Attenuation of the antiarrhythmic effects of ischaemic preconditioning by blockade of bradykinin B₂ receptors

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1 The possibility that bradykinin is involved in the pronounced antiarrhythmic effects of ischaemic preconditioning in anaesthetized mongrel dogs was examined with the use of the selective B₂ antagonist, icatibant (Hoe-140).

2 Preconditioning, achieved by two 5 min occlusions of the left anterior descending coronary artery, followed 20 min later by a 25 min occlusion of the same artery resulted, during this prolonged occlusion, in less severe arrhythmias (VF 0% versus 47% in control non-preconditioned dogs), reductions in the incidence and number of episodes of ventricular tachycardia (VT) and in the number of ventricular premature beats and increased survival following reperfusion (50% versus 0% in the controls).

3 Hoe-140 was given in a dose of 300 µg kg⁻¹ either before the preconditioning procedure or after preconditioning but before the prolonged occlusion. This dose of Hoe-140 had insignificant haemodynamic effects and failed to modify the increase in coronary blood flow that occurred in the circumflex coronary artery when the anterior descending branch was occluded.

4 It was difficult to precondition dogs in the presence of Hoe-140. There were more ventricular arrhythmias during the initial 5 min occlusion (44 ± 12 versus 10 ± 3) and a higher incidence of ventricular fibrillation (50% versus 21%) during the preconditioning procedure. There was also a more pronounced ST-elevation (recorded from epicardial electrodes) during the preconditioning occlusions in those dogs given Hoe-140.

5 In those dogs that survived to the long (25 min) occlusion, Hoe-140 prevented the antiarrhythmic effects of preconditioning (reduction in ventricular premature beats and in VT) although all the dogs survived the occlusion period. However on reperfusion, survival in the preconditioned dogs given Hoe-140 was less than in those dogs preconditioned without the B₂ antagonist.

6 Changes in the degree of inhomogeneity of conduction within the ischaemic area, which were markedly suppressed by preconditioning, were attenuated in those dogs preconditioned in the presence of Hoe-140.

7 These results suggest that bradykinin acts as both a 'trigger' for preconditioning and as one of the mediator protective (antiarrhythmic) substances generated by the myocardium under these conditions. Since the protection afforded both by preconditioning and by local intracoronary infusions of bradykinin is markedly attenuated by an inhibitor of the L-arginine nitric oxide pathway, we suggest that much of the protection afforded by ischaemic preconditioning results from the generation of nitric oxide, and that bradykinin, released early during ischaemia, acts as a stimulant for this generation.

Keywords: Ischaemic preconditioning; ventricular arrhythmias; bradykinin; Hoe-140 (icatibant); nitric oxide

Introduction

Apart from the reduction in myocardial ischaemic damage (Murry *et al.*, 1986), one of the most pronounced consequences of ischaemic preconditioning is a marked reduction in the severity of those ventricular arrhythmias that occur soon (i.e. minutes) after coronary artery occlusion (Vegh *et al.*, 1990; 1992a; Lawson *et al.*, 1993a,b; Piacentini *et al.*, 1993; Parratt & Vegh, 1994) and reperfusion. Particularly impressive in the canine model is the complete abolition of ventricular fibrillation and a marked reduction in the incidence and number of episodes of ventricular tachycardia (Vegh *et al.*, 1992a). The most likely mechanisms of this protection, recently reviewed by Lawson & Downey (1993) and by Parratt (1994a) would appear to be a reduction in the severity of ischaemia and its metabolic consequences, a reduction in the release of substances such as noradrenaline which induce arrhythmias under ischaemic conditions, and the production of some endogenous myocardial protective substance.

We have recently suggested that nitric oxide plays an important role in the antiarrhythmic effects of preconditioning, at least in dogs. The evidence for this is the marked attenuation of this protection by an inhibitor of the L-

arginine nitric oxide pathway (Vegh *et al.*, 1992b) and by methylene blue (Vegh *et al.*, 1992c), which is both an inhibitor of the pathway and of soluble guanylyl cyclase. Because bradykinin is antiarrhythmic in this model (Vegh *et al.*, 1991), an effect which, since it is prevented by an inhibitor of the L-arginine nitric oxide pathway is presumably mediated by nitric oxide (NO) release (Vegh *et al.*, 1993), we investigated whether bradykinin might be the trigger for the generation of nitric oxide under conditions of ischaemic preconditioning. We have therefore examined whether an antagonist of bradykinin B₂ receptors, activation of which is responsible for the release of prostacyclin and the generation of nitric oxide by bradykinin (Hecker *et al.*, 1992), modifies the antiarrhythmic effects of ischaemic preconditioning in anaesthetized open-chest mongrel dogs. A preliminary account of these results was presented at the joint meeting of the Italian and British Pharmacological Societies in Rome (Vegh *et al.*, 1994a).

Methods

These were similar to those already described in detail (Vegh *et al.*, 1992a,b). In brief, we used mongrel dogs, of either sex,

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with a body weight in excess of 17 kg (mean 26.6 ± 2.4 kg). They were anaesthetized with a mixture of chloralose and urethane (60 and 200 mg kg⁻¹, i.v. respectively), and ventilated with room air using a Harvard Respirator at a rate and volume sufficient to maintain arterial blood gases and pH within normal limits (Vegh *et al.*, 1992a). The animals were thoracotomised at the fifth intercostal space and the anterior descending branch of the left coronary artery (LAD) was prepared for occlusion just proximal to the first main diagonal branch. This gives an area at risk, as assessed by infusing patent blue V dye into the occluded artery at the end of the experiment, of between 34 and 42%.

Blood flow was measured on both the LAD (Doppler flow probe; Triton, U.S.A.) and left circumflex (LCX) coronary arteries (2.0 mm electromagnetic flow probe and a Statham SP 2202 flow meter). Epicardial ST-segment changes and the degree of inhomogeneity of electrical activation were measured from the left ventricular wall distal to the occlusion site using a 'composite' electrode described previously (Williams *et al.*, 1974; Vegh *et al.*, 1987; 1992a). 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 the summarized R-waves occurs indicating that adjacent fibres are not simultaneously activated because of inhomogeneity of conduction. We expressed this as the greatest delay in activation (ms) within the ischaemic area. This reflects, in part, local changes in myocardial blood flow.

All these parameters, together with a limb lead electrocardiogram, systemic arterial and left ventricular (LV) systolic (S) and end-diastolic (ED) pressures (Statham P23 Dp transducers) and LVdP/dt were recorded on an eight channel Medisor R81 recorder.

Ventricular arrhythmias during ischaemia and reperfusion were assessed and analysed as outlined previously (Vegh *et al.*, 1992a) i.e. total ventricular premature beats (VPBs), the incidence and number of episodes of ventricular tachycardia (VT) and the incidence of ventricular fibrillation (VF). All the data were analysed statistically as previously described (Vegh *et al.*, 1992a) i.e. data are expressed as means ($\pm$ s.e.mean) and differences between means were compared by a Student's *t* test corrected for multiple comparisons or, for arrhythmias, by the Mann-Whitney U-test. For comparison of the incidences of arrhythmias the chi-squared test for independence in a 2×2 table was used. Differences between groups was considered significant when $P < 0.05$.

Although these experiments were carried out in Szeged the protocol complied with U.K. Home Office regulations (Project Licence No. 60/00307).

There were four groups of animals. Some dogs were preconditioned as previously described (Vegh *et al.*, 1992a)

by occluding the LAD for two 5 min periods, with a 20 min reperfusion period between. Twenty min later, in fourteen of these dogs, the artery was reoccluded at the same site for 25 min and the ventricular arrhythmias assessed during this period; the myocardium was then reperfused by re-opening the occlusion. The second group of dogs ($n = 20$) were control animals which were subjected only to a 25 min coronary artery occlusion i.e. without preconditioning. Two other separate groups of dogs were preconditioned as described above but, in addition, received the bradykinin B₂ antagonist, Hoe-140 (icatibant; Wirth *et al.*, 1991), in a dose (300 μ g kg⁻¹, i.v.) chosen on the basis of unpublished studies from this laboratory in conscious dogs and which blocks the vasodepressor responses to injected bradykinin in doses of up to 10 μ g kg⁻¹ for a period of between 30 and 60 min. Icatibant was given either 10 min prior to the first preconditioning occlusion ($n = 10$) or after preconditioning but 10 min prior to the long (25 min) occlusion ($n = 10$).

Results

Haemodynamic effects of coronary artery occlusion and of icatibant

The haemodynamic effects of coronary artery occlusion are summarised in Table 1. There was a transient decrease in mean arterial pressure, an increase in LVEDP and reductions in both positive and negative LVdP/dt. These changes were not significantly modified by the prior administration of the B₂ antagonist, Hoe-140.

When the LAD is occluded there is invariably a compensatory increase in blood flow in the LCX coronary artery. This too was unchanged by icatibant (Figure 1). The only significant haemodynamic effect of icatibant itself was a slight reduction in mean arterial pressure (Table 1), which was slightly greater in those dogs which had been subjected to the preconditioning procedure (i.e. from 114 ± 12 to 104 ± 10 mmHg, $P < 0.05$).

Ventricular arrhythmias during the initial preconditioning coronary artery occlusions and the effects of the prior administration of icatibant

In normal dogs there are relatively few ventricular premature beats during a 5 min coronary artery occlusion. For example, in a total of 29 dogs subjected to preconditioning, including those used in the present study, there were less than a mean of 10 VPBs over the entire 5 min occlusion period. There was little difference between the number of VPBs during the first and second preconditioning occlusions (Figure 2). However, 6 out of 29 dogs fibrillated during reperfusion following the first occlusion (21%). Those dogs that were given icatibant

Table 1 Haemodynamic effects of Hoe-140 (0.3 mg kg⁻¹, i.v.) and of the subsequent occlusion of the LAD coronary artery (first preconditioning occlusion)

	Pre Hoe-140	Hoe-140	Δ	Pre-occlusion	5 min post-occlusion	Δ
Arterial blood pressure (systolic; mmHg)	152 $\pm$ 10	145 $\pm$ 1	-7 $\pm$ 3	155 $\pm$ 10	146 $\pm$ 11	-9 $\pm$ 2
Arterial blood pressure (diastolic; mmHg)	103 $\pm$ 7	97 $\pm$ 7	-6 $\pm$ 3	102 $\pm$ 7	96 $\pm$ 7	-6 $\pm$ 4
Arterial blood pressure (mean; mmHg)	119 $\pm$ 8	113 $\pm$ 9	-6 $\pm$ 2	119 $\pm$ 8	114 $\pm$ 8	-5 $\pm$ 3
LV systolic pressure (mmHg)	165 $\pm$ 11	159 $\pm$ 12	-6 $\pm$ 4	169 $\pm$ 11	156 $\pm$ 12	-12 $\pm$ 2
LVEDP (mmHg)	7 $\pm$ 0.8	8 $\pm$ 0.8	1 $\pm$ 0.6	8 $\pm$ 0.8	28 $\pm$ 0.9	20 $\pm$ 0.8*
Heart rate (beats min ⁻¹)	166 $\pm$ 6	164 $\pm$ 7	-2 $\pm$ 2	165 $\pm$ 7	170 $\pm$ 8	5 $\pm$ 1
LVdP/dt _{max} (+ve; mmHg min ⁻¹)	2962 $\pm$ 200	2791 $\pm$ 206	-126 $\pm$ 97	3088 $\pm$ 253	2324 $\pm$ 208	-632 $\pm$ 129*
LVdP/dt _{max} (-ve; mmHg min ⁻¹)	2470 $\pm$ 185	2726 $\pm$ 188	256 $\pm$ 108	2724 $\pm$ 188	2168 $\pm$ 181	-557 $\pm$ 65*

* $P < 0.05$; $n = 10-12$.

were much more difficult to precondition. There was an increased number of VPBs during the initial occlusion (44 ± 12 ; Figure 2) and a higher incidence of VF both during the initial occlusion and following reperfusion (50%). In each case VF was preceded by a brief period of VT. In those dogs that survived the initial occlusion in the presence of icatibant, the changes induced by a second occlusion were similar to those in control preconditioned dogs (Figure 2).

Figure 1 Changes in mean coronary blood flow and in coronary resistance in the left circumflex coronary artery which occur when the left anterior descending branch of that same artery is occluded for 5 min. Under control conditions there is a compensatory vasodilatation (increase in flow; decrease in resistance) which, since it is unaffected by Hoe-140, is unlikely to be due to the release of bradykinin.

Changes induced by icatibant on the antiarrhythmic effects of ischaemic preconditioning, on the degree of inhomogeneity of conduction within the ischaemic area and on the epicardial electrocardiogram

As we have shown previously (e.g. Vegh *et al.*, 1992a) ischaemic preconditioning markedly decreased the severity of the early, ischaemia-induced ventricular arrhythmias that results from coronary artery occlusion in this species. This is illustrated in Figure 3. Preconditioning markedly reduced the number of VPBs, and the incidence and duration of VT during the 25 min occlusion period. Ventricular fibrillation was reduced from 47% in control dogs to 0% in those subjected to the preconditioning protocol ($P < 0.05$) and 7/14 of these dogs survived reperfusion at 25 min. Those dogs that survived reperfusion had significantly fewer VPBs during the 25 min occlusion (20 ± 4) than those that did not (109 ± 27 ; $P < 0.05$). This protection was markedly attenuated by icatibant both when given prior to the preconditioning protocol and when given after preconditioning but before the

Figure 2 Ventricular premature beats during the two 5 min preconditioning coronary artery occlusion periods in dogs in the absence (open columns, $n = 29$) and presence (hatched columns, $n = 12$) of the bradykinin B₂ antagonist, Hoe-140. On the right is shown the incidence of ventricular fibrillation which occurred during this preconditioning procedure in the two groups of dogs.

Figure 3 The number of ventricular premature beats (VPBs), the incidence of ventricular tachycardia (VT) and the number of episodes of ventricular tachycardia which occurred during a 25 min occlusion of the left anterior descending coronary artery in control dogs (open columns, $n = 20$) and in preconditioned dogs (cross hatched columns, $n = 14$) in the absence, or presence, of the bradykinin B₂ antagonist Hoe-140. Hoe-140 was given either *before* the preconditioning procedures (hatched columns, $n = 6$) or *after* preconditioning but prior to the prolonged (25 min) occlusion (stippled columns, $n = 8$).

prolonged occlusion (Figure 3). Further, survival from the combined ischaemia/reperfusion insult was reduced, especially when icatibant was given before the start of the preconditioning protocol. This is illustrated in Figure 4.

Under conditions of myocardial ischaemia there is a marked inhomogeneity of conduction within the area supplied by the occluded vessel. This is greatly attenuated during the entire 25 min occlusion period in dogs that have been preconditioned (Figure 5). However, this inhomogeneity was markedly increased in those dogs given icatibant either before the preconditioning protocol or after the preconditioning protocol but before the prolonged occlusion. This too is

Figure 4 Survival from a combined 25 min occlusion period followed by reperfusion in control dogs (C) and in dogs subjected to preconditioning (PC) in the absence or presence of Hoe-140 given either before preconditioning (Hoe-140 + PC) or after preconditioning (PC + Hoe-140). The marked increase in survival from the combined ischaemia-reperfusion insult following preconditioning is attenuated by Hoe-140.

illustrated in Figure 5. Because the local intracoronary infusion of bradykinin also reduces the degree of inhomogeneity of conduction during ischaemia (Vegh *et al.*, 1991), we presume that this is due to the blockade of endogenous bradykinin.

Figure 5 Changes in the inhomogeneity of activation in the ischaemic area during a 25 min coronary artery occlusion in control dogs (O), in dogs administered bradykinin by intracoronary infusion (●), in preconditioned dogs (Δ) and in preconditioned dogs in the presence of the bradykinin B_2 antagonist Hoe-140: (□) Hoe-140 after preconditioning; (■) Hoe-140 before preconditioning. The bradykinin results are taken from Vegh *et al.* (1991).

Figure 6 Changes in ST-segment elevation, recorded from epicardial electrodes, during the two 5 min preconditioning coronary artery occlusions and during the subsequent prolonged (25 min) occlusion of that same artery. ST-segment elevation following preconditioning during the prolonged occlusion is markedly reduced (Δ) compared with control, non-preconditioned dogs (O). This reduction in this particular index of ischaemia is attenuated by the administration of Hoe-140 ($300 \mu\text{g kg}^{-1}$, i.v.) either before the preconditioning procedure (■) or after preconditioning but before the prolonged occlusion (□). Also included in the figure are results obtained following the local intracoronary administration of bradykinin (●), data obtained from Vegh *et al.* (1991).

Coronary artery occlusion leads to ST-segment elevation, as recorded from epicardial electrodes. This is less pronounced during a subsequent occlusion (Figure 6) and during a local intracoronary infusion of bradykinin (Vegh *et al.*, 1991). However, following icatibant administration, ST-segment elevation was significantly greater both during the two brief (5 min) preconditioning occlusions and during the prolonged (25 min) occlusion. This is also illustrated in Figure 6.

Discussion

The results from our previous studies using a similar experimental model (Vegh *et al.*, 1992b,c) suggested that the generation of nitric oxide is involved in the pronounced antiarrhythmic effects of ischaemic preconditioning in the canine. The present experiments were designed to explore the possibility that one stimulus for NO generation under these conditions might be bradykinin. The evidence that bradykinin should be considered as an endogenous myocardial protective substance has been recently reviewed (Parratt, 1993). This evidence includes: (a) its rapid generation under conditions of myocardial ischaemia; (b) that various indices of the severity of ischaemia are reduced by bradykinin and (c) that locally infused bradykinin, in doses too small to influence coronary blood flow, exerts a profound antiarrhythmic effect against the early, life-threatening ventricular arrhythmias that result from coronary artery occlusion. Since this antiarrhythmic effect of locally infused bradykinin is prevented by an inhibitor of the L-arginine nitric oxide pathway (Vegh *et al.*, 1993) it seemed at least possible that bradykinin 'triggers' the release of nitric oxide during ischaemic preconditioning. With the availability of a selective and potent antagonist at the relevant (B₂) bradykinin receptor we were able to investigate this possibility and the present results strongly suggest a primary role for bradykinin in mediating this protection. The evidence is that the reduction in arrhythmia severity and in the degree of inhomogeneity of conduction within the ischaemic area resulting from ischaemic preconditioning are both attenuated following blockade of B₂ bradykinin receptors, whether this achieved before or after the preconditioning procedures.

The effects of the initial preconditioning coronary artery occlusion were more marked if bradykinin B₂ receptors had first been blocked; ST-segment changes were accentuated (Figure 6) and arrhythmia severity was markedly increased (Figure 2). This suggests that bradykinin is generated during very short periods of ischaemia and has a protective role. Although it was not possible to sample coronary sinus blood in these particular experiments we do have data, from patients with coronary artery disease undergoing coronary angioplasty, that bradykinin levels in coronary sinus blood are elevated by up to 50 times during a 1 min balloon inflation (Oldroyd, Cobbe, Koide, Parratt and Zeitlin, unpublished observations), suggesting that this generation is extremely rapid. These levels are of a similar order of magnitude to those required to reduce arrhythmia severity during ischaemia when infused locally into a branch of the LAD coronary artery in anaesthetized dogs (Vegh *et al.*, 1991). We do not know how the rapid generation of bradykinin occurs but the precursor (kininogen) is present in or on the surface of vascular endothelial cells (Busse & Lamontagne, 1991) and we have described an acid-optimum kinin forming enzyme in the coronary vessels which can generate bradykinin from human kininogen (Zeitlin *et al.*, 1989) and which is depleted following ischaemia (Koide *et al.*, 1993).

These studies were designed to examine whether bradykinin acted as the 'trigger' for ischaemic precondition-

ing i.e. that it was released during the preconditioning period with the possibility that other protective mediators, such as nitric oxide and prostacyclin, might then be released as mediators of the protection afforded by preconditioning when the same coronary artery was occluded some time later. The present experiments show that whether icatibant is given before the preconditioning protocol or after it, the protection afforded by preconditioning was attenuated (e.g. Figures 3, 4, 5 and 6). This would suggest that bradykinin is both the 'trigger' and also one of the mediators of preconditioning, together with nitric oxide and prostacyclin. These results with a bradykinin B₂ antagonist are therefore quite different from those obtained with the non-selective adenosine receptor blocking agent 8-sulphophenyltheophylline (8-SPT) (Vegh *et al.*, 1994b). In the studies with 8-SPT, the severity of arrhythmias during the initial preconditioning occlusion, like those following the administration of icatibant, were much more severe than in dogs preconditioned in the absence of the drug. However, the protection afforded by preconditioning itself was not modified by 8-SPT whereas it clearly was with icatibant. This suggests that whereas both adenosine and bradykinin are potential endogenous myocardial protective (antiarrhythmic) agents (Parratt, 1993) it is only bradykinin that takes part in the protection afforded by ischaemic preconditioning. It would seem then that although the myocardium is able to generate a variety of potentially antiarrhythmic substances under conditions of ischaemia (including adenosine, bradykinin, nitric oxide and prostacyclin) it is only bradykinin, and the mediators released by it (nitric oxide, prostacyclin) that takes part in the protection afforded by ischaemic preconditioning, at least with regard to protection afforded against life-threatening ischaemia-induced ventricular arrhythmias.

As yet, we have no direct evidence that any of these potential mediators are released in increased amounts following a preconditioning protocol. Neither have we any clear explanation as to why the generation of nitric oxide, presumably from endothelial cells, results in such a marked attenuation of arrhythmia severity. One possibility might be through elevation of guanosine 3':5'-cyclic monophosphate (cyclic GMP) levels. This might influence arrhythmogenesis in several ways. First, nitric oxide depresses myocardial contractility (Brady *et al.*, 1992) which would improve the myocardial oxygen supply demand ratio and hence lead to 'energy sparing', which is a significant feature of preconditioning (Vander Heide *et al.*, 1993). Second, elevation of cyclic GMP inhibits calcium influx through L-type channels in the sarcolemma (Tohse & Sperelakis, 1991). Third, stimulation of a cyclic-GMP dependent cyclic-AMP phosphodiesterase should lead to a decrease in myocardial cyclic AMP levels, which would also result following direct stimulation of bradykinin B₂ receptors (Parratt, 1994a).

In conclusion, we would like to emphasize that these studies relate only to a possible mechanism of ischaemic preconditioning in reducing the severity of ventricular arrhythmias in one particular animal model. We cannot draw the conclusion that bradykinin is a 'universal trigger' for ischaemic preconditioning, although there is experimental evidence that inhibition of bradykinin breakdown with inhibitors of angiotensin converting enzyme has cardioprotective effects on the myocardium including suppression of ventricular arrhythmias (Parratt, 1994b). This may well have clinical implications.

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