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Regional myocardial ajmaline concentration and antiarrhythmic activity for ischaemia- and reperfusion-induced arrhythmias in rats

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1 Antiarrhythmic actions of ajmaline against ischaemia (left coronary artery occlusion for 15 min) and subsequent reperfusion-induced arrhythmias were investigated in anaesthetized rats.

2 Ajmaline ($2\,mg\,kg^{-1}$, i.v.) was effective in suppressing ischaemia-induced arrhythmias whether given pre- or post-occlusion.

3 Ajmaline diminished the reperfusion-induced arrhythmias completely when given pre-occlusion but had little effect when given post-occlusion.

4 Reperfusion-induced increases in plasma enzyme activities of lactate dehydrogenase, glutamate-oxaloacetate transaminase and creatine phosphokinase were prevented more effectively when ajmaline was given pre-occlusion rather than post-occlusion.

5 Fifteen min post-occlusion, the ajmaline concentrations in the ischaemic ventricle were 18.42 ± 1.66 and $1.18 \pm 0.15\,\mu g\,g^{-1}$ for pre- and post-occlusion administration, respectively. However, ajmaline concentrations in whole blood and normal ventricle were not significantly different between pre- and post-occlusion administration.

6 We suggest that the beneficial effect of ajmaline against reperfusion-induced arrhythmias is related to the ischaemic myocardial concentration of ajmaline which is markedly affected by the time of drug administration (i.e. pre- and post-occlusion).

Introduction

Arrhythmias associated with reperfusion of the ischaemic myocardium may be a possible progenitor for sudden cardiac death in man. In experimental animals, both myocardial ischaemia and subsequent reperfusion are associated with malignant ventricular arrhythmias. The mechanisms for the reperfusion-induced arrhythmias are not well understood but differ from the mechanisms for ischaemia-induced arrhythmias in their biochemical, ionic, adrenergic or electrophysiological aspects (Corbalan *et al.*, 1976; Corr & Witkowski, 1984). It is known that drug actions against these arrhythmias can be altered by several factors including animal species, duration of the occlusion, size of the ischaemic area, autonomic nervous activity, dose and time of drug administration (Bergey *et al.*, 1982; Kane *et al.*, 1984; Winslow, 1984; Crome *et al.*, 1986). As a result of the coronary artery occlusion, there are changes in the distribution of blood perfusion across the ventricular wall. Thus, from a pharmacokinetic viewpoint, the drug distribution within the cardiac tissue may be different if administered pre- rather than post-occlusion. Changes in drug distribution seem to have a potential effect on the antiarrhythmic activity of the drug. However, little is known about the regional myocardial drug concentration in the ischaemic heart or its relationship with the antiarrhythmic effect (Wenger *et al.*, 1980; Nattel *et al.*, 1981; Curtis *et al.*, 1984; Davis *et al.*, 1985).

In a previous study, we have shown that ajmaline given post-occlusion protects against ischaemia-induced arrhythmias in a dose-related manner (Hashimoto *et al.*, 1986). The present study was designed to compare the effect of ajmaline on ischaemia- and reperfusion-induced arrhythmias in rats. The primary aim of this study was to determine if differences in the time of drug administration could lead to differences in the antiarrhythmic activ-

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ity of ajmaline against ischaemia- or reperfusion-induced arrhythmias, and if so, to see if these differences were related to the regional myocardial drug concentration.

Methods

Experimental protocol

The method used to induce arrhythmias in the anaesthetized rat by left coronary artery occlusion and reperfusion has been described in detail by Kane *et al.* (1984). Male Wistar rats (310–390 g) were anaesthetized with sodium pentobarbitone, $60\,mg\,kg^{-1}$ administered as required. Systemic arterial blood pressure was recorded from the femoral artery by means of a capacitance transducer (Toyo Baldwin MPU-0.5-290-0-III). A catheter was placed in the femoral vein for administration of drugs, and the trachea was cannulated to allow artificial ventilation. The electrocardiogram (ECG) was recorded with bipolar standard limb leads. Arterial blood pressure and ECG were monitored continuously by use of a polygraph (San-ei Model 366) and a recorder (San-ei Model 8K21-L). Body temperature was maintained with appropriate heating lamps. The animals were ventilated with room air with a stroke of $15\,ml\,kg^{-1}$ and a rate of $54\,strokes\,min^{-1}$ (Harvard Rodent Respirator Model 683) to maintain normal Po_2, PCo_2 and pH parameters. The chest was opened by a left thoracotomy at the fourth intercostal space, and after opening the pericardium the heart was exteriorized by gentle pressure on the chest wall. A 6/0 braided silk suture attached to a 10 mm micro-pointed reverse cutting needle (Nesco ER1006s) was placed under the left coronary artery near its origin and the heart was then replaced in the chest cavity. Any animal in which this procedure itself produced arrhythmia or a sustained fall in mean arterial blood pressure to less than 70 mmHg before occlusion was immediately omitted from the study. After a stabilizing period of 15 min, left coronary artery occlusion was performed by application of tension to the suture passed through the catheter. Subsequent coronary reperfusion was performed by releasing the suture after a 15 min period of coronary artery occlusion.

Ajmaline (Gilurytmal, Nippon Chemiphar, $25\,mg\,ml^{-1}$) was diluted with isotonic saline, and $2\,mg\,kg^{-1}$ was administered intravenously as a bolus injection at $1\,ml\,kg^{-1}$ either just after coronary artery occlusion or 1 min before occlusion. Rats not treated with ajmaline served as controls. ECG was monitored for up to 30 min after coronary artery ligation in both permanently coronary occluded and subsequently reperfused rats. At 30 min after coronary occlusion, 5 ml of arterial blood was taken for the determination of marker enzyme activities in the plasma.

In order to investigate the tissue distribution of ajmaline, tissue and blood concentrations of ajmaline were determined 15 min post-occlusion after $2\,mg\,kg^{-1}$ i.v. given either pre- or post-occlusion in coronary artery-ligated rats. After a 15 min period of coronary occlusion, 1 ml of blood was collected and the heart was removed. On the basis of visual inspection, the ventricle was divided into three regions, i.e. normal, ischaemic and the other tissue (about 10, 10 and 80% of the whole ventricle weight respectively). Each tissue sample was weighed and homogenized in nine volumes of isotonic saline for the determination of ajmaline.

ECG analysis

From the ECG recordings run at a paper speed of $10\,mm\,s^{-1}$, only those beats of apparent sinus origin were considered to be normal sinus beats. All other ventricular complexes were classified as premature ventricular complexes (PVCs). The severity of arrhythmias was assessed by noting the mortality, the incidence and duration of ventricular fibrillation (VF) and ventricular tachycardia (VT, defined as any run of seven or more consecutive PVCs) and by counting the total number of PVCs for a defined time period post occlusion.

Determination of marker enzyme activities in plasma

The blood obtained at 30 min after coronary artery occlusion was immediately heparinized and centrifuged. The plasma activities of lactate dehydrogenase (LDH), glutamate-oxaloacetate transaminase (GOT) and creatine phosphokinase (CPK) were assayed by an automated assay system (JEOR JCA-SIM 6R).

Ajmaline assay

A specific reverse phase high performance liquid chromatographic (h.p.l.c.) method described previously (Hori *et al.*, 1984) was used to measure ajmaline in blood and tissue homogenate. The assay involved extraction of a 0.5 ml sample and 2.0 ml glycine buffer (pH 10, 0.1 M, saturated with sodium chloride) with 5 ml of diethylether, and re-extraction of the organic phase with 0.2 ml of 0.85% phosphoric acid solution. A 50 μl sample of the water phase was subjected to h.p.l.c.

Statistics

Data are expressed as mean $\pm$ s.e. mean. Statistical significance of difference between mean values was

Table 1 Effect of ajmaline on ischaemia-induced arrhythmias (0–15 min) in anaesthetized rats

Treatment	*Number of PVCs* (complexes)	*Duration of VT* (s)	*Duration of VF* (s)	*Mortality*
Control	1137 ± 204 (22/22)	64 ± 13 (22/22)	24 ± 7 (12/22)	1/22
Post-occlusion	79 ± 30† (9/11)	6 ± 4† (4/11)†	0 ± 0* (0/11)†	0/11
Pre-occlusion	281 ± 84* (10/10)	1 ± 1† (3/10)†	0 ± 0* (0/10)***	0/10

PVCs = premature ventricular complexes, VT = ventricular tachycardia and VF = ventricular fibrillation. Control = not treated with ajmaline, Post-occlusion = ajmaline ($2\,mg\,kg^{-1}$)-treated just after occlusion and Pre-occlusion = ajmaline ($2\,mg\,kg^{-1}$)-treated 1 min before occlusion. Values are expressed as mean ± s.e. mean for the survivors. The incidence of each type of arrhythmia is given in parentheses. * $P < 0.05$, *** $P < 0.01$ and † $P < 0.005$ denote significant difference from the control group.

calculated using a non-paired t test provided that the variances of groups were similar. If this was not the case, Mann-Whitney's U-test was applied. Multiple comparison was performed by use of a Scheffé-type test following Kruskal-Wallis analysis. Difference of incidences was compared by Fisher's exact test. P values of less than 0.05 (two-tailed) were considered to be significantly different.

Results

Effect of ajmaline on ischaemia-induced arrhythmias

In control rats, the onset of tachyarrhythmias was generally 5 min post-occlusion and the arrhythmias usually lasted for 10 min following left coronary artery occlusion. VF usually ceased with spontaneous reversion to sinus rhythm, and only one rat died with sustained fibrillation.

Table 1 summarizes the effect of ajmaline on the ischaemia-induced arrhythmias for a period of 15 min after coronary occlusion. Both post- and pre-occlusion administration of ajmaline significantly reduced the number of PVCs and the duration and incidence of VT and VF. In rats given ajmaline post-occlusion, the number of PVCs was slightly less than that in rats given ajmaline pre-occlusion, but this difference was not statistically significant.

Effect of ajmaline on reperfusion-induced arrhythmias

Reperfusion of the coronary artery after a 15 min occlusion period results in the burst of the ventricular arrhythmias, including VT and VF, in control rats. In this study, none of the animals died. Table 2 shows the effect of ajmaline on the reperfusion-induced arrhythmias. We compared the arrhythmias that occurred during the 15 min period after reperfusion with those that occurred during the corresponding time period, but with the occlusion still present. This was done to investigate any differences in ajmaline effect between ischaemia- and reperfusion-induced arrhythmias. Ajmaline diminished the reperfusion-induced arrhythmias completely when given pre-occlusion, but had little effect when given post-occlusion.

Table 2 Effect of ajmaline on reperfusion-induced arrhythmias (15–30 min) in anaesthetized rats

Treatment	*Number of PVCs* (complexes)	*Duration of VT* (s)	*Duration of VF* (s)
Control			
Occlusion	7 ± 6 (3/12)	1 ± 1 (1/12)	0 ± 0 (0/12)
Reperfusion	171 ± 35† (9/9)†	13 ± 3† (8/9)†	5 ± 3* (3/9)
Post-occlusion			
Occlusion	2 ± 2 (1/6)	0 ± 0 (0/6)	0 ± 0 (0/6)
Reperfusion	155 ± 89** (5/5)*	12 ± 8* (3/5)	1 ± 1 (1/5)
Pre-occlusion			
Occlusion	54 ± 31 (3/5)	0 ± 0 (0/5)	0 ± 0 (0/5)
Reperfusion	66 ± 44 (4/5)	0 ± 0 (1/5)	0 ± 0 (0/5)

Occlusion = rats subjected to permanent coronary artery occlusion, Reperfusion = rats subjected to coronary occlusion for 15 min and subsequent reperfusion. The other abbreviations are the same as in Table 1. Values are expressed as mean ± s.e. mean for the survivors. The incidence of each type of arrhythmia is given in parentheses.
* $P < 0.05$, ** $P < 0.025$ and † $P < 0.005$ denote significant difference from each Occlusion group.

Table 3 Effect of ajmaline on marker enzyme activities 30 min after coronary artery occlusion

Treatment	n	LDH ($iu\,l^{-1}$)	GOT ($iu\,l^{-1}$)	CPK ($iu\,l^{-1}$)
Control				
Occlusion	8	545 ± 55	108 ± 11	960 ± 180
Reperfusion	9	3689 ± 613†	749 ± 131†	2986 ± 190†
Post-occlusion				
Occlusion	4	362 ± 51	82 ± 14	543 ± 79
Reperfusion	5	2399 ± 781**	551 ± 193**	1998 ± 610*
Pre-occlusion				
Occlusion	5	586 ± 67	109 ± 14	849 ± 220
Reperfusion	5	864 ± 103	192 ± 19*	1025 ± 124

LDH = lactate dehydrogenase, GOT = glutamate-oxaloacetate transaminase and CPK = creatine phosphokinase. The other abbreviations are the same as in Tables 1 or 2. Values are expressed as mean ± s.e. mean for n animals. * $P < 0.05$, ** $P < 0.025$ and † $P < 0.005$ denote significant difference from each Occlusion group.

Effect of ajmaline on marker enzyme activities in plasma

In order to assess the myocardial damage induced by coronary artery occlusion or subsequent reperfusion, three kinds of marker enzyme activities in plasma were measured at 30 min after occlusion. As shown in Table 3, release of the occlusion in control rats resulted in a significant rise in all three enzyme activities compared to permanently occluded animals. Ajmaline did not show any marked effect on the enzyme activities of permanently occluded rats, whether given pre- or post-occlusion. On the other hand, ajmaline prevented reperfusion-induced increases in marker enzyme activities more effectively when given pre-occlusion than post-occlusion.

Distribution of ajmaline in coronary occluded rat heart

Table 4 shows the tissue and blood concentrations of ajmaline determined after a 15 min period of coronary artery occlusion. There was no significant difference in ajmaline concentrations between pre- and post-occlusion dose in both blood and normal ventricle. However, after the pre-occlusion dose, ajmaline concentrations in the ischaemic ventricle were about 16 fold higher than after the post-occlusion dose.

Discussion

This study examined the effects of the Class I antiarrhythmic agent, ajmaline, on the incidence of arrhythmias during coronary artery occlusion and reperfusion. It is known that in anaesthetized rats, ischaemic arrhythmias start 5 min after coronary artery occlusion and last for about 10 min (Hashimoto *et al.*, 1986). On the other hand, the incidence of reperfusion-induced arrhythmias is dependent on the duration of the preceding occlusion. It is well established that the most severe reperfusion-induced arrhythmias occur after an occlusion period of 5 or 15 min and the extension of an occlusion period to 20 min results in a marked decrease in the incidence of arrhythmias (Kane *et al.*, 1984; Crome *et al.*, 1986). In the present study, the coronary artery was occluded for 15 min before release in order to examine the effects of ajmaline on both ischaemia- and reperfusion-induced arrhythmias in the same

Table 4 Effect of the time of ajmaline administration on the drug concentrations in the blood and heart tissues 15 min after coronary artery occlusion

Sample		Post-occlusion (n = 4)	Pre-occlusion (n = 4)
Blood	($\mu g\,ml^{-1}$)	0.45 ± 0.01	0.41 ± 0.03
Normal ventricle	($\mu g\,g^{-1}$)	1.99 ± 0.21	1.98 ± 0.18
Ischaemic ventricle	($\mu g\,g^{-1}$)	1.18 ± 0.15	18.42 ± 1.66*

Post-occlusion = ajmaline $2\,mg\,kg^{-1}$ given just after occlusion, Pre-occlusion = ajmaline $2\,mg\,kg^{-1}$ given 1 min before occlusion. Values are expressed as mean ± s.e. mean for n animals. * $P < 0.05$ denotes significant difference from the Post-occlusion group.

animal. The ECG was continuously recorded for 30 min after occlusion. The first 15 min period was used for the assessment of ischaemic arrhythmias and the second half for the assessment of reperfusion-induced arrhythmias. Though most of the ischaemic arrhythmias usually occur within 15 min after occlusion, in the presence of ajmaline their time course may be different. To minimize possible misinterpretation of results referable to ischaemic arrhythmias rather than to reperfusion-induced ones, the effects of ajmaline on reperfusion-induced arrhythmias were investigated by comparing the arrhythmias after reperfusion with those that occurred during the corresponding time period in rats subjected to permanent occlusion.

We found a marked difference between pre- and post-occlusion administration of ajmaline with regard to the suppressive effect on reperfusion-induced arrhythmias. Ajmaline ($2\,mg\,kg^{-1}$, i.v.) diminished the reperfusion-induced arrhythmias completely when given pre-occlusion, but had little effect when given post-occlusion (Table 2). Supportive evidence was provided by the measurement of marker enzyme activities in the plasma. Reperfusion-induced increases in plasma enzyme activities of LDH, GOT and CPK were prevented more effectively when the ajmaline was given pre-occlusion rather than post-occlusion (Table 3). Recently Crome *et al.* (1986) showed a similar relationship between the time of nifedipine administration and its effect against reperfusion-induced arrhythmias in anaesthetized rats. They found that nifedipine given 10 min prior to occlusion reduced the incidence of reperfusion-induced VF following 5 min coronary occlusion while its administration 1 min before reperfusion afforded no protective effect.

In contrast to reperfusion-induced arrhythmias, the incidence of ischaemic arrhythmias was effectively suppressed by ajmaline whether given pre- or post-occlusion (Table 1). Thus, the antiarrhythmic effects of ajmaline differ, depending on the type of arrhythmias and the time of drug administration relative to the time of coronary artery occlusion.

We investigated the mechanisms of these differences from a pharmacokinetic standpoint. The heart is generally considered to be in rapid distribution equilibrium with the central pharmacokinetic compartment because it is highly perfused. In fact, the ajmaline concentration in the heart was reported to be rapidly equilibrated with blood in the normal mouse (Iven, 1977). However, if the rate of drug equilibration between blood and myocardium is related to perfusion, then ischaemic areas of myocardium should behave as a kinetically different compartment. As shown in Table 4, when ajmaline was given post-occlusion, myocardial concentrations of ajmaline in the ischaemic region were lower than those in the normal region, which indicated that the coronary occlusion performed before ajmaline administration delayed the appearance of ajmaline in the ischaemic zone. Similar results were demonstrated in dogs with procainamide (Wenger *et al.*, 1980) and lidocaine (Davis *et al.*, 1985). Wenger *et al.* (1980) showed a difference between normal and ischaemic myocardium with regard to the regional concentration of procainamide obtained after a single i.v. bolus administration and concluded that the ischaemic myocardium represents a tissue compartment whose pharmacokinetic distance from the central compartment is a function of the severity of ischaemia. On the other hand, when ajmaline was given pre-occlusion, ajmaline concentrations in the ischaemic myocardium were much higher than those in the normal region (Table 4). These results suggest that coronary occlusion performed after ajmaline administration markedly slows the removal of ajmaline from the ischaemic zone. Thus, rats given ajmaline pre-occlusion had about a 16 fold higher concentration of ajmaline in the ischaemic myocardium than rats given ajmaline post-occlusion, despite similar ajmaline concentrations in the blood and normal myocardium. This difference was very probably related to the difference in activity of ajmaline against reperfusion-induced arrhythmias between pre- and post-occlusion administration.

Reperfusion-induced arrhythmias are believed to be triggered by increased automaticity in the ischaemic myocardium and reperfusion in an ischaemic zone that includes reversibly and irreversibly injured cells, leads to severe electrophysiological non-homogeneity and hence increases the incidence of arrhythmias (Corr & Witkowski, 1984; Winslow, 1984). Therefore, the ischaemic myocardium seems to be the main site of drug action. Our results indicated that the pre-occlusion administration of ajmaline might produce a high enough concentration of ajmaline in this region to afford a prophylactic effect against reperfusion-induced arrhythmias.

Both pre- and post-occlusion administration of ajmaline caused similar effects against ischaemic arrhythmias in spite of marked differences in the ischaemic myocardial concentrations of ajmaline. This may be inconsistent with the results of Davis *et al.* (1985), who showed that the lidocaine concentration in the ischaemic myocardium but not in the plasma or normal myocardium correlates with the lidocaine effect against ischaemic arrhythmias in dogs. Our results suggest that low ajmaline concentrations in the ischaemic region obtained after the post-occlusion administration may have been enough to cause a maximal effect against ischaemic arrhythmias or that the ajmaline effect might have been related to the drug concentrations in the blood or normal myocardium. Winslow (1984) recently suggested that the majority of ischaemia-induced PVCs in rats are due to re-entry through the atrioventricular (AV) node. Hence the AV conduction system might also be a possible site of drug action in rats subjected to coronary artery occlusion; and drugs which slow AV conduction might exert an antiarrhythmic effect. In this respect we have already shown that ajmaline slows AV conduction and that left coronary artery occlusion does not affect the negative dromotropic activity of ajmaline (Hashimoto *et al.*, 1986).

Finally, our results may be interpreted as indicating a limitation of drug plasma concentration monitoring in antiarrhythmic therapy, since blood concentrations of ajmaline after pre- and post-occlusion administration were almost the same and did not reflect the difference in the ischaemic myocardial concentrations. However, the present investigation was performed under the non-steady state conditions of using a single i.v. bolus. Studies evaluating the concentration-effect relationships in animals on chronic drug therapy before coronary artery occlusion are necessary to assess further the clinical relevance of these observations.

References

BERGEY, J.L., NOCELLA, K. & MCCALLUM, J.D. (1982). Acute coronary artery occlusion-reperfusion-induced arrhythmias in rats, dogs and pigs: antiarrhythmic evaluation of quinidine, procainamide and lidocaine. *Eur. J. Pharmacol.*, **81**, 205–216.

CORBALAN, D., VERRIER, R.L. & LOWN, B. (1976). Differing mechanisms for ventricular vulnerability during coronary artery occlusion and release. *Am. Heart J.*, **92**, 223–230.

CORR, P.B. & WITKOWSKI, F.X. (1984). Arrhythmias associated with reperfusion: basic insights and clinical relevance. *J. Cardiovasc. Pharmacol.*, **6**, s903–909.

CROME, R., HEARSE, D.J. & MANNING, A.S. (1986). Ischemia- and reperfusion-induced arrhythmias: Beneficial actions of nifedipine. *J. Cardiovasc. Pharmacol.*, **8**, 1249–1256.

CURTIS, M.J., MACLEOD, B.A. & WALKER, M.J.A. (1984). Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rat. *Br. J. Pharmacol.*, **83**, 373–385.

DAVIS, R.F., DEBOER, L.W.V., YASUDA, T., RUDE, R.E., RIBEIRO, L.G.T. & MAROKO, P.R. (1985). Regional myocardial lidocaine concentration determines the antidysrhythmic effect in dogs after coronary artery occlusion. *Anesthesiology*, **62**, 155–160.

HASHIMOTO, Y., HORI, R., OKUMURA, K. & YASUHARA, M. (1986). Pharmacokinetics and antiarrhythmic activity of ajmaline in rats subjected to coronary artery occlusion. *Br. J. Pharmacol.*, **88**, 71–77.

HORI, R., OKUMURA, K., INUI, K., YASUHARA, M., YAMADA, K., SAKURAI, T. & KAWAI, C. (1984). Quinidine-induced rise in ajmaline plasma concentration. *J. Pharm. Pharmacol.*, **36**, 202–204.

IVEN, H. (1977). The pharmacokinetics and organ distribution of ajmaline and quinidine in the mouse. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **298**, 43–50.

KANE, K.A., PARRATT, J.R. & WILLIAMS, F.M. (1984). An investigation into the characteristics of reperfusion-induced arrhythmias in the anaesthetized rat and their susceptibility to antiarrhythmic agents. *Br. J. Pharmacol.*, **82**, 349–357.

NATTEL, S., PEDERSEN, D.H. & ZIPES, D.P. (1981). Alterations in regional myocardial distribution and arrhythmogenic effect of aprindine produced by coronary artery occlusion in the dog. *Cardiovasc. Res.*, **15**, 80–85.

WENGER, T.L., BROWNING, D.J., MASTERTON, C.E., ABOU-DONIA, M.B., HARRELL, F.E., BACHE, R.J. & STRAUSS, H.C. (1980). Procainamide delivery to ischemic canine myocardium following rapid intravenous administration. *Circ. Res.*, **46**, 789–795.

WINSLOW, E. (1984). Methods for the detection and assessment of antiarrhythmic activity. *Pharmacol. Ther.*, **24**, 401–433.

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