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The effect of modification of sympathetic activity on responses to ligation of a coronary artery in the conscious rat

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1 Ligation of a coronary artery was performed in conscious rats whose sympathetic system activity had been altered by various treatments.

2 β-Adrenoceptor blockade with acute ($0.2\,mg\,kg^{-1}$ plus $0.1\,\mu g\,kg^{-1}\,min^{-1}$) or chronic ($50-60\,mg\,kg^{-1}$ daily for 12 days) propranolol treatment had little effect on arrhythmias, or other responses to ligation.

3 Abrupt withdrawal of chronic propranolol two days before ligation was also without effect.

4 Reduction of sympathetic activity acutely with labetalol ($5\,mg\,kg^{-1}$), or chronically with adrenomedullectomy and 6-hydroxydopamine treatment, accentuated the adverse effects of ligation.

5 The results of this study suggest that, while activity of the sympathetic system is not detrimental during ligation in the conscious rat, it may be important for survival.

Introduction

Despite the widespread use of β-adrenoceptor blocking drugs to control arrhythmias following myocardial infarction, the role of neuronally released or extraneuronal catecholamines on the pathological sequelae of myocardial infarction is unclear.

Studies with experimental models of myocardial infarction have indicated a protective effect of infusions of adrenaline, noradrenaline and phenylephrine against dysrhythmias, and a worsening produced by isoprenaline (Marshall, Muir & Winslow, 1981). The latter provides support for the clinical use of β-blocking drugs, as do the observations of Campbell & Parratt (1981) that these drugs reduce arrhythmias following ligation of coronary arteries in rats. Further, β-receptor blocking drugs have been shown to reduce infarct size in the dog, provided that the blocking drug is administered soon after the experimental induction of myocardial ischaemia (Burmeister, Reynolds & Lee, 1981).

Many of the experimental models of myocardial ischaemia suffer from the disadvantage that the coronary ligation is carried out under anaesthesia. The following work is an evaluation of the effects of chronic and acute β-receptor blockade, combined α- and β-adrenoceptor blockade, adrenomedullectomy followed by chemical sympathectomy with 6-hydroxydopamine, and cardiac hypersensitivity, induced by withdrawal of chronic propranolol administration, on the conscious rat model of myocardial infarction.

Methods

Coronary ligation in conscious rats was performed as described by Johnston, MacLeod & Walker (1981) and MacLeod, Augereau & Walker (1982). Wistar rats (250–350 g) were anaesthetized with halothane and the chest opened under positive pressure respiration. A polypropylene suture was passed around the left anterior descending coronary artery and then through a polythene tube which was exteriorised behind the neck. Stainless steel ECG leads were placed subcutaneously in the pectoral muscle and in each limb and were exteriorized near the occluder. In the same operation permanent aortic and external venous cannulae were inserted for recording blood pressure after the technique of Weeks (1981).

Ten days after operation, animals were placed in a

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cage, the ECG leads connected to a Grass polygraph and the aortic cannula connected to a pressure transducer. ECG and blood pressure were recorded for 30 min before the coronary ligature was then tightened by pulling on the suture whilst holding the polythene guide. The ECG and blood pressure were recorded for 4 h post-ligation. The aortic cannula was disconnected from the transducer and heat-sealed, the ECG leads were disconnected and the animal returned to the animal house.

Variables recorded during the 4 h were heart rate, number of premature ventricular contractions (PVC), incidence and duration of spontaneously reversible or non-spontaneously reversible ventricular tachycardia (VT) or fibrillation (VF) and bradycardia. Also recorded was the time for appearance of a Q wave and the elevation of the 'S–T' segment. An arrhythmia score was recorded for each animal as follows: (1) 50–500 PVC; (2) >500 PVC or one episode of spontaneously reversible VF; (3) more than one episode of spontaneously reversible VT and/or VF, or one or more episodes of non-spontaneously reversible VT and/or VF lasting less than 60 s; (4) reversible VT and/or VF lasting 60–120 s; (5) VT and/or VF lasting more than 120 s; (6) irreversible VF causing death within 15–240 min of ligation; (7) fatal VF within 4–15 min; (8) fatal VF within 4 min.

Twenty-four hours later the blood pressure and ECG were re-recorded for 30 min and the animal was then killed and the heart rapidly removed and perfused retrogradely with saline through the severed aorta. When the perfusate was free of blood, 2 ml of fast green FCF dye (1 mg ml^{-1} in Krebs solution) was injected to differentiate between perfused (green) and occluded (pink) tissue. The occluded tissue was dissected free from the ventricle and weighed. Occluded zone (OZ) was recorded as percentage of total ventricular weight. Subsequently the ventricular tissue was cut into 1 mm slices and incubated in tetrazolium dye (10 mg triphenyltetrazolium chloride ml^{-1} of sodium phosphate buffer, pH 8.5) at 37°C for 30–45 min. Slices were then placed in formaldehyde (10% w/v normal saline) for two days. Infarcted tissue (white) was dissected from viable tissue (purple) and expressed as percentage total ventricular weight (infarcted zone IZ).

Drug treatments

Groups of 8 to 11 animals were treated as follows: (1) labetalol, 5 mg kg^{-1} intravenously 5 min before ligation; (2) adrenomedullectomy (under halothane anaesthesia) followed by 6-hydroxydopamine (2×50 mg kg^{-1} i.v.) nine days later, followed by a further 2×100 mg kg^{-1} six days after this. The surgical preparation was made seven days after adrenomedullectomy; (3) propranolol 0.2 mg kg^{-1} i.v. just before ligation, then 0.1 μg kg^{-1} min^{-1} throughout the experiment. (This gives a maintained dose-ratio for isoprenaline of 20); (4) propranolol 50–60 mg kg^{-1} day^{-1} for 12 days (including the period of ligation) in the drinking water (gives a dose ratio for isoprenaline of 24); (5) animals subjected to the chronic propranolol treatment for 12 days, then given normal drinking water for two days before ligation. Control animals (20) were used randomly throughout the experiment.

Where possible the design was a random and blind one. Complete details of this method of producing ligation in conscious rats, the measurement of arrhythmias and data analysis have been described in detail (Johnston, MacLeod & Walker, 1981; 1982).

Statistical treatment

Results for drug treatments were compared with the accumulated controls by analysis of variance and Duncan's mean test (Gregg & Osterlin, 1977).

Results

Arrhythmias

The incidence of PVC was not normally distributed but showed significant skewness and kurtosis. The logarithm to the base 10 of the number of PVC did show Gaussian distribution and statistical analysis was then performed on $\log_{10}$ PVC. The arrhythmias score (AS) also showed a normal distribution. The number and duration of the major arrhythmic events of VT or VF were expressed as $\log_{10}$ values. Such values were normally distributed.

Values are recorded for the 0–30 min and 0–4 h post-ligation periods as arrhythmias occur in two phases with peaks at 10 min and 2.5 h post-ligation.

None of the treatments caused alteration in arrhythmia score (Figure 1a) or in the incidence of PVC (Figure 1b). There was also no change in the overall incidence of ventricular tachycardia or fibrillation between the groups (Figure 1c) nor was there a difference in the number or duration (expressed as $\log_{10}$ values) of spontaneously reversible or non-spontaneously reversible VT or VF. Chronic propranolol treatment caused impairment of atrioventricular conduction (Figure 1c) seen as periods of bradycardia, and this was also observed in some animals whose coronary arteries were ligated two days after withdrawal of the chronic propranolol treatment.

Occluded and infarcted zone, mortality

Figure 2 summarizes the effects of the treatments on the size of the occluded and infarcted zones and on mortality. Whereas none of the treatments affected the extent of the occluded or infarcted zones (the infarcted zone in 6-hydroxydopamine-treated animals could not be measured due to early death) labetalol, or adrenomedullectomy followed by 6-hydroxydopamine caused increased mortality ($P < 0.05$) following coronary ligation. Most animals so treated died within 5 min of ligation either due to intractable ventricular fibrillation or, more often, of non-arrhythmic cardiac output failure. With such deaths, blood pressure fell below 25 mmHg and animals stopped breathing.

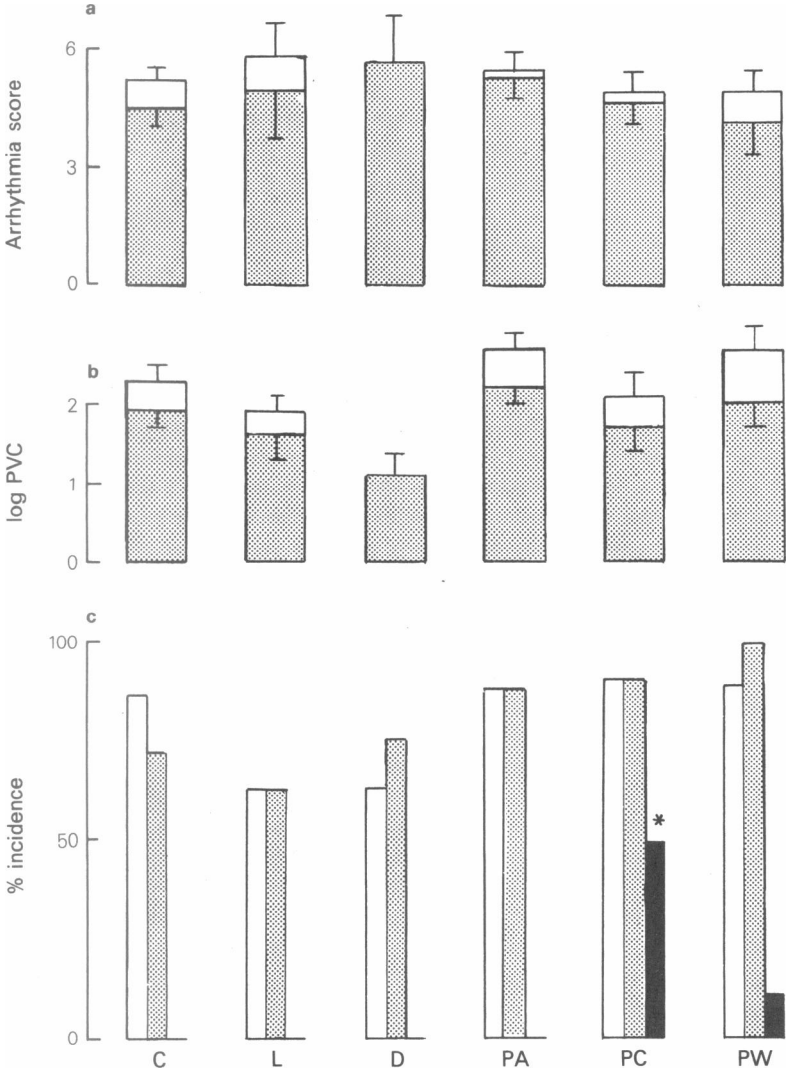

Figure 1 The effect of drug treatments on post-ligation arrhythmias: (a) arrhythmia score at 30 min (stippled) and 4 h (open columns); (b) log PVC at 30 min (stippled) and 4 h (open columns); (c) Incidence in group of ventricular tachycardia (open columns), ventricular fibrillation (stippled) and bradycardia (black) over four-hour experimental period. Vertical lines indicate s.e.mean. C, control; L, labetalol-treated; D, 6-hydroxydopamine treated; PA, acute and PC, chronic propranolol treatment; PW, chronic propranolol treatment withdrawn for 2 days (for doses, see Methods). Values are mean with s.e.mean for $n = 8-11$ except for accumulated control.
*Significant at $P < 0.05$ by ANOVA or Chi^2 from controls.

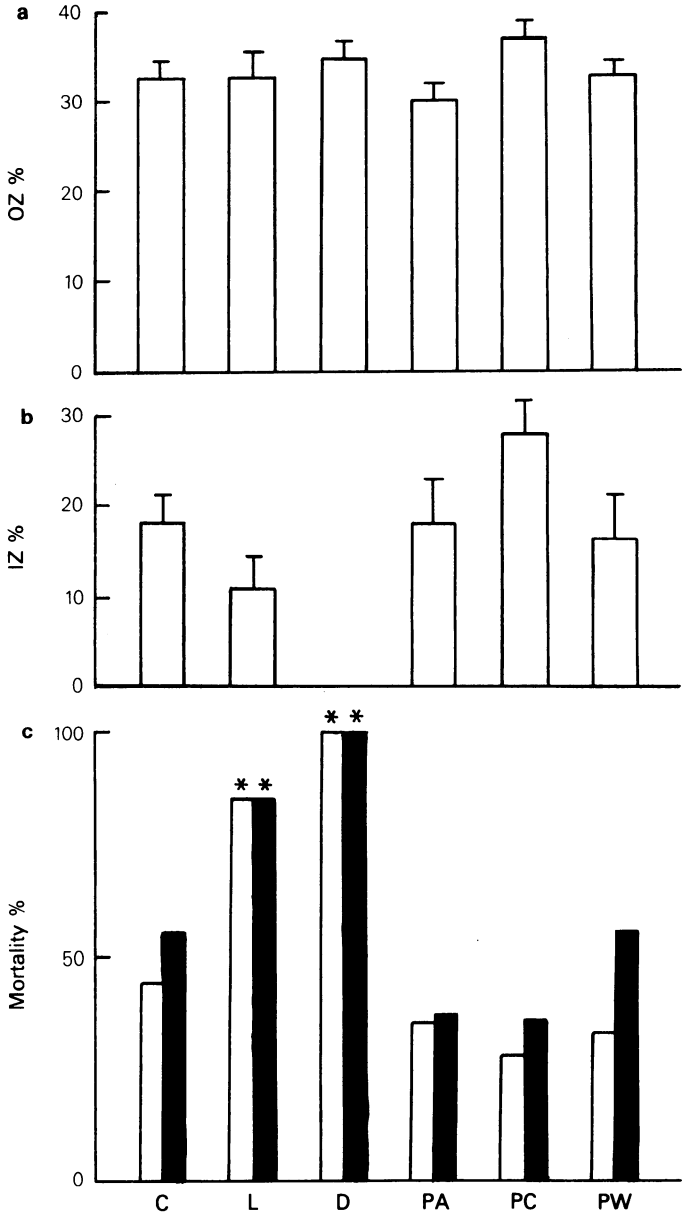

Figure 2 The effect of drug treatments on occluded zone (OZ), infarcted zone (IZ) and mortality. (a) Occluded zone and (b) infarcted zone as percentage of total ventricular weight; (c) mortality (percentage) in group at 4 (open columns) and 24 h (black columns) after ligation. Treatments denoted as Figure 1. Values are mean for $n = 4-20$; vertical lines indicate s.e.mean.
*Significant at $P < 0.05$ by ANOVA.

Blood pressure and heart rate

Labetalol and adrenomedullectomy, followed by 6-hydroxydopamine, reduced systolic and diastolic pressure at least for the period after ligation (Figure 3). Values for 6-hydroxydopamine-treated animals could only be recorded at -1 and $+5$ min post-ligation since the animals died soon after this time. Preligation heart rate was reduced ($P < 0.05$) in the labetalol- and 6-hydroxydopamine-treated animals,

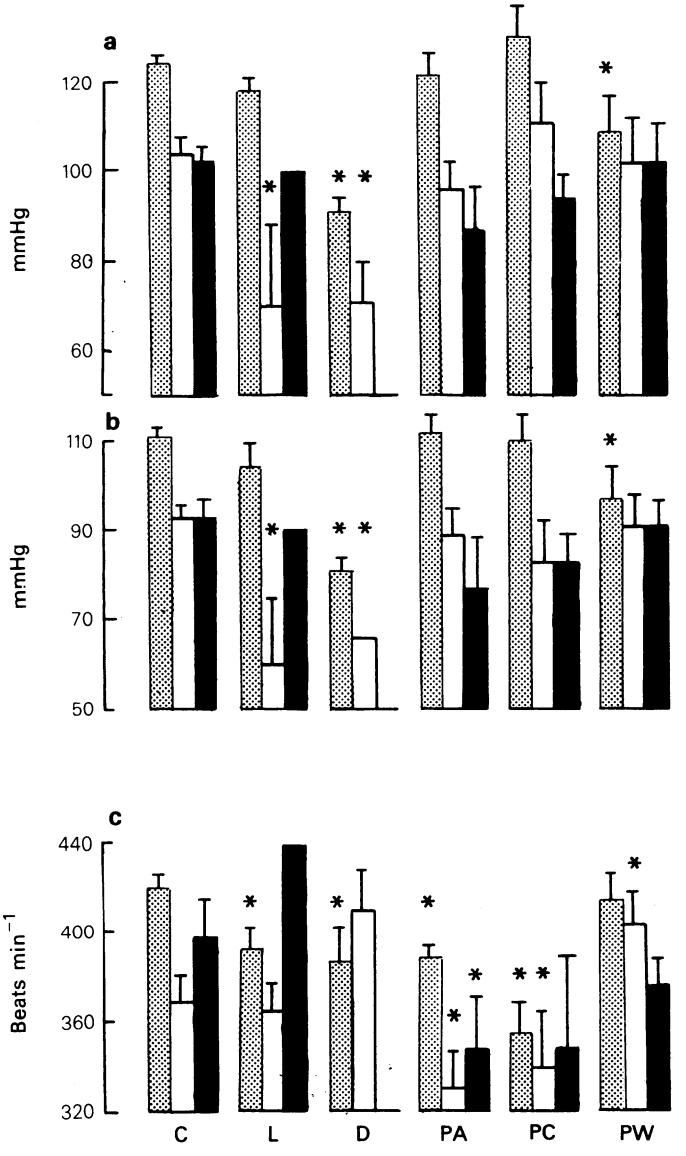

Figure 3 The effect of treatments on systolic (a) and diastolic (b) blood pressure and heart rate (c) 1 min before (stippled), 30 min after (open columns) and 4 h after (black) coronary ligation. Treatments denoted as for Figure 1. Four-hour values for D not recorded due to early death. Values are mean for $n = 4-20$; vertical lines indicate s.e.mean.
*Significant from controls at $P < 0.05$.

and heart rate at all periods was reduced in those animals on chronic and acute propranolol treatments.

ECG pattern

None of the drug treatments produced significant changes in ST segment elevation or R wave height following ligation. No effect on the incidence of a Q wave was observed but in labetolol or 6-hydroxydopamine-treated animals early death precluded the measurement of Q wave incidence, since a Q wave was rarely observed before 30 min post-ligation.

Discussion

Clinical studies suggest that administration of β-adrenoceptor blocking agents following myocardial infarction lowers the incidence of sudden death and reinfarction, and Parratt (1980) cites evidence that these drugs reduce the severity of the early dysrhythmias that occur after ligation of coronary arteries in experimental studies. The present studies in conscious animals show no protective action of propranolol whether it was acutely administered or after chronic treatment with effective blocking doses.

The absence of a protective effect of propranolol in our experiments could be explained by the fact that in other experimental studies ligation was carried out under anaesthesia. Various anaesthetics have profound effects on the pattern of arrhythmias and death following coronary artery ligation in rats. For example, Au, Collins, MacLeod & Walker (1979) showed that the incidence of arrhythmias was significantly less in halothane-anaesthetized animals compared with nitrous oxide/pethidine or pentobarbitone anaesthesia. Also, fentanyl anaesthesia tends to increase arrhythmias and mortality when compared with conscious controls, whereas halothane decreases arrhythmias and mortality (MacLeod *et al.*, 1982). Thus, the protective effect of propranolol may only be evident in such models if post-infarction arrhythmias are affected in some way by the anaesthesia. Alternatively, β-blocking drugs with some intrinsic sympathomimetic activity may be more effective antidysrhythmics as is suggested by the work of Campbell & Parratt (1981).

Where the protective effect of β-adrenoceptor blocking drugs can be shown in experimental studies it has been suggested that this is due to β-receptor blockade itself, rather than other actions shared by these drugs such as membrane stabilization (Campbell & Parratt, 1981). Thus, procedures leading to increased sensitivity of the myocardium to β-adrenoceptor agonists might be expected to make post-ligation dysrhythmias worse (see also Marshall *et al.*, 1981). Such a procedure is abrupt withdrawal of chronic administration of propranolol, which was shown by Botting & Crook (1981) to result in a four fold increase in sensitivity to isoprenaline in rat myocardium two days after withdrawal. However, such an induced hypersensitivity, had no significant effect on post-ligation mortality or dysrhythmias.

Whereas modification of β-adrenoceptor sensitivity unexpectedly caused no change in the pathological sequelae of ligation in conscious animals, quite striking effects were caused by reduction in total sympathetic activity produced by administration of the combined α- and β-adrenoceptor blocking drug labetalol, or by adrenomedullectomy followed by destruction of adrenergic nerves with 6-hydroxydopamine. In such animals mortality after ligation was significantly increased. Systolic and diastolic blood pressure was reduced by these treatments, at least post-ligation, and this lowered blood pressure per se may have been the reason for the increase in mortality, for Au, Collins, Harvie & Walker (1979) showed that infusions of prostacyclin in concentrations that were depressor increased incidence of arrhythmias, whereas lower concentrations of prostacyclin have an antiarrhythmic effect (Coker & Parratt, 1981). The lower blood pressures in these animals also contributed to the increase in deaths due to non-arrhythmogenic heart failure.

Our finding of an exacerbation of post-ligation mortality by labetalol is not in accord with the observations of Chiarello, Brevetti, De Rosa, Acunzo, Petillo, Rengo & Condorelli (1980) who demonstrated a reduction by labetalol of the infarcted tissue 24 h after coronary ligation in rats.

Overall the results described suggest little influence of β-adrenoceptor stimulation in the genesis of arrhythmias following coronary ligation and in fact suggest that an adequately functioning sympathetic system is to some extent protective in this experimental model. Such a view is in part agreement with the observations of Marshall *et al.* (1981).

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