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EFFECTS OF RESERPINE TREATMENT ON ARRHYTHMOGENESIS DURING ISCHAEMIA AND REPERFUSION IN THE ISOLATED RAT HEART

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SUMMARY

1. The effects of reserpine treatment on the myocardial contents of catecholamines and enkephalins and the incidence of ventricular arrhythmias during ischaemia and reperfusion in the isolated rat heart were studied.

2. Reserpine treatment almost completely depleted the heart of noradrenaline (NA). It also significantly depleted the heart of adrenaline and dopamines. It did not, however, alter the myocardial contents of enkephalins.

3. Reserpine-treatment attenuated significantly, but did not abolish, cardiac arrhythmias induced by ischaemia and reperfusion in the isolated heart preparation.

4. The results of the present study indicate that myocardial catecholamines especially NA are a contributing factor to arrhythmogenesis during ischaemia and reperfusion.

Key words: catecholamines, enkephalins, ischaemia, isolated rat heart, noradrenaline, reperfusion, reserpine.

INTRODUCTION

It is generally believed that myocardial catecholamines play an important role in the genesis of cardiac arrhythmias during ischaemia and reperfusion. One of the most important pieces of evidence is a reduction in the incidence of arrhythmias following chemical sympathectomy by 6-OH-dopamine or reserpine treatment or after surgical sympathectomy. 6-OH-Dopamine reduces the myocardial noradrenaline (NA) content by 90% both in the cat (Sheridan *et al.* 1980) and guinea-pig (Culling *et al.* 1984) as well as the incidence of cardiac arrhythmias. However, it has been shown that 6-OH-dopamine also reduces the myocardial content of leu-enkephalin by 70% in the guinea-pig (Lang *et al.* 1983), indicating that this treatment also disturbs the myocardial opioid system, which has been implicated in arrhythmogenesis during myocardial ischaemia and reperfusion (Zhan *et al.* 1985; Wong & Lee 1987). Surgical sympathectomy may

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have the same problem as chemical sympathectomy with 6-OH-dopamine as this treatment may also reduce the myocardial contents of enkephalins, which have been suggested to be present in the sympathetic nerve endings (Lang *et al.* 1983). Reserpine treatment was employed in earlier studies on ischaemia-induced arrhythmias. The results are, however, controversial. Maling and coworkers (1959) were unable to find any change in the spontaneous ventricular activities after coronary artery ligation in conscious dogs pretreated with reserpine. Ebert and coworkers (1970), also working on the dog, found that reserpine with or without vagotomy reduces ventricular arrhythmias, but only after vagotomy is the difference statistically significant.

Another important piece of evidence in favour of an important role of endogenous myocardial catecholamines in arrhythmogenesis during myocardial ischaemia and reperfusion is the attenuation by adrenoceptor blockers of arrhythmias induced by ischaemia and reperfusion. However, the actions of different α-antagonists on ischaemia-induced arrhythmias are different with some being antiarrhythmic and some not (Bralet *et al.* 1985) and the structural isomers of propranolol are equally antiarrhythmic (Daugherty *et al.* 1986). The results suggest that the antiarrhythmic actions of adrenoceptor blockers may be due at least partly to membrane stabilization rather than adrenoceptor blockade.

A third line of evidence, which is indirect, is an increased activity in the sympathetic nervous system. It has been well documented that there is an increased adrenergic activity during acute ischaemia (Corr *et al.* 1978; Pudzuweit *et al.* 1978; Abrahamsson *et al.* 1981; Holmgren *et al.* 1981) and reperfusion (Abrahamsson *et al.* 1983; Bralet *et al.* 1985).

However, whether NA really contributes to arrhythmogenesis, and if so how important its contribution is, needs further study.

In order to assess again the role of endogenous myocardial catecholamines in arrhythmogenesis during ischaemia and reperfusion, we depleted the myocardium of catecholamines by reserpine, which has been shown in our preliminary studies not to disturb the enkephalinergic system in the heart (Table 1), and studied the effects on arrhythmias induced by ischaemia and reperfusion in an isolated perfused rat heart preparation. The isolated rat heart preparation was used to avoid unnecessary systemic influences.

METHODS

Female Sprague-Dawley rats of 210–230 g were used. They were allowed free access to water and food. The procedure of Edoute and coworkers (1982) for reserpine treatment was adopted with a slight modification. Reserpine (CIBA) at doses of 5 and 1 mg/kg bodyweight was injected intraperitoneally at 18 and 2 h before sacrifice. The same volume of 0.9% of NaCl solution was injected to the control rat via the same route according to the same schedule.

The Langendorf isolated heart preparation was employed. Immediately after decapitation, the heart was removed and mounted within 1 min on the apparatus for the isolated heart preparation. The heart was perfused retrogradely at a pressure of 70 mmHg and at a flow rate of 8–10 mL/min with Krebs Ringer solution gassed by a mixture of 95% O_2 and 5% CO_2 which kept the pH at 7.4. The temperature was maintained at 31–32°C by a water jacket. Electrocardiogram (ECG) was monitored with a positive electrode hooked at the apex of the heart and a negative electrode at the aorta.

The procedure for the induction of arrhythmias by ischaemia and reperfusion of Zhan and co-workers (1985) was employed. Immediately after mounting, the heart was subject to ischaemia for 20 min by stoppage of flow, which was followed by perfusion for another 20 min. ECG was monitored throughout the experiment. When there were six ventricular premature contractions (VPC) of regular QRS complexes occurring in succession, it was considered to exhibit ventricular

tachycardia (VT). When the QRS complexes were irregular, it was considered to be ventricular fibrillation (VF). The numbers of hearts in each group exhibiting these ventricular arrhythmias were recorded.

The myocardial contents of catecholamines were determined by high performance liquid chromatography (HPLC). After decapitation the heart was removed immediately, washed in 0.9% saline, frozen on dry ice and stored at −70°C. The hearts were weighed just before extraction. The hearts were homogenized in 3 mL of 0.2 mol/L perchloric acid containing 0.002% w/v ascorbic acid using a polytron. For the adsorption of catecholamines, alumina (40 mg) was added to the supernatants (0.4 mL) after the addition of 1 mL of Tris buffer at pH 8.6. The alumina was washed three times with distilled water. Catecholamines were then eluted with 20 μL of 0.1 mol/L perchloric acid prior to HPLC. The HPLC system consisted of a Biophase ODS 5 μm 25 cm column (BAS, W. Lafayette, Indiana) with a mobile phase of 0.15 mmol/L monochloroacetate buffer, pH 3.0, containing 2 mmol/L NaEDTA and 30 mg/L sodium octyl sulphate. The injection volume was 20 μL and the flow rate was 2 mL/min. The electrochemical detector (BAS, W.Lafayette, Indiana) was set to a potential of 0.65 V. The retention times for NA, adrenaline and dopamine were 1.4, 4.2 and 8.2 min, respectively. The myocardial contents of these substances were calculated from known amounts of NA, adrenaline and dopamine and 3,4-dihydroxybenzylamine (200 pg) was used as an internal standard.

The myocardial contents of enkephalins were measured by radioimmunoassays. The hearts were homogenized in hot 0.1 mol/L hydrochloric acid by a polytron and then boiled for 10 min. After centrifugation, the supernatant was purified by Sep-Pak C18 cartridges (Waters) according to the method of Lang *et al.* (1983). The methanol was dried under a stream of nitrogen at 25°C and the aqueous extract was then lyophilized. Immediately before assays, the residues were dissolved in 0.8 mL assay buffer (0.1 mol/L phosphosaline at pH 7.5 containing 0.1% gelatin, 0.01% bovine serum albumin and 0.01% thimerosal) at 37°C for 10 min with vortexing. Both leu- and met-enkephalins were iodinated by the chloramine T method and purified by biogel P2 chromatography as described previously by Tang *et al.* (1988). Radioimmunoassays were performed according to the method of Hong *et al.* (1983), using activated charcoal to separate free and bound peptides. The antiserum for leu-enkephalin (Peninsula, Belmont) cross-reacted 0.9% with met-enkephalin, 0.02% with dynorphin (1–17), 0.01% with dynorphin (1–8), and 0.01% with dynorphinB/ Rimorphin. The antiserum for met-enkephalin (Immunonuclear, Stillwater) cross-reacted 2.8% with leu-enkephalin, <0.002% with substance P and β-endorphin, porcine dynorphin (1–13) and alpha-neoendorphin and alpha-endorphin (61–76). Pooled heart extracts measured at three different dilutions showed parallelism to the standard curve. The sensitivities of the RIAs were 5 pg/tube. To avoid intra-assay variation, all samples were measured in the same assay.

Chi-square test and Student's *t*-test were used to analyse the difference in the incidence of cardiac arrhythmias and in the myocardial contents of catecholamines and enkephalins, respectively. A difference at the level $P<0.05$ was considered statistically significant.

RESULTS

Table 1 shows the effect of pretreatment with reserpine on the myocardial contents of catecholamines and enkephalins. The myocardial contents of NA, adrenaline and dopamine were significantly reduced by 98.5%, 87.3% and 84.8%, respectively. The myocardial contents of enkephalins were unchanged by the treatment.

The effects of reserpine treatment on ischaemia- and reperfusion-induced ventricular arrhythmias are depicted in Table 2. During ischaemia, eight of 10 hearts exhibited VPC and four

Table 1. Effects of reserpine treatment on myocardial contents of catecholamines and enkephalins

	n	Catecholamines (ng/g) Noradrenaline	Adrenaline	Dopamine	Enkephalins (pg/g) Methionine-	Leucine-
Control	10	665 ± 55.2	27.8 ± 7.87	16.5 ± 1.06	293 ± 16.8	419.84 ± 20.80
Reserpine-treated	9	10.3 ± 0.75***	3.64 ± 0.64**	2.50 ± 0.32***	279 ± 18.6	421.15 ± 22.83
Percentage of control		1.50	12.7	15.2	95.2	100

, * indicate significant difference to the corresponding control group at the levels, $P<0.01$ and $P<0.001$, respectively by Student's t-test.

Table 2. Effects of reserpine treatment on incidence of ventricular arrhythmias during myocardial ischaemia and reperfusion

	n	Incidence of ventricular arrhythmias Ischaemia VPC	VT	VF	Total	Reperfusion VPC	VT	VF	Total	Recovery
Control	10	8	4	0	8	10	10	10	10	4 (40%)
Reserpine-treated	13	5*	0*	0	5*	11	7*	3***	12	7 (54%)

n, number of hearts; VPC, ventricular premature contraction; VT, ventricular tachycardia; VF, ventricular fibrillation.

*, *** signify statistical difference to the control group to the levels $P<0.05$, $P<0.001$, respectively, by Chi-squared test.

of 10 hearts exhibited VT in the control group. Altogether there were eight hearts exhibiting ventricular arrhythmias. In the reserpine-treated rats, only five of 13 hearts exhibited VPC. The reduction both in the incidence and severity of arrhythmias was statistically significant. During reperfusion both the incidence and severity of arrhythmias were greater than in the ischaemia period. All 10 hearts in the control group exhibited VPC, VT and VF during this period. In the reserpine-treated rats, 11, 7 and 3 of 13 hearts showed VPC, VT and VF, respectively and 12 of 13 hearts had ventricular arrhythmias. The reductions in the incidence of VT and VF in the reserpine-treated group were statistically significant.

DISCUSSION

The use of reserpine for depleting the myocardium of catecholamines has two advantages over 6-OH-dopamine. First, in accord with the finding of previous workers (Maling *et al.* 1959), reserpine depleted more than 98% of the myocardial NA in the rat (Table 1), whereas 6-OH-dopamine depleted only 90% of the myocardial NA in the cat (Sheridan *et al.* 1980) and guinea pig (Culling *et al.* 1984) and 83% in the rat (F. Tang, unpubl. data). Second, reserpine specifically depleted catecholamines without affecting the myocardial contents of enkephalins (Table 1). This suggests that the endogenous opioid system, which has been shown to be involved in reperfusion-induced arrhythmias (Zhan *et al.* 1985; Huang *et al.* 1986; Wong & Lee 1987), remained undisturbed, whereas 6-OH-dopamine removed 70% of the leu-enkephalin content in the heart (Lang *et al.* 1983), suggesting that the cardiac opioid system was affected.

In the present study, we found that pretreatment with reserpine reduced the incidence of arrhythmias during ischaemia and reperfusion. The results are at variance with the finding of Maling *et al.* (1959), who found no change in spontaneous ventricular activities in reserpine-treated conscious dogs following coronary artery ligation. On the other hand, our results are in agreement with the findings that reserpine treatment reduces the ischaemia-induced ventricular

arrhythmias in anaesthetized dogs except that the reduction in their experiment was significant only after vagotomy (Ebert *et al.* 1970). It is, however, difficult to explain the discrepancy as the contribution of the vagus nerve was not assessed in the isolated heart preparation. The lack of statistical difference in the groups with intact vagus nerves may be related partly to the small number of observations in the study. Our results are also in line with the finding that surgical sympathectomy significantly reduces the incidence of arrhythymias in dogs (Ebert *et al.* 1970). These findings indicate that myocardial catecholamines play a contributory role in arrhythmogenesis during ischaemia and reperfusion.

It has been shown that the incidence of arrhythmias was reduced significantly during ischaemia and reperfusion in 6-OH-dopamine-treated dogs in which the myocardial NA, measured at the end of 10 min of reperfusion following 30 min of ischaemia, was about 15% of the normal level, whereas no arrhythmias occurred when the myocardial NA was reduced to less than 5% of the normal levels (Culling *et al.* 1984), suggesting that myocardial NA is probably a dominant mediator of ischaemia- or reperfusion-induced arrhythmias. On the contrary, in the present study we found that despite an almost complete depletion of NA from the heart (more than 98%), there was still a significant number of arrhythmias both during ischaemia and reperfusion. While the results of the present study confirm that the reduction in incidence of ventricular arrhythmias during ischaemia and reperfusion is indeed due to the depletion of endogenous myocardial NA, the occurrence of arrhythmias in hearts with a NA level of less than 2% of the normal indicates that NA is not the only mediator of ischaemia- or reperfusion-induced arrhythmias. This explains the observation that ischaemia-induced arrhythmias are not always associated with increased myocardial levels of cAMP (Corr *et al.* 1978) or an increased release of NA (Daugherty *et al.* 1986), both of which are indications of increased adrenergic activities.

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