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Preconditioning of the ischaemic myocardium; involvement of the L-arginine nitric oxide pathway

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1 Short periods of coronary artery occlusion protect the heart against the effects of a subsequent prolonged period of ischaemia. This phenomenon is known as preconditioning of the ischaemic myocardium.

2 In mongrel, chloralose-urethane anaesthetized open-chest dogs, within a restricted body weight range, two 5 min periods of occlusion of the anterior descending branch of the left coronary artery markedly reduced the severity of the early ischaemic arrhythmias resulting from a prolonged (25 min) occlusion of the same coronary artery starting 20 min later. Thus, the number of ventricular premature beats (VPBs) was reduced from 528 ± 140 in controls to 78 ± 27 in preconditioned dogs, the incidence of ventricular fibrillation (VF) was reduced from 47% to 0% and the incidence of ventricular tachycardia (VT) from 100% to 20%. ST-segment elevation recorded from electrodes within the ischaemic area, and the degree of inhomogeneity of conduction within the ischaemic area were markedly reduced in these preconditioned dogs.

3 The incidence of VF following reperfusion of the ischaemic myocardium at the end of the 25 min occlusion period was reduced in the preconditioned dogs from 100% to 60%; there was thus a 40% survival from the combined ischaemia-reperfusion insult compared with 0% in the controls.

4 N^G-nitro-L-arginine methyl ester (L-NAME) an inhibitor of the L-arginine nitric oxide pathway, given in a dose of 10 mg kg^{-1} intravenously on two occasions, both before the initial preconditioning occlusion and then again before the prolonged occlusion, partially attenuated the protective effects of preconditioning. There were more VPBs (220 ± 75), a higher incidence of VT (60%) and more episodes of VT (11.5 ± 6.0 compared to 0.7 ± 0.3 episodes in the preconditioned dogs not given L-NAME); none of the animals survived reperfusion (incidence of VF 100%). The improvement in the severity of the degree of inhomogeneity which resulted from preconditioning was abolished by L-NAME administration.

5 L-NAME itself elevated blood pressure (from 96 ± 5 mmHg diastolic to 119 ± 7 mmHg), reduced heart rate (from 155 ± 7 to 144 ± 4 beats min^{-1}) but did not change LVEDP, $LVdP/dt_{max}$, coronary blood flow, ST-segment elevation or the degree of inhomogeneity of conduction. When given 10 min before the prolonged coronary artery occlusion in dogs not subjected to preconditioning, L-NAME had no significant effect on the severity of arrhythmias except for more periods of VT (a mean of 11.7 ± 4.7 episodes per dog).

6 It is concluded from these studies that the generation of nitric oxide contributes to the marked antiarrhythmic effects of preconditioning in the canine myocardium, probably through elevation of cyclic GMP.

Keywords: Nitric oxide; N^G-nitro-L-arginine methyl ester (L-NAME); preconditioning; ventricular arrhythmias; myocardial ischaemia; reperfusion; inhomogeneity of conduction; endogenous myocardial protective substances

Introduction

There has been considerable recent interest in the possibility that the heart is capable of rapidly adapting to brief periods of ischaemic stress, whether induced by transient coronary artery occlusion (Murry *et al.*, 1986; Henrichs *et al.*, 1987; Komori *et al.*, 1990; Li *et al.*, 1990; Vegh *et al.*, 1990; 1992a) or by rapid ventricular pacing (Vegh *et al.*, 1991a) in such a way that the severity of subsequent, more prolonged, periods of ischaemia (and the arrhythmic consequence of reperfusion; Shiki & Hearse, 1987) is much reduced. This phenomenon is known as preconditioning of the ischaemic myocardium. The mechanism(s) of this protection are unknown. Suggestions have included the opening up of coronary collateral vessels by the preconditioning occlusions (such that myocardial blood flow is higher during the subsequent prolonged occlusion), a reduction in the rate of utilization of high-energy phosphates, myocardial 'stunning', enhanced potassium uptake, the opening of ATP-dependent K^+ channels and inhibition of cardiac responsiveness to sympathetic neurotransmitters. The evidence for these, which is not completely convincing, has been recently reviewed (Vegh *et al.*, 1992b). An alternative suggested mechanism is that brief periods of ischaemia stimulate the heart to produce 'endogenous myocardial protective substances' (Parratt, 1987) which, in some way, protect the heart against subsequent, more severe ischaemic episodes. There is recent evidence for a major role of adenosine in the reduction in ultrastructural damage resulting from preconditioning (Van Winkle *et al.*, 1991; Liu *et al.*, 1991) and, since the antiarrhythmic effects of preconditioning are largely lost if the cyclo-oxygenase pathway of arachidonic acid metabolism is inhibited (Vegh *et al.*, 1990), the generation of prostanoids, and perhaps especially prostacyclin, also seems to be involved in this protection.

Because of the close interrelationship between adenosine, prostanoids, bradykinin (which is also cardioprotective; Martorana *et al.*, 1990; Vegh *et al.*, 1991b) and nitric oxide (NO), we have examined, using an inhibitor of the L-arginine nitric

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oxide pathway, N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME; Moore *et al.*, 1990) whether this substance might also play a role in ischaemic preconditioning. Preliminary accounts of these results have been given to meetings of the International Society for Heart Research (Vegh *et al.*, 1991c) and the Physiological Society (Vegh *et al.*, 1992c).

Methods

We used mongrel dogs, mainly Hungarian alsations, of either sex (evenly distributed between the groups in a ratio of 2.5 males:1 female) and with a body weight in excess of 17 kg (mean 22.8 ± 1.5 kg). The dogs were anaesthetized with a mixture of chloralose and urethane (60 and 200 mg kg^{-1} respectively given intravenously) and ventilated with room air using a Harvard Respirator at a rate/volume sufficient to maintain arterial blood gases and pH within normal limits (Vegh *et al.*, 1990). The temperature was measured from the oesophagus and maintained, by a heating pad, between 36.8 and 37.5°C.

A thoracotomy was performed at the fifth intercostal space and the anterior descending branch of the left coronary artery (LAD) prepared for occlusion just proximal to the first main diagonal branch. Epicardial ST-segment changes and the degree of inhomogeneity of activation were measured from the left ventricular wall distal to the proposed coronary artery occlusion with unipolar electrodes and a 'composite electrode' previously described (Vegh *et al.*, 1987). This gives a summarised recording of R-waves from 30 epicardial measuring points. In the adequately perfused and oxygenated myocardium all sites are activated simultaneously, resulting in a single large spike. However, following occlusion, widening and fractionation of this summarized R-wave occurs indicating that adjacent fibres are not simultaneously activated because of inhomogeneity of conduction. We expressed inhomogeneity of conduction as the greatest delay in activation (in ms) within the ischaemic area, i.e. between the first and last burst. This reflects in part, local changes in blood flow.

Blood flow in the left circumflex coronary artery was measured in some of the experiments with a 2.0 mm electromagnetic flow probe and a Statham SP2202 flow meter. These parameters, together with a limb lead electrocardiogram, systemic arterial pressure and left ventricular (LV) pressure (Statham P23Dp transducers) and LV$dP/dt$ were recorded on an eight channel Medicor R81 recorder. At the end of the experiment patent blue V dye was infused into the occluded LAD coronary artery to estimate the area at risk. This was expressed as a percentage of the left ventricular free wall.

Ventricular arrhythmias during ischaemia and reperfusion were analysed as outlined by Vegh *et al.* (1992a). No distinction was made between couplets and salvos, which were included as single ventricular ectopic (premature) beats (VPBs), and we defined ventricular tachycardia (VT) as a run of four or more ectopics at a rate faster than the resting sinus rate. We also estimated the number of episodes of ventricular tachycardia during coronary artery occlusion in each animal. To limit the variability in the severity of arrhythmias during coronary artery occlusion, we used dogs with a body weight in excess of 17 kg since smaller dogs have less severe arrhythmias following occlusion. For example, in a separate control group using dogs with a body weight less than 14 kg (mean weight 11.8 ± 0.3 kg) the number of premature beats was 280 ± 152 (compare 497 ± 109 in the present study; $P<0.01$) and the incidence of VF was 20% (compare 47% in the present series; $P<0.05$).

Data are expressed as means (± s.e.mean) and differences between means were compared by Student's t test, corrected for multiple comparisons, or by the Mann-Whitney U test (for arrhythmias). To compare between-group differences in VT and ventricular fibrillation (VF) and in survival from the combined ischaemia-reperfusion episode, the Fisher exact probability test was used. Differences between groups were considered significant at a level of $P<0.05$. Although these experiments were carried out in Szeged the protocol complies with the UK Home Office requirement (Project Licence No. 60/00307).

The protocols were as follows:-

(1) *Group 1 (controls)* These 15 animals served as controls and were allowed to stabilize after surgery for 1 h; the LAD coronary artery was then occluded for 25 min, after which the ischaemic area was reperfused.

(2) *Group 2 (preconditioned)* These 10 animals were preconditioned by two 5 min coronary artery occlusions, with a 20 min reperfusion period between, followed 20 min after the second preconditioning coronary artery occlusion, by a prolonged (25 min) occlusion. The ischaemic area was then reperfused.

(3) *Group 3 (preconditioned + L-NAME)* These 10 animals, were preconditioned in the same manner as the Group 2 dogs but also received L-NAME (10 mg kg^{-1} intravenously) both 10 min before the first 5 min preconditioning occlusion and 10 min before the prolonged (25 min) occlusion. These dogs were also reperfused at the end of the 25 min prolonged occlusion period.

(4) *Group 4 (controls plus L-NAME)* These ten dogs were given L-NAME (10 mg kg^{-1}) 10 min before a prolonged LAD occlusion in order to determine whether inhibition of NO generation itself modified post-occlusion arrhythmias. One of these animals died before occlusion. In this group the occlusion period was maintained for 60 rather than 25 min in order to determine if ventricular ectopic activity was maintained beyond the usual 20–25 i.e. whether inhibition of NO generation 'spread' the early ischaemic arrhythmias over a longer time span (Vegh *et al.*, 1992a).

Results

Haemodynamic changes induced by coronary artery occlusion and by L-NAME

Occlusion of the LAD coronary artery resulted, during the first 5 min, in a small decrease in mean arterial blood pressure (of 5.4 ± 1.8 mmHg; i.e. from 100 ± 5 to 95 ± 4 mmHg; $P<0.01$) and a marked increase in LVEDP (of 8.9 ± 1.3 mmHg i.e. from 5.6 ± 0.7 to 14.5 ± 1.2; $P<0.001$). Heart rate was unchanged by occlusion (132 ± 3 to 131 ± 3 beats min^{-1}). There was also a transient and significant ($P<0.05$) decrease in LV$dP/dt$ (of − 180 ± 71 mmHg s^{-1}). One of the most pronounced and immediate effects of occlusion of the anterior descending coronary artery was a 'compensatory' increase in blood flow in the circumflex coronary artery (of 6.3 ± 1.5 ml min^{-1} from a resting value of 37 ± 3 ml min^{-1}) and a decrease in coronary vascular resistance (diastolic arterial pressure divided by diastolic coronary blood flow) of − 0.7 ± 0.14 units.

The administration of L-NAME resulted in a marked, and long lasting, increase in systemic arterial blood pressure (from 140 ± 8 mmHg systolic and 96 ± 5 mmHg diastolic to 160 ± 8 mmHg and 119 ± 7 mmHg after 5 min; $P<0.05$) and a reduction in heart rate (from 155 ± 7 to 144 ± 4 beats min^{-1}; $P<0.05$). There were no significant changes in LVEDP (8.5 ± 1 mmHg to 10.0 ± 1.1 mmHg) or in LV$dP/dt_{max}$ (2139 ± 115 to 2203 ± 133 mmHg s^{-1}). There was also no significant change in coronary blood flow (35 ± 3 ml min^{-1} to 37 ± 3 ml min^{-1}); calculated vascular resistance was thus increased by L-NAME from 3.3 ± 0.3 to 3.9 ± 0.3 units ($P<0.05$). The administration of L-NAME resulted in no change either in epicardial electrocardiograms or in the degree of inhomogeneity of electrical activation. In those dogs (Group 3) given 2 doses of L-NAME, the haemodynamic effects of the second dose were significantly less than

those of the first (e.g. increase in mean arterial blood pressure 7.0 ± 1.7 mmHg (cf 22 ± 3 mmg Hg; $P<0.01$) and a reduction in heart rate of −2 ± 1 beats min^{-1} (cf 11 ± 3 beats min^{-1}; $P<0.001$). This perhaps implies that NO generation was still markedly inhibited at the commencement of the prolonged occlusion.

The haemodynamic effects of coronary artery occlusion in dogs treated with L-NAME were similar to those in the controls. For example, there was a small decrease in mean arterial blood pressure (of 5.3 ± 2.7 mmHg) and an increase in LVDEP of 4.0 ± 0.8 mmHg. Heart rate was unchanged (137 ± 6 to 144 ± 7 beats min^{-1}).

Ventricular arrhythmias during the preconditioning occlusions and the influence of L-NAME

There were occasional ventricular premature beats (VPBs) during the preconditioning occlusions in the Group 2 dogs. Thus, during the first 5 min preconditioning occlusion there was a mean of 14 ± 13 ventricular premature beats (including 1 episode of VT) and 2 ± 1 VPBs (and 1 period of VT) during reperfusion; during the second occlusion there were 12 ± 7 VPBs (2 episodes of VT) and a mean of 8 VPBs (1 episode of VT) during reperfusion. The administration of L-NAME before the preconditioning occlusions (Group 3 dogs) did not significantly modify the incidence, or severity, of these arrhythmias. For example, there were 24 ± 6 VPBs during the first preconditioning occlusion and 15 ± 5 VPBs during the second preconditioning occlusion (NS), with a 20% incidence of VT (0.3 ± 0.2 episodes per dog) both during the first preconditioning occlusion and during the second preconditioning occlusion (0.2 ± 0.1 episodes per dog). During reperfusion there were 19 ± 8 and 21 ± 10 VPBs respectively ($P<0.01$ in comparison with those dogs without L-NAME) following release of the first and second occlusions. VT occurred during reperfusion in one animal following the first preconditioning occlusion and in two animals following the second.

Ventricular arrhythmias during the prolonged coronary artery occlusion and during reperfusion; protective effects of preconditioning and the influence of L-NAME

Occluding the LAD coronary artery for 25 min resulted in severe ventricular arrhythmias in the control dogs (Figure 1). The incidence of VF was 47%; no attempt was made to defibrillate. All the animals exhibited VT with a mean of 5.1 ± 1.6 episodes per dog. The number of ventricular premature beats, in those dogs that survived, was 528 ± 140. Of these 212 ± 74 occurred during the first 10 min of occlusion (phase 1a arrhythmias) and 316 ± 27 during the 10–25 min period (phase 1b arrhythmias). All the dogs that survived the 25 min occlusion period fibrillated within 1 min, and usually within 15 s, of reperfusion. There were thus no survivors from the combined ischaemia-reperfusion insult in this control group (Figure 1).

The severity of these arrhythmias was markedly reduced if the 25 min occlusion period was preceded by two 5 min occlusions of the same coronary artery i.e. by preconditioning (Figure 1). Thus, there were only 78 ± 27 VPBs over the 25 min occlusion period ($P<0.01$ compared with controls). Of these, 20 ± 16 occurred during phase 1a and 58 ± 20 during phase 1b (both $P<0.001$ versus controls). There was also a lower incidence of VT (20%; $P<0.05$ versus controls; 0.7 ± 0.3 episodes per dog, $P<0.001$ versus controls), no animal fibrillated during occlusion ($P<0.05$ versus controls) and 4 of the 10 dogs that were reperfused at the end of the occlusion period survived. There was thus a survival from this combined ischaemia-reperfusion insult of 40% ($P<0.05$ versus controls).

The antiarrhythmic effects of preconditioning were less marked in those dogs given L-NAME. For example, there were more VPBs than in dogs preconditioned without L-NAME (220 ± 75 versus 78 ± 27; $P<0.05$), a higher incidence of VT (60% versus 20%) more episodes of VT (9.3 ± 4.3 versus 0.7 ± 0.3; $P<0.001$), a higher incidence of VF on reperfusion (100% versus 60%) and a lower survival from the combined ischaemia-reperfusion insult (0% versus 40%; $P<0.05$). The differences in response between those (Group 3) dogs preconditioned in the presence of L-NAME and those subjected to preconditioning, and to the prolonged coronary artery occlusion, in the absence of L-NAME (Group 2 dogs) are illustrated in Figure 2.

The general conclusion then from these L-NAME studies is that the protective effects of preconditioning are reduced by inhibition of the L-arginine NO pathway during the preconditioning period. However, protection by preconditioning is not completely lost if this pathway is inhibited because even in the presence of L-NAME the number of VPBs (220 ± 75) and the incidence of VF (0%) during occlusion were significantly reduced compared to those in control dogs (528 ± 140 VPBs and 47% incidence of VF; $P<0.01$ and $P<0.05$ respectively).

We also examined, in nine (Group 4) dogs, whether the administration of L-NAME itself modified ventricular arrhythmias during coronary artery occlusion. The haemodynamic changes induced by L-NAME were similar to those already outlined above, e.g. an increase in arterial blood pressure (from 148 ± 10 mmHg systolic and 94 ±

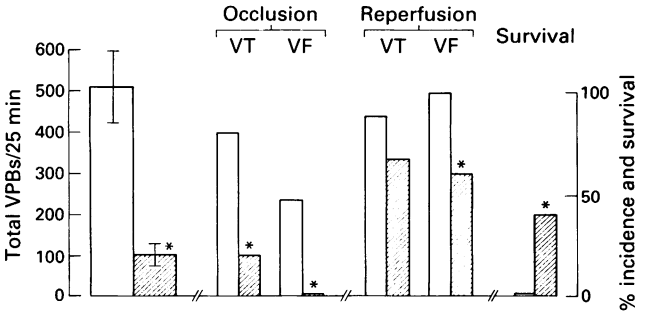

Figure 1 Ventricular arrhythmias (total number of ventricular premature beats (VPBs); incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF)) in control dogs subjected to a 25 min coronary artery occlusion followed by reperfusion (incidence of VT and VF, $n = 15$; open columns) and in dogs preconditioned by two 5 min periods of coronary artery occlusion ($n = 10$, hatched columns). Preconditioning causes a marked reduction in the severity of arrhythmias and a significant increase in survival from the combined ischaemia-reperfusion insult.
*$P<0.05$ versus control.

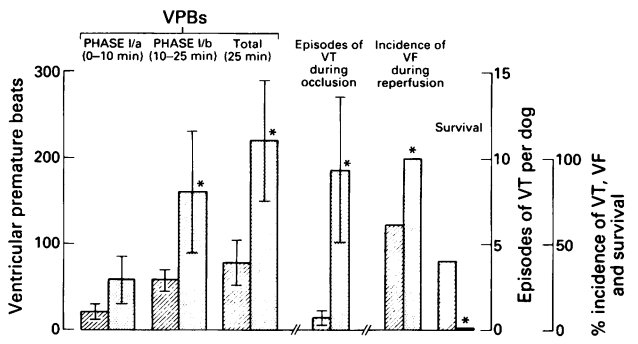

Figure 2 A comparison of the ventricular arrhythmias occurring during a 25 min coronary artery occlusion in dogs subjected to preconditioning ($n = 10$, hatched columns) and in dogs also subjected to preconditioning but in the presence of N^{G}-nitro-L-arginine methyl ester (L-NAME) ($n = 10$, stippled columns). Inhibition of the L-arginine NO pathway attenuates the protective effect of preconditioning (compare Figure 1).
*$P<0.05$ versus preconditioned group.

7 mmHg diastolic to 183 ± 9 and 118 ± 6 mmHg respectively; $P<0.05$), and in LVEDP (from 6 ± 0.6 to 10 ± 1.6 mmHg; $P<0.05$). LV$dP/dt_{max}$ was unchanged (2788 ± 237 and 2786 ± 211 mmHg s^{-1}). There was a small reduction in heart rate (from 160 ± 9 to 144 ± 10 beats min^{-1}; $P<0.05$). Five of these animals (i.e. 55%) fibrillated on occlusion (four between 3 and 10 min of the commencement of occlusion and one between 10 and 15 min); all (except one) had periods of VT. There were more episodes of VT (11.7 ± 4.7) and fewer VPBs (318 ± 104) than in the controls.

Although, in our hands, and with a group of over 40 dogs, there was no significant relationship between area at risk following coronary occlusion (when this was between 30 and 50% of the free left ventricular wall) and the severity of ischaemic arrhythmias (Coker & Parratt, 1985), we did determine risk area in all dogs. There was no significant difference between the two preconditioning groups (Groups 2 and 3); these were 40.4 ± 0.9% and 41.2 ± 3.4%. The risk area in the control Group (1) was somewhat less i.e. 34.2 ± 3.1% ($P<0.05$ compared to the preconditioned groups). In the dogs given an inhibitor of the L-arginine NO pathway (L-NAME) but not preconditioned, it was 39.6 ± 0.8%.

Changes in ST-segment elevation and in the degree of inhomogeneity during coronary artery occlusion: the effect of L-NAME

Preconditioning led to less marked ischaemic epicardial ST-segment changes and there was a significant delay in the development of these changes, especially during the first 5 min of the prolonged occlusion (Figure 3). This slower rate of development was also seen in those dogs that were preconditioned in the presence of L-NAME (Figure 3); only at one time point (15 min into the occlusion period) was the ST-segment elevation significantly different from that in dogs preconditioned without L-NAME.

The modification in the delay of activation by preconditioning was especially pronounced (Figure 4). In those dogs preconditioned in the presence of L-NAME this protective effect of preconditioning was abolished; the inhomogeneity of conduction within the ischaemic area was as pronounced in these dogs in the controls and remained so until reperfusion. This may account for the similar incidence in the severity of arrhythmias on reperfusion; in both these groups all the animals fibrillated on reperfusion (Figures 1 and 2). In contrast, there was a significantly lower incidence of VF in those dogs preconditioned in the absence of L-NAME (Figure 2).

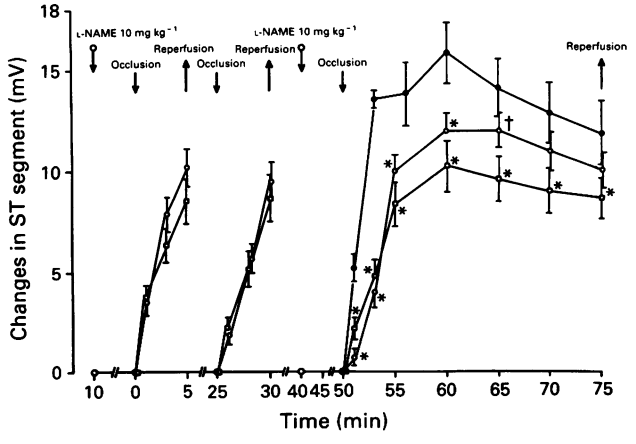

Figure 3 Changes in ST-segment elevation following coronary artery occlusion in control dogs ($n = 15$, ●), in dogs subjected to preconditioning ($n = 10$, □) and in dogs also subjected to preconditioning but in the presence of N^G-nitro-L-arginine methyl ester (L-NAME) given both before the preconditioning occlusion and before the prolonged occlusion ($n = 10$, ○). Values are means from 10–15 experiments; bars = s.e.mean.
*$P<0.05$ versus control; †$P<0.05$ versus preconditioned group.

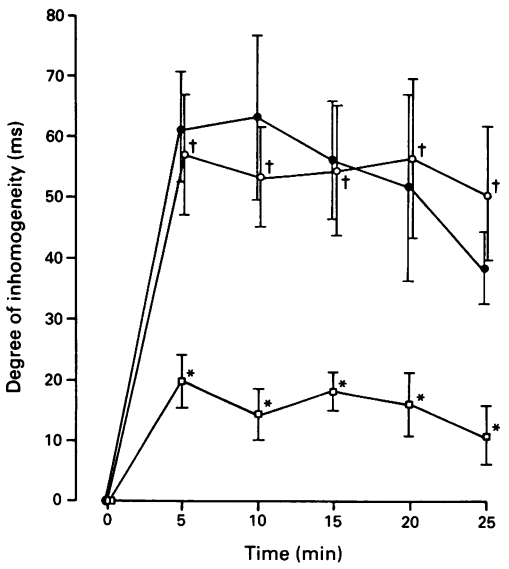

Figure 4 The degree of inhomogeneity of conduction (ms) during a 25 min occlusion period of the left anterior descending coronary artery in control dogs ($n = 15$, ●), in dogs subjected to preconditioning ($n = 10$, □) and in dogs subjected to preconditioning but in the presence of N^G-nitro-L-arginine methyl ester (L-NAME) ($n = 10$, ○). Values are means of up to 15 observations; bars = s.e.mean.
*$P<0.05$ versus control; †$P<0.05$ versus preconditioned group.

Discussion

These studies were concerned with the possibility that NO is involved in the antiischaemic and antiarrhythmic effects of preconditioning. We have previously suggested that brief periods of myocardial ischaemia induced by complete coronary artery occlusion (Vegh *et al.*, 1990) or by rapid ventricular pacing (Vegh *et al.*, 1991a) induce the generation and release of 'endogenous myocardial protective substances' which, in some way, then modify the myocardial response to later, more severe ischaemic episodes. In accord with this hypothesis are the findings that (i) the protective response wanes with time (for example in dogs it is largely lost if the period between the second preconditioning occlusion and the commencement of the prolonged occlusion is increased from 20 to 60 min (Vegh *et al.*, 1992a)) and (ii) that the antiarrhythmic effect of preconditioning is markedly attenuated following inhibition of the cyclo-oxygenase pathway of arachidonic acid metabolism (Vegh *et al.*, 1990).

When an inhibitor of the L-arginine NO pathway was administered before the first of the two preconditioning coronary artery occlusions, some of the marked protective effects of this procedure was lost. This perhaps implies that the generation of NO during the preconditioning period in some way contributes to the protection. Because the profound antiarrhythmic effects of preconditioning are likely to be related to the reduced severity of ischaemia during a subsequent prolonged coronary artery occlusion, as suggested for example by the less pronounced epicardial ST-segment changes (Figure 3), this protective effect of NO could involve dilatation of microvessels or inhibition of platelet adherence to endothelial cells, both of which could contribute to an antiarrhythmic and antiischaemic action of preconditioning. These actions are thought to be largely responsible for the antiischaemic effect of NO 'donors' such as molsidomine and nitroglycerin. It is not possible, at this stage, to eliminate possible extravascular protective effects of NO, for example, on ventricular myocytes which contain a Ca^{2+}-dependent NO synthase enzyme (Schultz *et al.*, 1992). Indeed, the reduction by L-NAME of the beneficial effects of preconditioning on the degree of inhomogeneity of conduction within the

ischaemic area (Figure 4) is probably too great to be accounted for solely by a vascular effect of NO.

How is it possible for NO generation during the preconditioning occlusions to modify the effects of a coronary artery occlusion 20 min later? Although it is possible that brief periods of ischaemic stress might stimulate the generation of NO over such a period (we have no means of evaluating this in the experimental model we have used) it is more likely that the protection involves the resultant stimulation of guanylyl cyclase and the elevation of guanosine 3':5'-cyclic monophosphate (cyclic GMP) within the vascular wall, or in ventricular myocytes. Several years ago Opie (1982) suggested that an elevation of cardiac cyclic GMP could be an antiarrhythmic procedure, a view substantiated by the more recent studies of Billman (1990). In mongrel dogs with a healed myocardial infarction he showed that carbachol and 8-bromo cyclic GMP both substantially reduced the incidence of VF that occurred during a combination of exercise and a brief coronary artery occlusion. It may be no coincidence that those substances which have been suggested as endogenous myocardial protective substances (Parratt, 1987) participating in preconditioning, namely prostanoids (Vegh *et al.*, 1990), adenosine (Van Winkle *et al.*, 1991; Liu *et al.*, 1991) and bradykinin (Vegh *et al.*, 1991b) all stimulate guanylyl cyclase either directly or through NO production. Further evidence for this mechanism comes from studies involving methylene blue, an inhibitor of soluble guanylyl cyclase. The local intracoronary administration of methylene blue reverses completely the antiarrhythmic effect of preconditioning (Vegh *et al.*, 1992d).

The mechanism, whatever it is, deserves investigation because the antiarrhythmic effects of preconditioning, although short lived, are pronounced. Indeed, they are probably as marked in this experimental model as those of pharmacological approaches using standard antiarrhythmic drugs. If, as we suggest, the generation of endogenous myocardial protective substances contributes to the beneficial effects of preconditioning then modification of these substances, by prolonging their action or facilitating their release, could be an alternative approach to the treatment or prevention of ischaemia-induced life-threatening ventricular arrhythmias.

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References

BILLMAN, G.E. (1990). Effect of carbachol and cyclic GMP on susceptibility to ventricular fibrillation. *F.A.S.E.B.*, **4**, 1668–1673.

COKER, S.J. & PARRATT, J.R. (1985). Relationships between the severity of myocardial ischaemia, reperfusion-induced ventricular fibrillation, and the late administration of dazmegrel and nifedipine. *J. Cardiovasc. Pharmacol.*, **7**, 327–334.

HENRICHS, K.J., MATSUOKA, H. & SCHAPER, J. (1987). Influence of repetitive coronary occlusions on myocardial adenine nucleosides, high energy phosphates and ultrastructure. *Basic Res. Cardiol.*, **82**, 557–565.

KOMORI, S., PARRATT, J.R., SZEKERES, L. & VEGH, A. (1990). Preconditioning reduces the severity of ischaemia and reperfusion-induced arrhythmias in both anaesthetised rats and dogs. *J. Physiol.*, **423**, 16P.

LI, G.C., VASQUEZ, J.A., GALLAGHER, K.P. & LUCCHESI, B.R. (1990). Myocardial protection with preconditioning. *Circulation*, **82**, 609–619.

LIU, G.S., THORNTON, J., VAN WINKLE, D.M., STANLEY, A.W.H., OLSSON, R.A. & DOWNEY, J.M. (1991). Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. *Circulation*, **84**, 350–356.

MARTORANA, P.A., KETTENBACH, B., BREIPOHL, G., LINZ, W. & SCHOLKENS, B.A. (1990). Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a bradykinin antagonist. *Eur. J. Pharmacol.*, **182**, 395–396.

MOORE, P.K., AL-SWAYEH, O.A., CHONG, N.W.S., EVANS, R.A. & GIBSON, A. (1990). L-N^G-nitro arginine (L-NOARG), a novel L-arginine-reversible inhibitor of endothelium-dependent vasodilation *in vitro*. *Br. J. Pharmacol.*, **99**, 408–412.

MURRY, C.E., JENNINGS, R.B. & REIMER, K.A. (1986). Preconditioning with ischemia; a delay of lethal cell injury in ischemic myocardium. *Circulation*, **74**, 1124–1126.

OPIE, L.H. (1982). Role of cyclic nucleotides in heart metabolism. *Cardiovasc. Res.*, **16**, 483–507.

PARRATT, J.R. (1987). Reperfusion arrhythmias, platelet-vessel wall interactions and endogenous antiarrhythmic substances. In *Cardiovascular Pharmacology '87: Results, Concepts and Perspectives*. ed. Papp, J.G. pp. 3–16. Akademiai Kiado: Budapest.

SCHULZ, R., NAVA, E. & MONCADA, S. (1992). Induction and potential biological relevance of a Ca^{2+}-independent nitric oxide synthase in the myocardium. *Br. J. Pharmacol.*, **105**, 575–580.

SHIKI, K. & HEARSE, D.J. (1987). Preconditioning of ischemic myocardium; reperfusion-induced arrhythmias. *Am. J. Physiol.*, **253**, H1470–H1476.

VAN WINKLE, D.M., THORNTON, J.D., DOWNEY, D.M. & DOWNEY, J.M. (1991). The natural history of preconditioning, cardioprotection depends on duration of transient ischaemia and time to subsequent ischaemia. *Coronary Artery Disease*, **2**, 613–619.

VEGH, A., KOMORI, S., SZEKERES, L. & PARRATT, J.R. (1992a). Antiarrhythmic effects of preconditioning in anaesthetised dogs and rats. *Cardiovasc. Res.*, **26**, 487–495.

VEGH, A., SZEKERES, L. & PARRATT, J.R. (1990). Protective effects of preconditioning of the ischaemic myocardium involve cyclooxygenase products. *Cardiovasc. Res.*, **24**, 1020–1022.

VEGH, A., SZEKERES, L. & PARRATT, J.R. (1991a). Transient ischaemia induced by rapid cardiac pacing results in myocardial preconditioning. *Cardiovasc. Res.*, **25**, 1051–1053.

VEGH, A., SZEKERES, L. & PARRATT, J.R. (1991b). Local intracoronary infusions of bradykinin profoundly reduce the severity of ischaemia-induced arrhythmias in anaesthetised dogs. *Br. J. Pharmacol.*, **104**, 294–295.

VEGH, A., SZEKERES, L. & PARRATT, J.R. (1991c). Does nitric oxide play a role in ischaemic preconditioning? *J. Mol. Cell. Cardiol.*, **23** (Suppl V); P48.

VEGH, A., SZEKERES, L. & PARRATT, J.R. (1992b). Preconditioning, an early protective response to myocardial ischaemia. In *Myocardial Response to Acute Injury*. ed. Parratt, J.R. pp. 110–127. London: Macmillan.

VEGH, A., SZEKERES, L. & PARRATT, J.R. (1992c). Nitric oxide involvement in preconditioning of the ischaemic myocardium in anaesthetised dogs. *J. Physiol.*, **446**, 541P.

VEGH, A., SZEKERES, L., PAPP, J. Gy. & PARRATT, J.R. (1992d). Preconditioning of the ischaemic myocardium is reversed by methylene blue; further evidence for the role of cGMP. *J. Mol. Cell. Cardiol.*, **24**, (in press).

VEGH, A., SZEKERES, L. & UDVARY, E. (1987). Effects of the blood supply to the normal non-infarcted myocardium on the incidence and severity of early post-occlusion arrhythmias in dogs. *Basic Res. Cardiol.*, **82**, 159–171.

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