysis, data from drug-treated animals were compared with those from 8–10 control experiments, carried out over the same time period.
*$P < 0.05$, **$P < 0.01$, compared with control values.

to a pre-cooled tube and homogenized in 1 ml of ice-cold 6% trichloroacetic acid with an 8N ultra-turrax (TP18/10, 8N shaft) cell disrupter for a period of 90 s (9 × 10 s bursts with a 20 s delay between bursts) at 4°C. The remainder of the extraction and assay procedures for cyclic AMP and cyclic GMP were identical to those described by Rodger & Shahid (1984). This experimental procedure yielded cyclic nucleotide levels in sham-operated animals within the range of those previously reported in both the rat (Dobson & Meyer, 1973) and the dog (Wollenberger *et al.*, 1969) *in vivo*.

Statistics

The data are expressed as means ± the standard error of the mean (s.e.mean). Statistical significance of differences between mean values of ectopic count, onset time and duration of arrhythmias was calculated using a Student's *t* test, provided that the number of observations was not less than 4. Analysis of variance together with a modified *t* test was used to calculate statistical significance of differences between mean values of heart rate and blood pressure at various time points. A χ-squared test was used to analyse the statistical significance of differences in the incidences of a given event.

Results

Post-ligation arrhythmias in control and drug pretreated anaesthestized rats

Table 1 shows the severity of the arrhythmias and the resultant mortality in control and drug pretreated

Table 2 The effects of pretreatment with quazodine, isobutyl methylxanthine (IBMX), dibutyryl cyclic AMP and dibutyryl cyclic GMP on the time of onset of arrhythmias and on the number of ventricular extrasystoles occurring in the first 30 min following coronary artery ligation in anaesthetized rats

	n	*Time of onset of first arrhythmias* (min)	*Number of ventricular extrasystoles in consecutive 5 min periods after ligation* *0–5*	*6–10*	*11–15*	*16–20*	*21–25*	*26–30*	*Total number of ventricular extrasystoles*
Control	28	4.6 ± 0.5	18 ± 9	609 ± 119	527 ± 113	142 ± 37	68 ± 28	55 ± 30	1407 ± 229
Quazodine	3	2.7 ± 0.7	45 ± 7	855 ± 3	1689 ± 313	162 ± 65	2 ± 2	0	2753 ± 346
IBMX	2	1, 1	67, 204	754, 833	1590, 2828	440, 1531	193, 2	0	3044, 5398
db cyclic AMP	4	2 ± 1.1*	29 ± 13	1263 ± 242*	1549 ± 242**	819 ± 340	48 ± 40	0	3708 ± 677**
db cyclic GMP	6	1.8 ± 0.7**	37 ± 22	965 ± 141	875 ± 277	135 ± 42	169 ± 124	341 ± 298	2522 ± 408*

n is the number of animals surviving the initial 30 min period of coronary artery ligation. Individual values are quoted when *n* is < 3. For the statistical analysis, data from drug-treated animals were compared with that from 6–8 control experiments (survivors), carried out over the same time period.
*$P < 0.05$, *$P < 0.01$, compared with control values.

Table 3 Drug- and coronary artery ligation-induced effects on heart rate and mean arterial blood pressure in anaesthetized rats

		Heart rate (beats min^{-1})					*Mean arterial blood pressure* (mm Hg)				
		Drug infusion			*Coronary ligation*		*Drug infusion*			*Coronary ligation*	
	n	*0 min*	*5 min*	*15 min*	*1 min*	*5 min*	*0 min*	*5 min*	*15 min*	*1 min*	*5 min*
Control	36			402±6	404±8	403±8			81±2	69±1†	76±2
Quazodine	8	400±18	433±15	487±17*	495±18	503±13	79±3	73±3	81±3	62±3†	72±3
IBMX	11	401±12	402±13	451±17*	454±18	455±19	77±3	68±3	79±3	71±3	76±3
		0 min	*10 min*	*30 min*	*1 min*	*5 min*	*0 min*	*10 min*	*30 min*	*1 min*	*5 min*
db-cyclic AMP	8	382±10	365±10	374±9	380±8	380±8	76±3	77±3	87±6	74±5	79±6
db-cyclic GMP	9	397±14	381±14	381±15	389±17	390±14	79±3	70±6	84±4	74±4	77±6

n is the number of animals subjected to coronary artery ligation.
*Significantly different from pre-infusion value, $P<0.05$.
†Significantly different from pre-ligation value, $P<0.05$.

animals subjected to coronary artery occlusion. Although 58% of control animals fibrillated, mortality was less than 25% since spontaneous reversion to sinus rhythm is common in the rat. Pretreatment of animals with quazodine, IBMX, dibutyryl (db) cyclic AMP or cyclic GMP exacerbated the arrhythmias resulting in ventricular fibrillation in all animals. Mortality was also increased in the drug pretreated groups, statistically significant increases being observed following administration of quazodine and

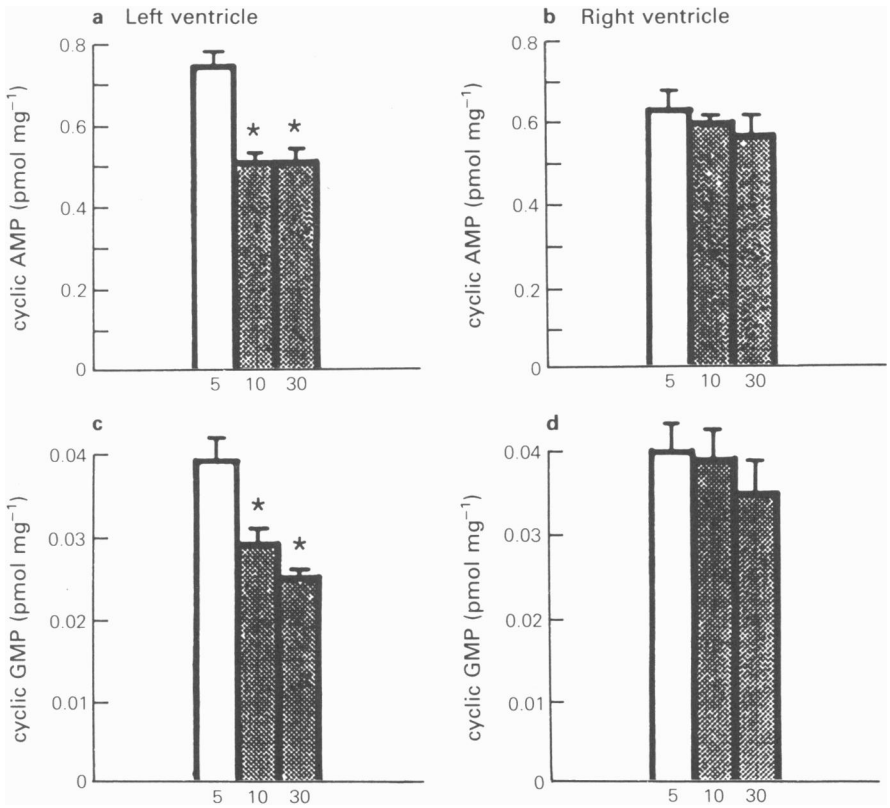

Figure 1 Cyclic AMP (a and b) and cyclic GMP (c and d) content of ischaemic (left ventricle) and normal (right ventricle) myocardium of sham-operated hearts, $n=4$, (open columns) and of those ligated for 10, $n=8$, or 30 min, $n=4$, (shaded columns). *$P<0.05$.

IBMX. In those animals surviving the 30 min ligation period, the duration of ventricular tachycardia was longer in db-cyclic AMP and db-cyclic GMP pretreated groups (Table 1). Since only a few animals treated with quazodine or IBMX survived coronary artery occlusion, statistical analysis on the durations of the arrhythmias in these animals could not be performed.

The total number of ventricular extrasystoles which occurred in animals surviving the 30 min ligation period was also increased following pretreatment with all four drugs (Table 2). It can be seen from Table 2, however, that although the first arrhythmia occurred earlier following ligation in drug pretreated animals, subsequent extrasystoles followed the characteristic time distribution observed in control animals. Namely, the peak number of extrasystoles occurred during the 6–15 min post-ligation period. However, in none of the drug-treated animals, was the mean time to the onset of the first episode of ventricular fibrillation changed from that observed in the control group, i.e. 7.9 ± 1.1 min.

Drug-and coronary artery ligation-induced haemodynamic effects in anaesthetized rats

The effects of drug administration and of coronary artery ligation on heart rate and mean arterial blood pressure are summarized in Table 3. Before ligation, heart rate was significantly elevated following quazodine and IBMX but not db cyclic AMP and db cyclic GMP administration. None of the four drugs markedly affected mean arterial blood pressure. The haemodynamic effects of coronary artery ligation in control animals consists of a transient fall in mean arterial blood pressure at 1 min post-ligation but no alterations in heart rate are observed. Similar haemodynamic effects of coronary artery ligation were observed in animals pretreated with all four drugs.

Cyclic AMP and cyclic GMP levels in the myocardium

Two separate sets of experiments were carried out. In the first, both cyclic AMP and cyclic GMP levels in the left and right ventricular free wall of sham-operated hearts and of hearts ligated for either 10 or 30 min were measured. These results are shown in Figure 1. At 10 min post-ligation, the peak of the arrhythmic activity, both cyclic AMP and cyclic GMP levels in the left, but not the right, ventricular free wall, were decreased when compared with sham-operated animals, It is of interest to note that in 3 out of 8 of the animals in this group, ventricular fibrillation had commenced just before the heart was excised, but levels of both cyclic nucleotides in these hearts were in the mid range of those obtained for the group as a whole. A similar reduction in both nucleotide levels was observed at 30 min post-ligation, when the arrhythmias had ceased.

In the second series of experiments the same protocol was followed but sham-operated hearts were compared with those in which the ligature was tied for 5 min. In 5 sham-operated hearts the mean cyclic AMP levels in the right and left ventricular free walls were 0.550 ± 0.023 and 0.631 ± 0.008 pmol mg^{-1} wet weight respectively, whereas in hearts subjected to coronary occlusion for 5 min the respective values were increased to 0.760 ± 0.050 ($n = 5$) and 0.693 ± 0.032 ($n = 5$) pmol mg^{-1} wet weight. In these hearts, cyclic GMP levels in right venticular tissue were also significantly higher when compared with those in sham-operated animals (0.037 ± 0.003 vs 0.029 ± 0.002 pmol mg^{-1} wet weight), whereas in left ventricular tissue the levels were similar (ligated, 0.033 ± 0.003 vs sham, 0.034 ± 0.004 pmol mg^{-1} wet weight).

In the second series of experiments 5 animals were also administered IBMX, in the dose used in the arrhythmia study, and cyclic nucleotide levels measured in the left ventricle at the end of the infusion period. A marked increase in both cyclic AMP (IBMX treated, 0.920 ± 0.003 vs sham, 0.631 ± 0.01 pmol mg^{-1}wet weight) and cyclic GMP (IBMX treated, 0.052 ± 0.006 vs sham, 0.034 ± 0.004 pmol mg^{-1} wet weight) content of the left ventricle was observed.

Discussion

In this study, we have demonstrated that in the pentobarbitone-anaesthetized rat, subjected to coronary artery ligation, both cyclic AMP and cyclic GMP levels in the right (normal) and left (ischaemic) ventricular free wall undergo time-dependent changes. At 5 min post ligation, cyclic AMP levels were elevated in both normal and ischaemic myocardium, this effect being more pronounced in the normal tissue. At 10 and 30 min post-occlusion, however, cyclic AMP levels had fallen below those observed in sham-operated animals in the ischaemic but not in the normal myocardium. Thus, before the development of marked arrhythmic activity, i.e. at 5 min post-ligation, cyclic AMP levels were elevated in both ventricles whereas during the peak arrhythmias, at 10 min post-ligation, they had fallen in ischaemic but not in normal myocardium.

In this particular model, therefore, there does not appear to be a direct relationship between the rise in cyclic AMP levels in ischaemic tissue and the occurrence of marked ectopic activity. Nor did the initiation of ventricular fibrillation appear to be related to elevations in cyclic AMP since in 3 out of 8 hearts,

studied at 10 min post-ligation, the heart had just fibrillated prior to excision yet cyclic nucleotide levels were in the mid range of those measured for that group. These results differ from those described in the cat (Corr *et al.*, 1978) and the baboon (Podzuweit *et al.*, 1978) in which a rise in cyclic AMP levels in ischaemic myocardium was found to precede the onset of fibrillation and also in the pig (Podzuweit & Lubbe, 1977; Podzuweit *et al.*, 1981) in which a relationship was found between the accumulation of cyclic AMP and the occurrence of ventricular extrasystoles. In the rat isolated heart, conflicting results have been obtained. Using glucose as a substrate, no change (Bricknell & Opie, 1978) and an increase in ischaemic myocardial cyclic AMP levels (Lubbe *et al.*, 1981) have both been reported. A recent study, carried out at the Rayne Institute, St. Thomas' Hospital, London, has shown a profile of changes in cyclic AMP levels following ischaemia in the rat heart *in vitro* similar to that which we have observed *in vivo* (R. Crome, unpublished ovservations). In these studies, cyclic AMP levels in ischaemic tissue did rise, the peak effect being observed after 3 min, whereas by 10 min post-ischaemia they had fallen substantially below control values. Thus it may be that in our studies too, the peak rise in cyclic AMP levels occurred earlier than 5 min post-ligation, i.e. before the onset of severe arrhythmias. Certainly during the peak of the arrhythmias, cyclic AMP levels were below those found in sham-operated animals; this reduction presumably being caused by depletion of ATP stores in the ischaemic region. Preliminary work in our laboratory has, indeed, shown that in this experimental model, at 15 min post-ligation, ATP levels are reduced to 15% of pre-ligation values (F. Williams, unpublished observations). Therefore, this model is one of severe ischaemia and this in itself may explain why a sustained rise in cyclic AMP is not observed. It may also be of importance that a generalized increase in sympathetic drive to the heart is not observed under these experimental conditions as there is a lack of alteration in heart rate following ligation.

The changes in myocardial cyclic GMP levels, for the most part, paralleled the changes in cyclic AMP seen on ligation. In the right ventricular free wall, cyclic GMP levels were elevated at 5 min post-ligation but following longer periods of ischaemia were similar to those observed in sham-operated animals. In ischaemic tissue there was a reduction in cyclic GMP content both at 10 and 30 min post-ligation, whereas after 5 min of ischaemia no change was observed. In the rat isolated heart, a peak elevation of cyclic GMP has been reported after 1 min of hypoxia (Busuttil *et al.*, 1978) but following longer hypoxic periods (30 min) the levels fall to below control values (Nesher *et al.*, 1977). It may be, therefore, that in designing an experimental protocol to correlate changes in cyclic nucleotide levels with the occurrence of arrhythmias, we have failed to observe a possible rapid and transient rise in cyclic GMP in the ischaemic myocardium. The cause of the rise in cyclic GMP content of the non-ischaemic myocardium is not known but it is of interest to note that a similar post-ligation elevation of this nucleotide in non-ischaemic dog myocardium was prevented by bilateral vagotomy (Krause *et al.*, 1978).

Although in this study there was no direct relationship between the occurrence of arrhythmias and a rise in cyclic AMP in the ischaemic myocardium, we did nevertheless observe that the severity of such arrhythmias was exacerbated by pretreatment with drugs which increase the intracellular concentration of this nucleotide. Thus, pretreatment with the phosphodiesterase inhibitors, quazodine and IBMX, or with dibutyryl cyclic AMP, the more lipid soluble acylated form of cyclic AMP, resulted in an increased incidence of ventricular fibrillation after coronary artery occlusion. In the drug-treated animals that survived the initial 30 min of ischaemia, there were also more ventricular extrasystoles, an effect that could not be attributed to any marked haemodynamic effect of the pretreatment. A positive chronotropic effect was observed following pretreatment with both phosphodiesterase inhibitors but not with dibutyryl cyclic AMP. This lack of effect of dibutyryl cyclic AMP on heart rate is in agreement with the results of Lubbe *et al.* (1976) who showed that in concentrations which increased the vulnerability of rat isolated heart to fibrillation, heart rate remained unchanged.

These results are in accord with those obtained in a number of other studies showing the arrhythmogenic activity of agents which may act by increasing the myocardial concentration of cyclic AMP (Sugiura *et al.*, 1979; Podzuweit *et al.*, 1981). In this study we also demonstrated that at the time of ligation, cyclic AMP levels in the left ventricle were elevated by pretreatment with IBMX. However, IBMX also inhibits the hydrolysis of cyclic GMP (Mushlin *et al.*, 1981b) as indicated in our experiments by its augmentative effect on the myocardial levels of this nucleotide. This, together with the fact that infusion of dibutyryl cyclic GMP also appeared to enhance ischaemia-induced arrhythmias, raises the possibility of an arrhythmogenic effect of cyclic GMP in this experimental model. Unfortunately, research in this area has focussed upon the relationship between myocardial cyclic AMP and arrhythmias and to our knowledge there is no information in the literature about the effect of dibutyryl cyclic GMP and ischaemia-induced arrhthmias with which we can compare our findings.

We conclude, therefore, that in the

pentobarbitone-anaesthetized rat subjected to coronary artery occlusion there is no evidence to support the hypothesis that a rise in cyclic AMP content of the ischaemic myocardium occurs at the same time as the resultant arrhythmias. It remains a possibility that the elevations in cyclic AMP, observed in both normal and ischaemic myocardium to precede the onset of severe arrhythmias, may rather have initiated a chain of further intracellular events which could subsequently lead to the genesis of arrhythmias. We did, however, observe that drug interventions designed to raise the myocardial levels of both cyclic AMP and cyclic GMP exacerbated the ischaemia-induced arrhythmias, suggesting that both of these nucleotides can be arrhythmogenic during ischaemia. Further work on the levels of myocardial cyclic GMP in the rat and in other species is required before its role in the genesis of cardiac arrhythmias can be fully elucidated.

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