Delayed cardioprotective effects of exercise in dogs are aminoguanidine sensitive: possible involvement of nitric oxide

László BABAI*, Zsolt SZIGETI*, James R. PARRATT† and Ágnes VÉGH*
*Department of Pharmacology and Pharmacotherapy, University of Szeged, Albert Szent-Györgyi Faculty of Medicine, Dóm tér 12, P.O. Box 427, H-6701 Szeged, Hungary, and †Department of Physiology and Pharmacology, Strathclyde Institute for Biomedical Sciences, 27 Taylor Street, Glasgow G4 ONR, U.K.

ABSTRACT

Dogs were subjected to exercise on a treadmill, using a protocol in which the speed and slope were increased every 3 min, and which elevated both heart rate (to a mean of 198 ± 14 beats min⁻¹) and mean arterial blood pressure (to 150 ± 4 mmHg). Then, 24 or 48 h later, the dogs were anaesthetized with a mixture of α-chloralose and urethane and subjected to a 25 min occlusion of the left anterior descending coronary artery. The control dogs (instrumented but not exercised) were subjected to the same procedure. In some dogs the nitric oxide synthase inhibitor aminoguanidine (50 mg kg⁻¹; intravenous) was administered 30 min before occlusion. Baroreflex sensitivity (BRS) was determined by the rapid bolus injection of phenylephrine 60 min before, and again 3 min after, the onset of occlusion. Exercise markedly reduced the consequences of coronary artery occlusion 24 h (but not 48 h) later, without modifying myocardial tissue blood flow. In the exercised dogs there were reductions in arrhythmia severity [ventricular fibrillation (VF) during occlusion, 0%; survival from the combined ischaemia/reperfusion insult, 70%] compared with controls (VF during occlusion, 36%; survival, 9%). BRS was preserved during occlusion in the exercised dogs (before occlusion, 1.60 ± 0.54 ms mmHg⁻¹; 3 min after occlusion, 1.37 ± 0.4 ms mmHg⁻¹), but not in controls (before occlusion, 1.28 ± 0.29 ms mmHg⁻¹; 3 min after occlusion, 0.45 ± 0.12 ms mmHg⁻¹; \( P < 0.05 \)), and other ischaemic changes (inhomogeneity of electrical activation and changes in the ST-segment, recorded over the ischaemic region) were also less marked in the exercised dogs. Exercise-induced cardioprotection was abolished by aminoguanidine (VF during occlusion, 25%; survival, 0%). The results show that even a single period of exercise affords delayed protection against ischaemia/reperfusion-induced VF and other ischaemic changes. Since this protection is abolished by aminoguanidine, and since (inducible) NO synthase activity was elevated 3-fold in left ventricular samples 24 h after exercise, we suggest that this protection is mediated by nitric oxide.

INTRODUCTION

There is evidence that physical activity reduces cardiac mortality, especially sudden death [1,2], but the conclusion that ‘the protective effect of exercise requires continued exertion’ [3] implies that the duration of this protection is short-lived. The intensity of the exercise required to induce this protection, and its time course, are matters of ongoing debate and are difficult to assess clinically. There have been few attempts to examine these...
questions using large-animal experiments. Further, the mechanisms of this protection, for example against sudden (arrhythmic) cardiac death, are undefined. Perhaps the most relevant investigations in this regard are those using trained and conscious dogs [4,5]. For example, Billman et al. [4] showed that, in dogs with a healed anterior wall infarct, daily exercise for 6 weeks protected against the effects of coronary artery occlusion when this was induced at the end of a further exercise period. This was attributed to a shift in the autonomic balance favouring increased cardiac vagal activity, and to the preservation of baroreflex sensitivity (BRS) [5]. They did not determine whether regular exercise was also effective in dogs without a prior infarction, nor whether shorter periods of exercise were also effective.

Our own studies, also in dogs, involved right ventricular pacing to heart rates of 220 beats·min\(^{-1}\) [6,7] and showed that this protected hearts against the life-threatening ventricular arrhythmias that result from coronary artery occlusion when this was induced minutes later or on the following day. This protection, which could be extended to several days if the pacing stimulus was repeated [8,9], was lost in the presence of two different inhibitors of nitric oxide synthase (NOS) [10,11] or of dexamethasone [12], which prevents the formation both of NO and prostaglandins. There is thus experimental evidence that the heart can be protected, at least in the short term, by increasing the ventricular rate, and that this is probably mediated by NO.

There are, of course, important differences between the effects of right ventricular pacing during anaesthesia (as in the pacing experiments outlined above) and treadmill exercise in conscious animals. Further, the use of trained dogs with a healed anterior infarct [4] might not appropriately relate to the possible cardioprotective effects of exercise in untrained individuals who have yet to experience a myocardial infarction. There have been no attempts to determine just how much exercise might protect the heart, or how long this protection lasts. It was these questions that stimulated us to design studies, in untrained dogs, to examine the time course between an exercise stimulus and the effects of a subsequent coronary artery occlusion.

The aim of the first of such studies, the results of which are described in the present paper, was to determine whether a single period of exercise, sufficient to elevate heart rate to levels similar to those achieved in our cardiac pacing studies, would protect the heart against the effects of acute coronary artery occlusion at specified times (24 h and 48 h) after the exercise protocol, and, if so, what mechanisms might be involved. As in earlier exercise studies, we also assessed BRS using a modification [4,5] of the technique used in the original study, in human subjects, by Smyth et al. [13]. In clinical studies BRS has been shown by some workers to discriminate between post-infarction patients destined to survive and those destined to die (e.g. [14,15]).

A preliminary account of some of these findings was given at recent meeting of the Physiological Society [16].

**METHODS**

**Animals and surgical preparation for the exercise protocol**

We used adult mongrel dogs of either sex, weighing between 18 and 26 kg (mean 23 ± 1 kg). The origin and upkeep of these dogs were in accordance with Hungarian law (XXVIII, chapter IV, paragraph 31) regarding large experimental animals, which comply with the recommendations of the European Commission as described in the regulations dated December 16, 1991. Under light pentobarbitone anaesthesia [sodium pentobarbitone (Sigma); 30 mg·kg\(^{-1}\) intravenous], heparinized, saline-filled polyethylene catheters were introduced into the left common carotid artery (for the measurement of arterial blood pressure during exercise) and into the left external jugular vein (for the administration of drugs). After instrumentation, the dogs were allowed to adapt to laboratory conditions for 1 week. During this period the dogs were transported to the laboratory and made to stand on the treadmill, but were not exercised. Each day the catheters were flushed with heparinized saline. After surgery, the dogs were treated with 750 mg of cefuroxime (Eli Lilly) daily for 5 days.

**Exercise protocol**

After the 1-week adaptation period following instrumentation, dogs were subjected to the exercise protocol described by Tipton et al. [17], modified as outlined in Figure 1. Dogs were subjected to a total exercise period of 21 min. The slope and the speed of the treadmill were

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**Figure 1** Exercise protocol

Instrumented dogs were subjected to treadmill exercise for a period of 21 min. The slope and the speed of the treadmill were increased every 3 min, starting from 6.4 km·h\(^{-1}\) and a 0% grade during the first 3 min and finishing with a speed of 13 km·h\(^{-1}\) and a 13.5% slope during the last 3 min period.
increased every 3 min, reaching the maximum during the final 3 min period. Arterial blood pressure and heart rate, measured from a chest-lead surface ECG, were recorded during exercise. Data were collected by a computer-assisted system and analysed by the Advanced CODAS Analysing System using the Windaq Waveform Browser playback and analysing software (DATAQ Instruments).

Assessment of ventricular arrhythmias and area at risk

Ventricular arrhythmias during a 25 min coronary artery occlusion and following reperfusion were assessed [7,18] using the suggestions made in the 'Lambeth Conventions', except that we assessed not only the incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF), but also the number of episodes of VT. Following reperfusion, only the incidence of VF was assessed. The dogs were defined as survivors if they were still alive, and predominantly in sinus rhythm, 10 min after reperfusion. These animals were killed subsequently by an excess of anaesthetic.

The risk area following coronary artery occlusion was assessed in each dog at the end of the experiment by injecting Patent Blue V dye into the re-occluded coronary artery. Risk area was expressed as a percentage of the area of the left ventricular wall together with the septum [7].

Experimental protocol

This is illustrated in Figure 2. Six groups of dogs were used. Control dogs (groups 1 and 2) were accustomed to the laboratory conditions by standing them on the treadmill, but were not exercised. After 1 week, these dogs were anaesthetized as described above and subjected to a 25 min occlusion of the LAD; this was followed by the rapid re-opening of the occluded artery. During these experiments BRS was determined either once (group 1; \( n = 10 \)), 60 min before coronary artery occlusion, or twice (group 2; \( n = 11 \)), 60 min before and again 3 min after the commencement of coronary artery occlusion. Dogs subjected to the exercise protocol either 24 h (group 3; \( n = 10 \)) or 48 h (group 4; \( n = 9 \)) previously were also subjected to coronary artery occlusion. BRS was also measured twice in these dogs, 60 min before and 3 min after occlusion of the LAD. A further two groups of dogs either were subjected to exercise 24 h before ischaemia (group 5; \( n = 9 \)) or were allowed simply to stand on the treadmill, but without exercise (group 6; \( n = 9 \)). These dogs were then given aminoguanidine (as the hemisulphate salt; Sigma) intravenously at a dose of 50 mg · kg\(^{-1}\), 30 min before coronary artery occlusion. BRS was also determined twice in these dogs.

Because there is some evidence that phenylephrine can itself precondition the myocardium, and that preconditioning stimuli can summate, a further group of five dogs were exercised and subjected 24 h later to coronary artery occlusion without assessing BRS. These dogs did not receive phenylephrine.
Assessment of myocardial tissue blood flow
In a separate series of eight dogs (four exercised; four controls), myocardial tissue blood flow was measured, immediately before and again 4–5 min after coronary artery occlusion, both in the ischaemic area of the left ventricular wall (supplied by the LAD) and in the adjacent essentially normal area [supplied by the left circumflex coronary artery (LCX)]. The method was the coloured microsphere technique described by Heusch et al. [19]. Prior to coronary artery occlusion, $1 \times 10^7$ coloured microspheres (15 $\mu$m diam.) were injected into the left atrium, and blood samples were collected from the thoracic aorta. A similar number of microspheres, but of a different colour, were injected in the same way 4–5 min after coronary artery occlusion. At the end of the experiments the heart was sectioned and both endocardial and epicardial samples were taken from the area supplied by the occluded LAD and from the non-ischaemic area supplied by the LCX. These blood and tissue samples were frozen for further processing [19] at the Department of Pathophysiology, University of Essen, Germany.

Measurement of NOS activity
In separate groups of five to six dogs, NOS enzyme activity was determined in left ventricular tissue taken, after sacrifice, from dogs subjected to exercise 24 h previously, but not to ischaemia. The method involved the conversion of l-$[^{14}C]$arginine into l-$[^{14}C]$citrulline using modifications [20,21] of the method described by Salter et al. [22]. NOS activity was expressed in units of pmol $\cdot h^{-1} \cdot mg^{-1}$ protein.

Statistical evaluation
All data are expressed as means $\pm$ S.E.M., and the differences between means were compared using ANOVA for repeated measures or Student’s t test, as appropriate. A one-way ANOVA was undertaken to determine whether or not there were significant haemodynamic differences between the groups. Ventricular premature beats were compared using the Mann–Whitney Rank Sum test, and the incidence of arrhythmias was compared using the Fisher Exact test. Differences between groups were considered significant when $P < 0.05$.

RESULTS
Haemodynamic changes during exercise
Exercise resulted in marked increases in arterial blood pressure (e.g. mean arterial blood pressure from 98 $\pm$ 6 to 150 $\pm$ 4 mmHg; systolic pressure from 151 $\pm$ 4 to 222 $\pm$ 10 mmHg; $P < 0.05$) and heart rate (from 119 $\pm$ 8 to 198 $\pm$ 14 beats $\cdot min^{-1}$; $P < 0.05$) within 1 min of the start of exercise, which were maintained throughout the entire running period (Figure 3). Following exercise, these parameters returned to resting values within approx. 20 min.

Haemodynamic measurements before coronary artery occlusion
There were no significant differences in haemodynamic parameters between any of the groups either at baseline (30 min after completion of surgery) or immediately
arterial blood pressure (mmHg); MABP, mean arterial blood pressure (mmHg); HR, heart rate (beats·min⁻¹); SABP, systolic arterial blood pressure (mmHg); DABP, diastolic arterial blood pressure (mmHg). The administration of aminoguanidine resulted in a slight increase in arterial blood pressure; DABP, diastolic arterial blood pressure; LV, left ventricular; AG, aminoguanidine.

There were marked increases in both heart rate (beats·min⁻¹) and systolic arterial blood pressure (mmHg) within 1 min of the start of exercise, and these were maintained during the entire running protocol. Values are means ± S.E.M. Significance of differences: *P < 0.05 compared with baseline; † P < 0.05 compared with controls at a similar time; ‡ P < 0.05 compared with dogs exercised 24 h previously (Ex24 h). SABP, systolic arterial blood pressure; DABP, diastolic arterial blood pressure; LV, left ventricular; AG, aminoguanidine.

Table 1 Haemodynamic changes following coronary artery occlusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-occlusion</th>
<th>2 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SABP (mmHg)</td>
<td>135 ± 5</td>
<td>-7 ± 3*</td>
<td>0 ± 3</td>
</tr>
<tr>
<td>DABP (mmHg)</td>
<td>92 ± 3</td>
<td>-2 ± 2</td>
<td>-2 ± 4</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4.1 ± 0.4</td>
<td>+5.2 ± 0.6*</td>
<td>+9.3 ± 0.8*</td>
</tr>
<tr>
<td>LV (dP/dt)max (mmHg·s⁻¹)</td>
<td>2572 ± 120</td>
<td>-390 ± 96*</td>
<td>-188 ± 196</td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td>151 ± 6</td>
<td>+3 ± 1*</td>
<td>+4 ± 2</td>
</tr>
<tr>
<td>Ex24 h (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SABP (mmHg)</td>
<td>142 ± 8</td>
<td>-5 ± 3</td>
<td>-1 ± 5</td>
</tr>
<tr>
<td>DABP (mmHg)</td>
<td>92 ± 6</td>
<td>-7 ± 3*</td>
<td>+2 ± 4</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4.9 ± 0.8</td>
<td>+5.8 ± 0.7*</td>
<td>+5.8 ± 0.7*</td>
</tr>
<tr>
<td>LV (dP/dt)max (mmHg·s⁻¹)</td>
<td>2284 ± 220</td>
<td>-21 ± 211</td>
<td>-95 ± 190</td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td>138 ± 8</td>
<td>+5 ± 3</td>
<td>0 ± 3</td>
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<tr>
<td>Ex24 h + AG (n = 6)</td>
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<td></td>
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<tr>
<td>SABP (mmHg)</td>
<td>140 ± 9</td>
<td>-8 ± 4</td>
<td>-6 ± 4</td>
</tr>
<tr>
<td>DABP (mmHg)</td>
<td>87 ± 6</td>
<td>-6 ± 3</td>
<td>+5 ± 2</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.8 ± 0.8</td>
<td>+6.1 ± 0.4*</td>
<td>+12.8 ± 2.0†</td>
</tr>
<tr>
<td>LV (dP/dt)max (mmHg·s⁻¹)</td>
<td>2220 ± 310</td>
<td>-92 ± 217</td>
<td>+69 ± 327</td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td>134 ± 3†</td>
<td>+10 ± 3*</td>
<td>+4 ± 1</td>
</tr>
</tbody>
</table>

in a marked increase in systolic arterial blood pressure (mean increase of 47 ± 2 mmHg; *P < 0.05) and a reduction in heart rate (mean decrease of 22 ± 2 beats·min⁻¹; †P < 0.05) in all dogs; these values had returned to baseline before the start of coronary artery occlusion. There were no significant differences between the groups in blood pressure responses to phenylephrine measured either at baseline or during coronary artery occlusion (results not shown).

**Haemodynamic changes following aminoguanidine administration**

In two groups of dogs, either subjected to exercise 24 h previously (group 5) or not (group 6), aminoguanidine was administered intravenously at a dose of 50 mg·kg⁻¹, 30 min before coronary artery occlusion. The most important haemodynamic changes are illustrated in Figure 4. In control dogs (without exercise), aminoguanidine resulted in a slight increase in arterial blood pressure (mean of 8 ± 3 mmHg; not significant) and

before coronary artery occlusion (results not shown, but available from the authors). The administration of phenylephrine (for the determination of BRS) resulted
Changes in BRS following coronary artery occlusion

There was a marked reduction in BRS during coronary artery occlusion in control dogs (phenylephrine (PhE) control and AG control). This reduction in BRS was not seen in dogs subjected to exercise 24 or 48 h previously. Aminoguanidine attenuated the BRS-preserving effect of exercise. Significance of differences: *P < 0.05 compared with baseline; †P < 0.05 compared with controls.

Haemodynamic changes resulting from coronary artery occlusion

Only the most relevant data are shown in Table 1. In all groups, coronary artery occlusion resulted in similar decreases in arterial blood pressure and negative (dP/dt)max and a similar increase in LVEDP, when these were measured 2 min after the onset of occlusion. The decrease in positive (dP/dt)max following coronary artery occlusion was somewhat less pronounced in dogs subjected to exercise 24 h before occlusion compared with any other experimental group. In the exercised dogs, the increase in LVEDP during the later phase of ischaemia was significantly less pronounced than in either the controls or the dogs treated with aminoguanidine.

Changes in BRS before and after coronary artery occlusion in control dogs and in dogs subjected to exercise, and effects of aminoguanidine

BRS measurements at baseline (i.e. 60 min before ischaemia) and 3 min after the start of coronary artery occlusion are shown for each dog in Figure 5. There were no significant differences in the mean baseline BRS values increase in blood pressure of 41 ± 7 mmHg (P < 0.05) and a reduction in heart rate of 21 ± 8 beats·min⁻¹ (P < 0.05) (Figure 4b).

Figure 5
Changes in BRS (mmHg·ms⁻¹) in control dogs, in dogs subjected to exercise (Ex) 24 or 48 h previously and in dogs given aminoguanidine (AG; 50 mg·kg⁻¹, intravenous) with or without exercise are shown. Data are presented for each dog individually, and also as means ± S.E.M., measured at baseline and 3 min after the start of coronary artery occlusion. Changes in BRS before and after coronary artery occlusion in control dogs and in dogs subjected to exercise, and effects of aminoguanidine

Figure 6
Incidence of ventricular arrhythmias following coronary artery occlusion
The total number of ventricular premature beats (VPBs) (a), the number of episodes of VT (b) and the incidence of VT (c) during coronary artery occlusion are given for control (C) and phenylephrine control (PhC) dogs, for dogs exercised 24 h (Ex24 h) or 48 h (Ex48 h) previously, for exercised dogs given aminoguanidine (Ex24 + AG) and for non-exercised dogs given aminoguanidine (AG). Significance of differences: *P < 0.05 compared with PhC controls; †P < 0.05 compared with exercised (Ex24 h) dogs.
The incidence of VF during coronary artery occlusion (filled bars) and following reperfusion (open bars) is shown for control (C) and phenylephrine control (PheC) dogs, for dogs exercised 24 h (Ex24 h) or 48 h (Ex48 h) previously, for exercised dogs given aminoguanidine (Ex24 h $+$ AG) and for non-exercised dogs given aminoguanidine (AG). Also shown in the right-hand panel (hatched bars) is survival following the combined ischaemia/reperfusion insult. Significance of differences: $^* P < 0.05$ compared with Phe controls; $^g P < 0.05$ compared with exercised (Ex24 h) dogs.

between the six experimental groups. However, following occlusion there was an immediate reduction in BRS in the control dogs, whereas in dogs subjected to submaximal exercise 24 h or 48 h previously BRS was largely preserved. However, when aminoguanidine was administered to exercised dogs, BRS during occlusion was again reduced; in dogs not subjected to exercise, aminoguanidine did not modify the decrease in BRS that occurred during occlusion.

**Severity of ventricular arrhythmias during a 25 min coronary artery occlusion**

The severity of ventricular arrhythmias is illustrated in Figures 6 and 7. In control dogs (groups 1 and 2), occlusion of the LAD resulted in a large number of ventricular premature beats, and a high incidence and many episodes of VT (Figure 6). Furthermore, 40% and 36% respectively of these control dogs experienced VF during the occlusion period, and only one dog from each group survived the combined occlusion and reperfusion insult (Figure 7).

A single period of exercise markedly reduced the severity of ventricular arrhythmias that resulted from a 25 min occlusion and reperfusion insult 24 h later. Thus, compared with controls, the number of ventricular premature beats, the number of episodes of VT and the incidence of VT during occlusion were all markedly reduced (Figure 6). No dog subjected to exercise 24 h previously experienced VF during occlusion, and 70% of these dogs also survived rapid reperfusion of the ischaemic myocardium (contrasting with only 9% survival in the controls; Figure 7). Further, none of the five exercised dogs subjected to coronary occlusion 24 h later, but not given phenylephrine, experienced VF during the occlusion. The anti-arrhythmic effect of exercise had faded by 48 h (Figures 6 and 7).

These anti-arrhythmic effects of exercise were not seen in dogs treated with aminoguanidine, and aminoguanidine itself did not modify arrhythmia severity (Figures 6 and 7).

**Changes in other indices of ischaemia severity following coronary artery occlusion**

We used two other parameters to assess the cardioprotective effects of exercise; these were changes in the epicardial ST-segment and in the degree of inhomogeneity of electrical activation within the ischaemic area recorded from a composite electrode. In control dogs (groups 1 and 2) during coronary artery occlusion there were marked, and similar, increases in both the epicardial ST-segment and the degree of inhomogeneity of electrical activation; only changes in the latter are illustrated in Figure 8. A single period of submaximal exercise, either 24 h or 48 h before the onset of occlusion, markedly reduced the severity of ischaemia; this effect of exercise was attenuated in the presence of aminoguanidine, particularly during the first 5 min of occlusion.
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Figure 9 Changes in myocardial blood flow in endocardial (endo) and epicardial (epi) areas of the left ventricular wall supplied by the (occluded) LAD (upper panels) and by the (non-occluded) LCX (lower panels) in control dogs (left-hand panels) and exercised dogs (right-hand panels)

Myocardial blood flow is given in units of ml \( \text{min}^{-1} \cdot \text{g}^{-1} \). Before occlusion (filled bars) there was no difference between the two groups, and after occlusion (open bars) the changes in flow were similar. Following occlusion, the increase in flow in the essentially normal area of the left ventricular wall (supplied by the LCX; post-occlusion compensatory vasodilatation) was somewhat greater in the exercised dogs.

Measurement of myocardial tissue blood flow

The results are illustrated in Figure 9. Before coronary artery occlusion there were no significant differences in tissue blood flow, in the areas supplied by the LAD or the LCX, between the exercised and the control dogs. The endocardial/epicardial ratios were also similar (1.00 ± 0.05 and 1.06 ± 0.04 respectively). Following coronary artery occlusion, there were marked decreases in both endocardial and epicardial flows in the region supplied by the occluded LAD; these decreases were similar in both exercised dogs (95.3 ± 1.3% and 93.9 ± 1.2% respectively) and control dogs (96.1 ± 0.6% and 89.9 ± 1.8% respectively). Flow in the adjacent, essentially normal, region of the left ventricular wall supplied by the LCX was elevated after LAD occlusion (‘compensatory vasodilatation’), and this was slightly more pronounced in those dogs that had been exercised 24 h previously (increases of 26 ± 6% and 24 ± 5% in the endocardial and epicardial areas respectively in exercised dogs, compared with 16 ± 6% and 17 ± 6% respectively in the controls).

Area at risk

There were no significant differences in the area at risk among the groups. These values were 37 ± 2% in control dogs, 33 ± 2% in controls given phenylephrine, 33 ± 2% in dogs subjected to exercise 24 h before occlusion, 31 ± 2% in dogs subjected to exercise 48 h before occlusion, 32 ± 1% in dogs exercised 24 h previously and then given aminoguanidine, and 34 ± 3% in control dogs treated with aminoguanidine.

Measurement of NOS activity

Measurement of NOS activity in homogenized pieces of the left ventricular wall taken from exercised dogs showed a significant increase only in inducible NOS (iNOS) (from 0.32 ± 0.11 to 1.07 ± 0.21 pmol \( \cdot \text{h}^{-1} \cdot \text{mg}^{-1} \); \( P < 0.05 \)); constitutive NOS (cNOS) was not significantly changed from 2.72 ± 0.48 pmol \( \cdot \text{h}^{-1} \cdot \text{mg}^{-1} \). Neither iNOS nor cNOS was significantly up-regulated by exercise in non-cardiac tissue (aorta: iNOS, 0.70 ± 0.11 to 0.97 ± 0.11 pmol \( \cdot \text{h}^{-1} \cdot \text{mg}^{-1} \); cNOS, 0.77 ± 0.18 to 0.59 ± 0.13 pmol \( \cdot \text{h}^{-1} \cdot \text{mg}^{-1} \)).

DISCUSSION

The present studies were designed to determine whether exercise could protect the hearts of dogs, untrained but familiarized to laboratory conditions, against the effects of ischaemia. The results show that even a single period of
exercise reduced the severity of those life-threatening arrhythmias that occur soon after the onset of ischaemia when a coronary artery was occluded 24 h later. The degree of treadmill exercise elevated heart rates to values similar to those obtained in our right ventricular pacing studies, which also protected against the effects of coronary artery occlusion on the following day [7]. As with cardiac pacing, some protection was still apparent 48 h after exercise.

This ‘anti-arrhythmic’ effect of exercise probably results from a reduced severity of ischaemia following coronary artery occlusion. The evidence for this is the less pronounced ST-segment elevation (as recorded from epicardial electrodes) and the reduced degree of inhomogeneity of electrical activation within the ischaemic area in the exercised dogs as compared with controls. However, the degree of ischaemia is only one of several factors that influence arrhythmia severity after acute coronary occlusion and, in any case, the precise relationship between ischaemia severity, as recorded in these ways, and ventricular arrhythmias during occlusion is not defined. Other possibilities relevant to the present studies (and reviewed for example by Wit and Janse [23]) include the size of the ischaemic area, the degree of collateral circulation (which is variable in this species), heart rate and the activity of the autonomic nervous system. There were no significant differences in the present studies between the groups with respect to either the area of the left ventricular wall supplied by the occluded vessel or heart rate. Further, this degree of exercise did not alter myocardial tissue blood flow measured 24 h later, before coronary artery occlusion (Figure 9); the reduction in flow in the area supplied by the occluded coronary artery (LAD) was also similar in both groups. Thus, although it is recognized that the degree of coronary collateral development is an important factor in determining the severity of ischaemia-induced arrhythmias in this species [24], the present experiments show that a single period of exercise does not stimulate such development at a time when protection against the consequences of ischaemia is apparent. Of course, regular exercise might reduce arrhythmia severity by stimulating the coronary collateral circulation, because it certainly does so through an increased availability of NO [25].

Whatever the explanation for the decrease in arrhythmia severity and for the reduced ischaemic changes during coronary artery occlusion 24 h after a single period of exercise, the protection is aminoguanidine sensitive. Aminoguanidine inhibits a number of enzymes other than NOS, including catalase (reviewed in [26]), which is also up-regulated by exercise, and it is conceivable that prevention of the up-regulation of cellular antioxidants might also contribute to the observed protection. In favour of a role for NOS inhibition in the loss of protection following aminoguanidine administra-

This evidence may be added the finding that a more selective (and specific) iNOS inhibitor, S-(2-aminoethyl)methylisothiourea, also abolishes the protection against arrhythmias in dogs subjected to right ventricular pacing [11]. Regular (chronic) exercise, or exercise training, certainly results in the increased formation of NO (reviewed in [27]). For example, in dogs it results in enhanced endothelial NOS gene expression and coronary vascular NO production [28]. There is also increased NO-mediated coronary vascular sensitivity to vagal stimulation and to substances (e.g. acetylcholine and bradykinin) that release NO from endothelial cells [29,30]. In humans, NO is also responsible for at least part of the coronary vasodilatation that occurs during exercise and following cardiac pacing [31]. There is also evidence for increased NOS activity in muscle following exercise [32].

One other finding of interest was that BRS was maintained during coronary artery occlusion in those dogs that had been subjected to exercise 24 h previously. Although the role of this index as an independent predictor of mortality during ischaemia has been questioned [33], and the technique itself has inherent limitations (discussed in detail in [23]), there is impressive evidence, especially in the conscious canine model of the Schwartz group [4,5,34], to indicate that protection induced by exercise training from sudden death during acute myocardial ischaemia is associated with the maintenance of BRS. In the present study BRS was reduced during coronary artery occlusion in every control dog and the mean change was highly significant, a finding that confirms several previous studies [4,5,34,35]. In the exercised dogs BRS was relatively unchanged soon after occlusion; indeed, in some dogs it was increased. As with the other indices used to assess ischaemia severity, this maintenance of BRS was not observed in the presence of aminoguanidine. The reduced BRS during ischaemia was associated with a higher frequency of arrhythmic events, especially VF and VT (Figures 6 and 7), a finding that also accords with some clinical investigations in patients shortly (days) after acute myocardial infarction, when ‘baroceptor reflex testing was a very strong marker of arrhythmic risk’ [36].

If indeed NO is a key mediator of exercise-induced protection against life-threatening post-occlusion ventricular arrhythmias, how does this relate to the maintenance of BRS during ischaemia? We suggest that this is due to modulation of cardiac autonomic transmission. First, NO can attenuate the cardiac responses to sympathetic stimulation both by inhibiting neuronal noradrenaline release [37] at the prejunctional level [38] and
by inhibiting its effects on cardiac β-adrenoceptors [39]. This would be one explanation of why NO, released for example during preconditioning [40] and exercise or following the administration of NO donors [41], is able to reduce the severity of ischaemia-induced ventricular arrhythmias. Secondly, cardiac NO, by a presynaptic cGMP-dependent mechanism, increases the cardiac vagal release of acetylcholine [42]. This may be important, because blockade of the muscarinic effects of acetylcholine with atropine also abolishes the beneficial effects of ischaemic preconditioning [43].

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