The local intracoronary administration of methylene blue prevents the pronounced antiarrhythmic effect of ischaemic preconditioning

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Short periods of coronary artery occlusion (2×5 min) markedly reduce the severity of arrhythmias and the changes in ST-segment elevation and in the degree of inhomogeneity of conduction during a subsequent 25 min occlusion of the left anterior descending coronary artery in anaesthetized dogs. These changes were completely reversed if methylene blue (5 mg min^{-1}) was infused into a side branch of the coronary artery throughout both the preconditioning and prolonged occlusions. These results suggest that the pronounced antiarrhythmic effects of preconditioning result from activation of guanylyl cyclase and result in increased levels of guanosine 3':5'-cyclic monophosphate.

Keywords: Methylene blue; guanylyl cyclase; cyclic GMP; ischaemic preconditioning; ventricular arrhythmias; reperfusion; nitric oxide

Introduction When a prolonged occlusion of a coronary artery is preceded by either one or more shorter periods of occlusion of that same artery, both the degree of ischaemic damage (reviewed by Walker & Yellon, 1992) and the severity of life-threatening ventricular arrhythmias (Vegh *et al.*, 1990; 1992a) resulting from the prolonged occlusion are dramatically reduced. This phenomenon is known as preconditioning of the ischaemic myocardium. The mechanisms of this protection are unclear but one possibility is that the preconditioning stimulus 'primes' the heart to release 'endogenous myocardial protective substances' which may include adenosine (Liu *et al.*, 1991) and prostanoids (Vegh *et al.*, 1990). Recently, we have shown that the antiarrhythmic effects of preconditioning are attenuated by inhibition of the L-arginine/nitric oxide (NO) pathway (Vegh *et al.*, 1992b), suggesting that the generation of NO contributes to this protection. Since NO acts by stimulating soluble guanylyl cyclase and elevating guanosine 3':5'-cyclic monophosphate (cyclic GMP) levels, another way of examining the possible role of NO in this protection is to inhibit the activation of this enzyme. We now describe experiments in which we have infused methylene blue (Martin *et al.*, 1985) directly into a small branch of the left coronary artery in anaesthetized dogs to examine whether this modified the protective (antiarrhythmic) effect of preconditioning.

Methods These have been described in detail elsewhere (Vegh *et al.*, 1990; 1991; 1992a,b). In brief, we used mongrel dogs with a weight in excess of 17 kg. They were anaesthetized with a mixture of chloralose and urethane (60 and 200 mg kg⁻¹ respectively, given i.v.) and ventilated with room air. Following a left thoracotomy the anterior descending branch of the left coronary artery (LAD) was prepared for occlusion just proximal to the first main diagonal branch. We used a composite electrode to assess inhomogeneity of conduction and unipolar electrodes to measure epicardial ST-segment changes. A small branch of the LAD coronary artery immediately proximal to the proposed occlusion site (Vegh *et al.*, 1991) was catheterized for the local administration of methylene blue.

Forty four dogs were used in this study. Of these, 21 animals served as controls. In these, after stabilization following surgery, the LAD coronary artery was occluded for 25 min after which the ischaemic area was reperfused. Fourteen dogs were preconditioned as previously described (Vegh *et al.*, 1990; 1992a) by two 5 min coronary artery occlusions, with a 20 min reperfusion period between, followed 20 min after the second preconditioning occlusion by a prolonged (25 min) occlusion. The ischaemic area was then reperfused. A further group of 9 dogs were subjected to preconditioning as described above but methylene blue (MB; 10 mg ml^{-1}), infused at a rate of 0.5 ml min^{-1} , was infused throughout both preconditioning occlusions and throughout the prolonged occlusion. The protocol was to infuse MB for 5 or 10 min before each preconditioning occlusion, throughout the 5 min occlusion period and for 5 min after the start of reperfusion. MB was infused again for 5 min prior to the prolonged occlusion and throughout that occlusion period. The total doses administered were therefore 100 mg (for the first preconditioning occlusion), 75 mg (for the second preconditioning occlusion) and 150 mg for the prolonged occlusion.

Epicardial electrograms, the output from the composite electrode, systemic arterial and left ventricular (LV) pressures (Statham P23Dp transducers), and blood flow in the circumflex coronary artery (Statham SP 222 electromagnetic flow meter) were recorded on a Medisor R81 recorder. The assessment of ventricular arrhythmias during ischaemia and reperfusion, and the statistical tests used, were as outlined by Vegh *et al.* (1992a).

Results Local intracoronary administration of MB in the doses described above had no effect on arterial blood pressure (133 ± 9 mmHg systolic and 93 ± 5 mmHg diastolic before the infusions and 141 ± 10 mmHg and 93 ± 5 mmHg respectively at the end of the infusion period). Heart rate was also unchanged (164 ± 15 to 161 ± 11 beats min⁻¹). There were also no changes in LVEDP (7.2 ± 2.5 to 8.3 ± 0.3 mmHg), in diastolic coronary blood flow (46 ± 7 to $43 \pm 7 \text{ ml min}^{-1}$) or in the output from the composite electrode (38 ± 6 to 38 ± 6 ms) after MB administration.

The effects on ventricular arrhythmias during the occlusion period and following reperfusion are shown in Figure 1. Preconditioning markedly reduced the total number of ventricular premature beats (VPBs), the episodes of ventricular tachycardia (VT), the number of animals exhibiting VT and

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Figure 1 The total number of ventricular premature beats (VPB's), the number of episodes of ventricular tachycardia (VT), the incidences of VT and ventricular fibrillation (VF) and the survival from the combined-reperfusion insult in dogs subjected to a 25 min occlusion of the left anterior descending coronary artery. Preconditioning (solid columns, $n = 14$) markedly reduces the severity of these arrhythmias but this marked protection is not seen if preconditioning is carried out in the presence of methylene blue (hatched columns, $n = 7$). The incidence of VF is given both as total incidence throughout the 25 min occlusion period and during the first 5 min (stippled columns). Open columns = controls, $n = 21$.

ventricular fibrillation (VF) and the incidence of VF on reperfusion; 50% of these preconditioned animals survived the combined ischaemia-reperfusion insult. This marked protection afforded by preconditioning was completely reversed by MB (Figure 1) and there were no survivors from the combined ischaemia-reperfusion procedure.

The marked attenuation of ischaemia-induced changes in epicardial ST-segment elevation and in the degree of inhomogeneity of conduction within the ischaemic area (Vegh *et al.* 1992a; 1992b) were also reversed by MB. For example, ST-segment elevation at 1, 3, 5 and 10 min after the start of the prolonged occlusion in the control dogs was 5.2 ± 0.6 , 13.4 ± 0.9 , 13.6 ± 0.8 and 16.0 ± 1.0 mV respectively. In the preconditioned group the respective values at these times were 1.4 ± 0.4 , 6.4 ± 0.7 , 8.2 ± 0.8 and 10.0 ± 1.0 mV whereas in the preconditioned dogs given MB they were 2.1 ± 0.4 , 8.3 ± 1.9 , 12.5 ± 1.4 and 13.0 ± 1.5 mV. With inhomogeneity of conduction the changes at 1, 3 and 5 min following the start of occlusion were 20 ± 3 , 80 ± 9 and 90 ± 10 ms in the controls, 0 , 15 ± 4 and 36 ± 6 ms in the preconditioned group and 10 ± 7 , 72 ± 10 and 77 ± 10 ms in the preconditioned dogs administered MB. The differences for each of these values between the preconditioned group and the controls were significant at each time at a level of at least $P < 0.05$ but there were no significant differences between the control dogs and those preconditioned dogs given MB.

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Discussion The fact that when given throughout both the preconditioning periods and the prolonged occlusion, MB completely prevented the pronounced antiarrhythmic effects of preconditioning, suggests that it is the stimulation of soluble guanylyl cyclase that is responsible for this aspect of the cardioprotection afforded by preconditioning. It also indirectly supports the concept that nitric oxide is involved in this protection (Vegh *et al.*, 1992b). The abolition of the antiarrhythmic effect of preconditioning by MB cannot be explained by haemodynamic changes or by alterations in coronary blood flow. MB, in the doses used, was devoid of effects on blood pressure, heart rate, coronary blood flow and myocardial contractility, at least as assessed for measurements for LVEDP and $LVdP/dt$, neither of which was changed by the compound.

We suggest that this study throws light on the mechanisms of the pronounced antiarrhythmic effect of preconditioning. One possibility under consideration is that the ischaemia associated with the brief preconditioning occlusions results in the release of some activator of guanylyl cyclase such as nitric oxide. Certainly some NO 'donors' such as mol-sidomine and nitroglycerin are active against early ischaemia-induced ventricular arrhythmias. The 'priming' procedure of preconditioning might result in increased levels of cyclic GMP such that these were elevated before the onset of the prolonged occlusion. There is already evidence, albeit in a different model, for an antiarrhythmic effect of lipid soluble analogues of cyclic GMP which are also more resistant to breakdown by phosphodiesterase. Billman (1990) found, in conscious dogs with a left ventricular free wall infarct subjected to a combination of exercise and ischaemia (left circumflex artery occlusion), that the incidence of VF was drastically reduced following the intravenous administration of either 8-bromo cyclic GMP or dibutyryl cyclic GMP (from 17/17 in the control dogs to 1/14). The mechanisms of such a myocardial effect of elevated cyclic GMP are unclear but possibilities include a reduction in calcium influx and/or of intracellular calcium release or activation of sarcolemmal potassium channels (reviewed recently by Henderson *et al.*, 1992). It would be important to examine directly how preconditioning alters cyclic GMP, and in which cells, and to determine whether selective inhibition of the phosphodiesterase enzyme responsible for its breakdown is, like preconditioning, a powerful antiarrhythmic procedure.

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