Anti-arrhythmic protection by ischaemic preconditioning in isolated rat hearts is not due to depletion of endogenous catecholamines

C.S. Lawson a,*, D.J. Hearse b

a Wessex Cardiac Unit, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
b Cardiovascular Research, The Rayne Institute, St Thomas' Hospital, London SE1 7EH, UK

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Abstract

Objectives: The present study addresses whether the mechanism of anti-arrhythmic protection by ischaemic preconditioning involves depletion of myocardial catecholamines. Methods: In a randomised series of studies isolated rat hearts, perfused with whole blood, underwent episodes of regional ischaemia and reperfusion induced with a snare around the left coronary artery. Control hearts (Group 1, n = 12) were subjected to 40 min aerobic perfusion, 30 min ischaemia and 10 min reperfusion. Preconditioned hearts (Group 2, n = 12) were subjected to 10 min aerobic perfusion, three cycles of ischaemia and reperfusion (5 min each), 30 min ischaemia and 10 min reperfusion. Cardiac rhythm was recorded continuously and arrhythmias quantified during the final periods of ischaemia and reperfusion. At the end of the experiment samples of right ventricular (RV; non-ischaemic) and left ventricular (LV; ischaemic territory) tissue were separated and frozen. In 5 additional groups (n = 6/group) tissue samples were taken after 10 min aerobic perfusion, after 10 min aerobic perfusion followed by 1, 2 or 3 preconditioning cycles and after 40 min of aerobic perfusion. All tissue samples were analysed for catecholamine content. Results: Preconditioning resulted in reductions in the incidence of ischaemia-induced VF from 67% in Group 1 to 8% in Group 2, the incidence of ischaemia-induced VT from 100% to 17% and the number of ischaemia-induced VPBs from 246 ± 25 to 59 ± 19 (each P < 0.05). The mean content of noradrenaline and adrenaline was consistently higher in RV than LV tissue. Within the LV, however, neither preconditioning nor prolonged ischaemia had any significant effect upon tissue catecholamine content at any time in the experimental protocol. Conclusions: Depletion of myocardial catecholamines is not involved in the mechanism of anti-arrhythmic protection by ischaemic preconditioning in isolated rat hearts.

Keywords: Preconditioning; Arrhythmias; Rat, isolated heart; Catecholamines

1. Introduction

Ischaemic preconditioning can afford profound protection against necrosis [1–3] and arrhythmias [2–5] occurring as a consequence of myocardial ischaemia and reperfusion. The precise mechanisms underlying these protective phenomena remain to be firmly established. Although in most species protection against myocardial necrosis has been reported to involve the release of endogenous adenosine [6,7], possibly acting via inhibitory G proteins [8], this does not appear to be the case for the rat [9,10], the species in which anti-arrhythmic protection has been most consistently reported [2–5].

Additional evidence, although largely circumstantial, suggests that the mechanism of anti-arrhythmic protection by ischaemic preconditioning may be distinct from that responsible for protection against myocardial necrosis. Firstly, anti-arrhythmic protection can be demonstrated whilst myocardial injury is reversible rather than irreversible [4,11,12]. Secondly, preconditioning reduces the severity of both ischaemia- and reperfusion-induced arrhythmias without altering their temporal profiles [5,11,12] — this is contrary to what might be expected from the proven ability of preconditioning to delay the development of irreversible ischaemic injury. Thirdly, mechanistic studies utilising arrhythmias as the primary end-point [13,14]
have yielded results at odds with comparable studies of infarct size [15,16].

Recent data have suggested catecholamines may be involved in the mechanism of preconditioning; indeed, the protective phenomenon can be mimicked by \( \alpha_1 \)-adrenoceptor activation [17]. The importance of endogenous catecholamines in the genesis of arrhythmias during episodes of acute myocardial ischaemia and reperfusion has long been recognised. Depletion of endogenous catecholamines and sympathetic denervation can each result in protection against these arrhythmias [18–21]. In addition, ischaemia can induce the release of catecholamines from sympathetic nerve terminals within the heart wall, ultimately leading to their depletion [22,23]. In view of these findings an alternative possible mechanistic role for catecholamines has been suggested: that episodes of preconditioning ischaemia and reperfusion might result in the depletion of endogenous catecholamines [4] and thus protect the heart from arrhythmias during subsequent episodes of ischaemia and reperfusion.

It is not clear, however, if the very brief episodes of ischaemia used to induce preconditioning are sufficient to produce significant reductions in the content of endogenous catecholamines. Indeed, in dogs Miyazaki and Zipes [24] have shown that preconditioning results in preservation, rather than attenuation, of autonomic reflexes. A comparison of studies performed in different species, however, suggests that rats might be more prone than other species to ischaemia-induced catecholamine depletion [23,25–27]. In the present study, therefore, an established model of protection by ischaemic preconditioning was employed to investigate whether anti-arrhythmic protection is temporally associated with depletion of endogenous cardiac catecholamine stores.

2. Materials and methods

2.1. Animals

Wistar rats (Bantin and Kingman Ltd, Humberside, UK) weighing 300–350 g (donor animals, male) or 450–600 g (support animals, either sex) were used in all studies. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH No. 85-23, revised 1985).

2.2. The isolated blood-perfused rat heart preparation

Support rats, used to provide blood for the perfusion circuit, were anaesthetised with pentobarbitone (60 mg/kg, ip) and placed supine on a thermostatically controlled heated operating table to maintain rectal temperature at 37.0 ± 0.5°C. Animals breathed spontaneously (via a face mask) a mixture of oxygen and air, the proportions and flow rate of which were adjusted to maintain \( \text{PO}_2 \) and \( \text{pCO}_2 \) within the physiological range. The femoral artery and vein were cannulated and heparin administered (1000 U/kg, iv). Support animal heart rate and blood pressure were continuously recorded via a side arm on the arterial cannula. Support animals were used for no longer than 4 h and additional anaesthesia was administered as necessary using boluses of pentobarbitone (6 mg/kg, ip).

Donor rats were anaesthetised with pentobarbitone (60 mg/kg, ip), heparinised (1000 U/kg, iv) and their hearts rapidly excised. Cardiac arrest was induced by immersing the heart in cold (4°C) Haemaccel (Hoechst Ltd). A stainless steel perfusion cannula was inserted into the ascending aorta within 30 s of excision and perfusion in the Langendorff mode was initiated with arterial blood from the support animal. During periods of aerobic perfusion blood was pumped to the cannula at an initial rate of 2.5 ml/min (Minipuls 3 peristaltic pump, Gilson Ltd). Perfusion pressure was continuously monitored via a sidearm on the perfusion cannula. The pulmonary artery of the donor heart was cut to facilitate the drainage of coronary effluent which was collected in a reservoir and pumped back, through a blood filter (pore size 200 \( \mu \)m), into the femoral vein of the support animal. The donor heart was sealed in a temperature-regulated reservoir and the temperature of the blood and donor heart was maintained at 37.0 ± 0.5°C by a thermostatically regulated heat-exchange system.

The electrocardiogram (ECG) of the donor heart was continuously recorded via a silver electrode implanted in the left ventricular free wall with respect to a reference electrode attached to the aortic cannula. The signal was displayed continuously on a digital storage oscilloscope and recorded continuously on a pen recorder at a paper speed of 10 mm/s. The chart speed was increased to 50 mm/s during arrhythmic episodes. This system gave a clear P wave and allowed analysis of the morphology of the QRS complex.

2.3. Induction of regional ischaemia and reperfusion

A 4/0 braided silk suture was passed around the left main coronary artery and a snare was formed by passing both ends through a piece of polyethylene tubing. Regional ischaemia and reperfusion were produced by tightening and loosening the snare as described previously [28]. Occlusion and reperfusion were confirmed by ECG changes, instantaneous changes in perfusion pressure and the development and relief of cyanosis of the occluded zone. To maintain a constant flow of blood/gram tissue, during occlusions coronary flow was reduced so as to maintain the perfusion pressure at the pre-occlusion level. Similarly, flow was restored to 2.5 ml/min at the time of reperfusion.
2.4. Experimental protocols

The experimental protocol is shown in Fig. 1. A total of 54 rats were randomised into 7 groups. Control hearts (Group 1, n = 12) were subjected to 40 min of aerobic perfusion, 30 min of ischaemia and 10 min of reperfusion. Preconditioned hearts (Group 2, n = 12) were subjected to 10 min aerobic perfusion, three cycles of 5 min of ischaemia and 5 min of reperfusion, 30 min of ischaemia and 10 min of reperfusion. In 5 additional groups (n = 6/group) tissue samples were taken after 10 min of aerobic perfusion (Group 3), after 10 min of aerobic perfusion followed by 1, 2 or 3 cycles of 5 min of ischaemia and 5 min of reperfusion (Groups 4, 5 and 6 respectively), and after 40 min of aerobic perfusion (Group 7). Cardiac rhythm was recorded continuously in all hearts and arrhythmias quantified during the final periods of ischaemia and reperfusion in Groups 1 and 2.

At the end of perfusion all hearts from Groups 3-7 and 6 hearts selected at random from each of Groups 1 and 2 were taken for measurement of tissue catecholamine content. These hearts were perfused with oxygenated bicarbonate buffer for 20 s at 80 mmHg pressure to wash the blood from the coronary circulation. Occlusion of the left coronary artery, as described above, reliably results in ischaemia of the entire left ventricular free wall, in addition to a variable amount of the intraventricular septum, but with sparing of the right ventricular free wall which is supplied by the right coronary artery. To separate ischaemic from non-ischaemic tissue, therefore, hearts to be used for analysis of endogenous catecholamines were removed from the perfusion apparatus and their left and right ventricular free walls separately excised within 30 s. Tissue samples were rapidly frozen in liquid nitrogen (−196°C) and subsequently stored in liquid nitrogen until analysed.

2.5. Estimation of occluded zone size

The six hearts in Groups 1 and 2 that were not used for catecholamine analysis underwent measurement of occluded zone size by blue dye perfusion. At the end of the experimental period the hearts were perfused with oxygenated bicarbonate buffer for 2 min at 80 mmHg pressure and then sulphadiazine blue BPC (Disulfine Blue, ICI, 5 ml/l in 0.9% saline) was infused for 2 min at 80 mmHg pressure. The snare around the left main coronary artery was re-tightened and dye washed from the non-ischaemic zone by further perfusion with bicarbonate buffer for 2 min. The heart was removed from the cannula and the ventricles were separated from the atria and great vessels. The dyed and non-dyed regions were then separated by careful dissection. Both were lightly blotted and weighed. Dye staining was uniform and the occluded zone was transmural in all hearts examined.

The freezing of hearts for the assay of catecholamines at the end of each perfusion protocol precluded direct measurement of occluded zone size in all hearts. Estimates of the occluded zone sizes could be made, however, from (i) the initial increase in perfusion pressure (prior to the coronary flow rate being reduced) following coronary occlusion, and (ii) the reduction in flow rate necessary to return the perfusion pressure to the pre-occlusion level. These indices correlated well with the occluded zone size estimated by blue dye perfusion in Groups 1 and 2, and indicated occluded zones of between 37 and 51% for all hearts studied.

2.6. Exclusion criteria

Based on previous experience with the isolated blood-perfused rat heart preparation, predefined exclusion criteria were: (i) the development of significant arrhythmias (VF, VT or more than 6 VPBs per min) except during the final 30 min episode of ischaemia and subsequent periods of reperfusion in Groups 1 and 2, (ii) the perfusion pressure falling to < 50 mmHg for > 1 min, and (iii) occluded zone size representing < 30% of the ventricular mass. In the present study no heart was excluded on these, or any other, grounds.

2.7. Analysis of arrhythmias

Arrhythmias were quantified for all hearts in Groups 1 and 2. The incidences of ventricular fibrillation and ventricular tachycardia, and the number of ventricular prema-
ture beats occurring during the final 30 min episode of ischaemia, and the incidences of ventricular fibrillation and ventricular tachycardia during the final 10 min reperfusion period were determined. The diagnosis and quantification of these arrhythmias conformed with the Lambeth Conventions [29]. Ventricular tachycardia was defined as 4 or more consecutive ventricular premature beats and ventricular fibrillation as a signal in which individual QRS complexes could not be distinguished from one another and for which a rate could not be determined.

2.8. Catecholamine assays

Myocardial catecholamines were extracted by homogenising tissue samples in 0.1 M perchloric acid. Following centrifugation the concentrations of noradrenaline and adrenaline in the supernatant were determined using high pressure liquid chromatography [30].

2.9. Statistics

Data are presented as either percent incidence or mean ± standard error of mean. Binomially distributed data (arrhythmia incidences) were compared using contingency tables. For normally distributed data, comparisons between multiple groups were performed using a one-way analysis of variance followed by the Student-Newman-Keuls test where appropriate.

3. Results

3.1. Preconditioning and ischaemia-induced arrhythmias

Fig. 2 shows the effect of preconditioning on the incidences of ischaemia-induced VF and VT, and on the number of ischaemia-induced VPBs, during the 30 min episodes of ischaemia in Groups 1 and 2. As in previous studies in the isolated blood-perfused rat heart [12,31],

Table 1

<table>
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<th>Study Groups</th>
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<tr>
<td>LV Noradrenaline</td>
<td>4.1±0.4</td>
<td>4.4±0.4</td>
<td>4.1±1.1*</td>
<td>3.9±0.4*</td>
<td>4.9±0.6</td>
<td>4.5±0.4</td>
<td>5.0±0.3</td>
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<tr>
<td>RV Noradrenaline</td>
<td>4.6±0.4</td>
<td>5.8±0.5</td>
<td>6.9±0.2</td>
<td>6.1±0.3</td>
<td>6.0±0.4</td>
<td>5.3±0.5</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>LV Adrenaline</td>
<td>0.47±0.08*</td>
<td>0.52±0.09</td>
<td>0.46±0.23</td>
<td>0.22±0.08*</td>
<td>0.42±0.10</td>
<td>0.53±0.04</td>
<td>0.60±0.18</td>
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<tr>
<td>RV Adrenaline</td>
<td>0.82±0.09</td>
<td>0.80±0.18</td>
<td>0.47±0.19</td>
<td>0.46±0.06</td>
<td>0.74±0.22</td>
<td>0.53±0.16</td>
<td>0.98±0.18</td>
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LV = left ventricular free wall; RV = right ventricular free wall.
Group 1 = 40 min aerobic perfusion + 30 min ischaemia + 10 min reperfusion, Group 2 = 10 min aerobic perfusion + 3 cycles of preconditioning + 30 min ischaemia + 5 min reperfusion, Group 3 = 10 min aerobic perfusion, Group 4 = 10 min aerobic perfusion + 1 cycle of preconditioning, Group 5 = 10 min aerobic perfusion + 2 cycles of preconditioning, Group 6 = 10 min aerobic perfusion + 3 cycles of preconditioning, Group 7 = 40 min aerobic perfusion.

One cycle of preconditioning = 5 min ischaemia + 5 min reperfusion.

* P < 0.05 versus respective right ventricular tissue sample. n = 12/group.
Preconditioning had a profound protective effect on the severity of ischaemia-induced arrhythmias. The incidence of ischaemia-induced VF was reduced from 67% to 8% \((P < 0.05)\), the incidence of ischaemia-induced VT from 100% to 17% \((P < 0.05)\) and the mean number of VPBs from 246 ± 25 to 59 ± 19 \((P < 0.05)\).

3.2. Preconditioning and reperfusion-induced arrhythmias

Preconditioning also resulted in reductions in the severity of reperfusion-induced arrhythmias, although as a consequence of the extended period of ischaemia used in the present study these were not statistically significant. The incidence of reperfusion-induced VP was reduced from 42% to 8% and that of reperfusion-induced VT from 75% to 42% in Groups 1 and 2 respectively.

3.3. Tissue catecholamine content

The contents of noradrenaline and adrenaline in the left ventricular and right ventricular free walls in the various study groups at the end of perfusion are given in Table 1. In the left ventricular free wall the noradrenaline content ranged from 3.9 ± 0.4 pmol/g in Group 4 to 5.0 ± 0.3 pmol/g in Group 7, and the content of adrenaline ranged from 0.22 ± 0.08 pmol/g in Group 4 to 0.60 ± 0.18 pmol/g in Group 7. In the right ventricular free wall the noradrenaline content ranged from 4.6 ± 0.4 pmol/g in Group 1 to 6.9 ± 0.2 pmol/g in Group 3 and the adrenaline content ranged from 0.46 ± 0.06 pmol/g in Group 4 to 0.98 ± 0.18 pmol/g in Group 7. The tissue contents of both noradrenaline and adrenaline tended to be consistently lower in the left ventricular free wall than in the right, significantly so for noradrenaline in Groups 3 and 4 and for adrenaline in Groups 1 and 4. The only exception to this generalisation was the adrenaline content in Group 6 which was the same in both left and right ventricular free walls.

In none of the study groups was the left ventricular free wall tissue content of either noradrenaline or adrenaline significantly different from that in Groups 3 or 7 (i.e. those hearts subjected only to aerobic perfusion). Thus, there was no evidence that preconditioning, 30 min of extracorporeal perfusion, or 30 min of sustained ischaemia followed by 10 min of reperfusion, resulted in depletion of catecholamines from the left ventricular free wall.

3.4. Haemodynamic data

The initial blood pressure and heart rate of the support animals and the initial rate and perfusion pressure of the isolated hearts are shown in Table 2. There were no significant differences between the groups for any of these variables. The support animals remained haemodynamically stable throughout all experiments with no significant alterations in blood pressure or heart rate. Following reperfusion, the perfusion pressure stabilised within 5 min in all groups and there were no significant changes in this or in donor heart rate during the experiments. There was no significant difference between the occluded zone sizes, measured by blue dye perfusion, in the control and preconditioned groups (41 ± 4% in Group 1 and 44 ± 3% in Group 2).

4. Discussion

4.1. Preconditioning and arrhythmias

The results of the present study provide further confirmation that, in addition to its action in delaying the development of irreversible ischaemic myocardial injury [1], ischaemic preconditioning also has potent protective effects on the severity of ischaemia-induced arrhythmias. The nature of anti-arrhythmic protection differs from that provided against myocardial necrosis in that: (i) the end-point reflects reversible rather than irreversible injury, and (ii) the protection results in an absolute reduction in the severity of arrhythmias rather than a delay in their onset [5,11,12]. Thus, there is a strong possibility that the mechanism of anti-arrhythmic protection may be significantly different from that responsible for protecting against my-

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<tr>
<td>Donor HR (bpm)</td>
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<tr>
<td>Perfusion pressure (mmHg)</td>
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<tr>
<td>Support rat HR (bpm)</td>
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<td>Support rat MBP (mmHg)</td>
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HR = heart rate; MBP = mean blood pressure.

Group 1 = 40 min aerobic perfusion + 30 min ischaemia + 10 min reperfusion, Group 2 = 10 min aerobic perfusion + 3 cycles of preconditioning + 30 min ischaemia + 10 min reperfusion, Group 3 = 10 min aerobic perfusion, Group 4 = 10 min aerobic perfusion + 1 cycle of preconditioning, Group 5 = 10 min aerobic perfusion + 2 cycles of preconditioning, Group 6 = 10 min aerobic perfusion + 3 cycles of preconditioning, Group 7 = 40 min aerobic perfusion. One cycle of preconditioning = 5 min ischaemia + 5 min reperfusion.

Groups 1 and 2, n = 12/group, Groups 3–7, n = 6/group.
ocardial necrosis. In considering mechanisms that might underlie the anti-arrhythmic properties of ischaemic preconditioning a number of possibilities have been identified [13,14]. One contending mechanism relates to modifications of myocardial catecholamine status as a consequence of the brief initial cycles of preconditioning ischaemia and reperfusion.

4.2. Catecholamines and arrhythmias during ischaemia and reperfusion

During acute myocardial ischaemia circulating catecholamines are increased but these rarely exceed 5 times their basal concentration [32,33] whereas much higher levels of noradrenaline occur close to sympathetic nerve terminals [34]. The local handling of catecholamines is complex, but Schomig [35] has characterised three phases of release following the onset of acute myocardial ischaemia. During the first 10 min or so of ischaemia, release occurs by exocytosis and this is dependent on activity of efferent cardiac sympathetic nerves [22]. Although Wollenberger et al. [36] have described a massive release of catecholamines within 3 min of coronary occlusion, this does not result in extracellular accumulation until substantially later — this is because reuptake mechanisms remain active [37]. Between typically 10 and 40 min of ischaemia, however, massive accumulation of noradrenaline occurs in the extracellular space. This is thought to be due primarily to the energy-dependent failure of reuptake mechanisms and outward movement of catecholamines via the uptake-1 transport mechanism [22]. Beyond approximately 40 min of ischaemia structural damage of the sympathetic neurones results in a further depletion of catecholamines.

A number of lines of evidence suggest that catecholamines may be important in the genesis of both ischaemia- and reperfusion-induced arrhythmias. Increased levels of circulating catecholamines, and noradrenaline released locally in response to stellate ganglion stimulation, can each cause an increase in vulnerability to ventricular arrhythmias during acute myocardial ischaemia [38,39]. Conversely, surgical denervation, beta blockade and pharmacological depletion of myocardial noradrenaline stores can protect against VF [18,40,41]. In the context of reperfusion, it has also been suggested that release of endogenous catecholamines during the early moments of reflow might contribute to the genesis of various arrhythmias [19] and pharmacological depletion of myocardial catecholamines can markedly reduce the severity of reperfusion-induced arrhythmias [20,21].

4.3. Ischaemia-induced catecholamine depletion

Although ischaemic periods as brief as 3 min can result in substantial catecholamine release, most reports indicate that such short episodes of ischaemia do not result in significant catecholamine depletion [42–44]. This is confirmed by the results of the present study which showed no depletion of catecholamines with 5 min ischaemic periods typically used to precondition hearts. The precise duration of ischaemia required to cause detectable catecholamine depletion appears to vary with species and preparation. Studies in dogs and pigs in vivo have shown no significant depletion with ischaemic durations of less than 1 h [25–27]. In isolated crystalloid-perfused Wistar rat hearts some depletion has been detected after 30 min of ischaemia [23]. This was not confirmed with the preparation used in the present study. It is possible that the difference between these results is a consequence of the use of re-circulated blood as the perfusate, possibly because of increased resistance to ischaemia of blood-perfused isolated hearts [45] or the presence of catecholamines in re-circulated blood. One potential drawback of the isolated heart preparation employed in the present study is the absence of intact sympathetic reflex arcs which are required for the very early phase of ischaemia-induced catecholamine release, but these do not appear to be important in determining whether catecholamine depletion occurs [22].

4.4. Catecholamines and preconditioning

Amongst the many suggested mechanisms of ischaemic preconditioning is the possibility that transient exposure of the heart to released catecholamines might result in the induction of a cascade of protective metabolic changes. This has recently been examined in detail in isolated rat hearts by Bannerjee et al. [17] using post-ischaemic contractile dysfunction as the primary end-point. They showed that in this species protection could be mimicked by exogenous α1-receptor agonists (noradrenaline and phenylephrine) and prevented by α1-receptor antagonists or by catecholamine depletion with reserpine. This suggests that in rats the initiation of protection is a consequence of endogenous catecholamine release acting on α1-receptors, although the precise effector mechanism of this novel method of inducing protection remains to be established.

An alternative possible mechanism originally suggested by Shiki and Hearse [4], that the anti-arrhythmic protection conferred by preconditioning might be a result of ischaemia-induced depletion of catecholamines, was examined in the present study. Although ischaemic periods as brief as 3 min can result in substantial catecholamine release, most reports indicate that such short episodes of ischaemia do not result in significant catecholamine depletion [42–44]. However, we have previously demonstrated that protection against arrhythmias in isolated rat hearts increases with the number of preconditioning cycles [11,12]. Although initial studies in dogs indicated that such ‘frequency’-dependency did not occur for protection against necrosis [46], more recent studies in rabbits and rats have shown enhanced reduction in infarct size with the use of repeated preconditioning cycles [2,47]. We postulated, therefore, that repeated cycles might result in cumu-
lative depletion of catecholamines and thus explain the 'frequency'-dependency of protection by preconditioning. The results of the present study, however, suggest that this is not the case.

Our studies have shown that there was no depletion of endogenous catecholamines, even with repeated cycles of ischaemia and reperfusion. These results are in accordance with those of Miyazaki and Zipes [24] who demonstrated that preconditioning was associated with preservation of autonomic responses during acute myocardial ischaemia in dogs. Other canine studies have also claimed that repeated short ischaemic episodes do not result in impairment of responses to stellate ganglion stimulation [48].

An alternative, and equally plausible, possibility that we considered was that the anti-arrhythmic effect of ischaemic preconditioning in isolated rat hearts might be attributable, in part, to catecholamine depletion arising as a consequence of extra-corporeal perfusion. However, our results indicate that neither prolonged periods of extracorporeal perfusion nor an extended (30 min) period of ischaemia resulted in significant depletion of endogenous catecholamines. In addition, the fact that preconditioning protects against arrhythmias in isolated rat hearts indicates that any effect of preconditioning on cardiac autonomic function is unimportant in its protective action.

Although our results were negative, it is not possible to exclude completely some modification of the handling of endogenous catecholamines in the mechanism of preconditioning. One remaining possibility is that preconditioning may result in altered ischaemia- and/or reperfusion-induced release of catecholamines by exerting some specific effect on release and reuptake mechanisms. This could result in a reduction in catecholamine release, resulting in lower levels of noradrenaline close to the sympathetic terminals, in the absence of significant depletion.

4.5. Right and left ventricular catecholamine contents

It is interesting to note that the tissue contents of both adrenaline and noradrenaline were higher in the right ventricular free wall than in the left in virtually all of the study groups. We are not aware that such a comparison has been made previously, but presumably this result represents either a greater number of sympathetic nerve terminals per gram of tissue or a higher catecholamine content per terminal. From a functional point of view, it is not clear why the right ventricle should have a higher catecholamine content than the left. Nevertheless, the consistent finding of a higher catecholamine content in the right ventricular free wall in both control and study groups indicates that this pattern is due to intrinsic differences between the ventricles rather than an effect of ischaemia/reperfusion or prolonged extracorporeal perfusion.

4.6. Concluding comments

Although depletion of endogenous catecholamines is an attractive theory to explain the anti-arrhythmic actions of ischaemic preconditioning, the results of the present study indicate that no significant reduction in tissue content occurs in hearts subjected to a preconditioning protocol associated with marked protection against ischaemia-induced arrhythmias. Furthermore, we observed that tissue catecholamine levels were maintained throughout a subsequent sustained episode of ischaemia and reperfusion. Thus, depletion of endogenous catecholamines does not appear to be involved in the anti-arrhythmic action of ischaemic preconditioning in isolated rat hearts.

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