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Nicorandil suppressed ventricular arrhythmias in a canine model of myocardial ischaemia

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Abstract

These experiments were designed to explore the possibility that a K^+ channel opener which also donates nitric oxide to the myocardium (nicorandil) may modify ischaemia-induced ventricular arrhythmias in a large animal model. In mongrel dogs anaesthetised with chloralose-urethane and thoracotomised, a side branch of the left anterior descending artery was catheterised for the local intracoronary infusion of nicorandil (2.5 $\mu g\ kg^{-1}\ min^{-1}$ for 20 min prior to coronary artery occlusion and then continuing throughout the 25 min occlusion period). In this dose, nicorandil had no haemodynamic effects, increased coronary blood flow by up to 16% and significantly reduced the severity of ischaemia-induced arrhythmias (e.g. from nearly 500 ventricular premature beats in the controls to 160 ± 60 in the nicorandil group). There was a significant reduction in the number of episodes of ventricular tachycardia during the ischaemic period and a reduced incidence of ventricular fibrillation following reperfusion resulting in a 42% survival from the combined ischaemia-reperfusion insult (cf. 0% in the control; $P < 0.05$). The marked changes that occurred in ST-segment elevation (mapped with epicardial electrodes) and in the inhomogeneity of electrical activation within the ischaemic area in control dogs was markedly reduced in those dogs administered nicorandil. We conclude that the local intracoronary administration of nicorandil reduces the severity of both ischaemia and the life-threatening arrhythmias that result from an abrupt reduction in coronary blood flow in this canine model. Possible mechanisms include an increase in coronary blood flow, a reduction in the severity of myocardial ischaemia and an ability of the compound to 'donate' nitric oxide to the ischaemic area.

Keywords: Nicorandil; Myocardial ischemia; Ventricular arrhythmia; K_{ATP} channel; Nitric oxide (NO); Reperfusion

1. Introduction

Although there is much evidence for the cardioprotective effects of K^+ channel openers (recently reviewed by Gross (1993) and by Grover (1994)), as demonstrated by a reduction in myocardial ischaemic damage (necrosis) and by enhanced recovery of cardiac contractility following a period of ischaemia and reperfusion, there is still some debate regarding the possible arrhythmogenic effect of opening K^+ channels under conditions of pre-existing ischaemia or disease. Thus, 'on the basis of findings from animal experiments it is not possible to say whether K^+ channel activators, at therapeutically relevant dosages, could have an arrhythmogenic effect in patients with coronary disease' (Neuss, 1993). The question as to whether K^+ channel openers are likely to be pro-arrhythmic in the diseased human heart has been debated in a recent issue of Cardiovascular Research by Black and Lucchesi (1994) and by D'Alonzo and Grover (1994); it has been stated (Wilde, 1994) that 'there is simply insufficient experimental material to define potential risk'.

Much of the debate has centred upon the suitability, or otherwise, of experimental models for arrhythmias. Although studies with acetylstrophanthidin (Lathrop et al., 1990) and isolated hearts subjected to ischaemia and reperfusion (Kempsford and Hawgood, 1989) are of some interest, they do not contribute significantly to the debate. Similarly, studies in which large doses of activators have been used, resulting in marked decreases in systemic arterial pressure (as in the study by Chi et al., 1990) and the resultant activation of cardiac sympathetic nerves leading to noradrenaline release, may confound the direct activity of these compounds. A further complicating factor is that there are marked differences between different K^+ channel

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openers and these differences may lead to inappropriate general conclusions being drawn.

Nicorandil has the ability to open K^+ channels but has the additional property of nitrate-like activity leading to the stimulation of guanylyl cyclase and an increase in intracellular cGMP concentrations (Holzmann, 1983). This is a significant aspect of the pharmacology of the compound and is likely to modify an effect on ischaemia-induced arrhythmias especially as there is some evidence that, for example in preconditioning, the generation of cGMP in cardiac myocytes (resulting from the release of nitric oxide from endothelial cells) contributes to the antiarrhythmic effect of this phenomenon (Parratt and Vegh, 1996). In the present studies we have examined the effect of nicorandil given locally into a side branch of the left coronary artery in anaesthetised dogs in order to obviate the effects *marked* systemic hypotension might have on the generation of these arrhythmias through reflex activation of cardiac sympathetic nerves.

2. Materials and methods

2.1. Animals and experimental design

These were similar to those already described in detail elsewhere (Vegh et al., 1992a). In brief, we used 32 mongrel dogs, of either sex, with a body weight in excess of 17 kg (mean 28.3 ± 1.2 kg). They were anaesthetised with a mixture of chloralose and urethane (60 and 200 mg kg^{-1} i.v., respectively), 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), thoracotomised at the fifth intercostal space and the anterior descending branch of the left coronary artery prepared for occlusion just proximal to the first main diagonal branch. Area at risk was assessed at the end of each experiment by infusing patent blue V dye into the occluded artery and was 36.8 ± 1.7% of the left ventricular wall in the nicorandil-treated dogs and 38.5 ± 2.0% in the controls (ns).

Blood flow on the left circumflex artery was measured using an electromagnetic flow probe (2.2 mm coupled to a Statham SP 2202 flow meter) and on the LAD with a Doppler flow probe (Triton, California). 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 (Vegh et al., 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 virtually simultaneously, resulting in a single spike. Following occlusion however, widening and fractionation of the summarised 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.

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

A side branch of the left anterior descending coronary artery was catheterised as previously described (Vegh et al., 1992b) to allow the infusion of nicorandil in a dose of 2.5 $\mu g\ kg^{-1}\ min^{-1}$ (in a volume of 0.5 ml min^{-1}) or normal saline for 20 min prior to coronary artery occlusion and continuing throughout the 25 min occlusion period. There were 12 dogs in the nicorandil group and 20 in the control group.

Ventricular arrhythmias during a 25 min period of coronary artery occlusion (ischaemia) and reperfusion were assessed and analysed as outlined previously (Vegh et al., 1992a). We measured the total number of ventricular premature beats (VPBs) over the entire 25 min occlusion period, the incidence and number of episodes of ventricular tachycardia (VT) and the incidence of ventricular fibrillation (VF). Only the incidence of VF was measured during a 5 min reperfusion period. The data were analysed statistically as previously described (Vegh et al., 1992a) i.e. data are expressed as means ± S.E.M. and differences

Table 1
Haemodynamic effects of nicorandil (2.5 $\mu g\ kg^{-1}\ min^{-1}$ i.c.) and of subsequent coronary artery occlusion

	Control group ($n = 20$)			Nicorandil group ($n = 12$)	
	Pre-occlusion	5 min post-occlusion	Pre-infusion	Pre-occlusion [b]	5 min post-occlusion
Arterial blood pressure					
Systolic (mm Hg)	126 ± 5	109 ± 5 [a]	117 ± 9	114 ± 9	97 ± 9 [a]
Diastolic (mm Hg)	85 ± 4	72 ± 4 [a]	74 ± 5	70 ± 5	62 ± 5 [a]
Mean (mm Hg)	99 ± 5	94 ± 4 [a]	88 ± 6	85 ± 6	74 ± 6 [a]
LV systolic pressure (mm Hg)	138 ± 7	117 ± 7 [a]	135 ± 9	132 ± 10	114 ± 9 [a]
LVEDP (mm Hg)	5.6 ± 0.4	22.2 ± 1.9 [a]	5.0 ± 0.3	5.0 ± 0.4	17 ± 1.3 [a]
$LVdP/dt_{max}$ (+ve; mm Hg s^{-1})	3027 ± 355	1809 ± 319 [a]	2694 ± 251	2803 ± 297	2358 ± 254 [a]
(−ve; mm Hg s^{-1})	2269 ± 271	1698 ± 266 [a]	1915 ± 271	2035 ± 330	1816 ± 332
Heart rate (beats min^{-1})	146 ± 5	147 ± 5	160 ± 10	167 ± 10 [a]	167 ± 13

[a] $P < 0.05$; [b] 20 min after commencement of the infusion.

between means compared by a Student's *t*-test corrected for multiple comparisons using a two way ANOVA or, for arrhythmias, by the Mann-Whitney U-test. For comparison of incidences of arrhythmias, VT, VF and survival from the combined ischaemia-reperfusion insult the Chi-squared test for independence in a 2×2 table was used. Differences between groups were considered significant when $P < 0.05$.

Although the experiments were carried out in Szeged the protocol complies with UK Home Office Regulations, Project Licence No. 60/00307.

3. Results

3.1. The haemodynamic effects of intracoronary nicorandil

These are summarised in Table 1. Nicorandil in this dose had no effect on arterial blood pressure (88 ± 6 mm Hg prior to infusion and 85 ± 6 mm Hg (ns) immediately before coronary artery occlusion), or on left ventricular systolic and diastolic pressures and LVdP/dt. There was a slight increase in heart rate (160 ± 10 to 167 ± 10; $P < 0.05$) but no significant change in the inhomogeneity of electrical activation (74 ± 7 to 78 ± 7 ms), or on the epicardial ST-segment. The most significant haemodynamic effect of nicorandil was an increase in coronary blood flow and this is illustrated in Fig. 1. This increase in flow occurred in both the LAD coronary artery and also in the circumflex coronary artery and, since diastolic perfusion pressure was unchanged, this represents a reduction in coronary vascular resistance.

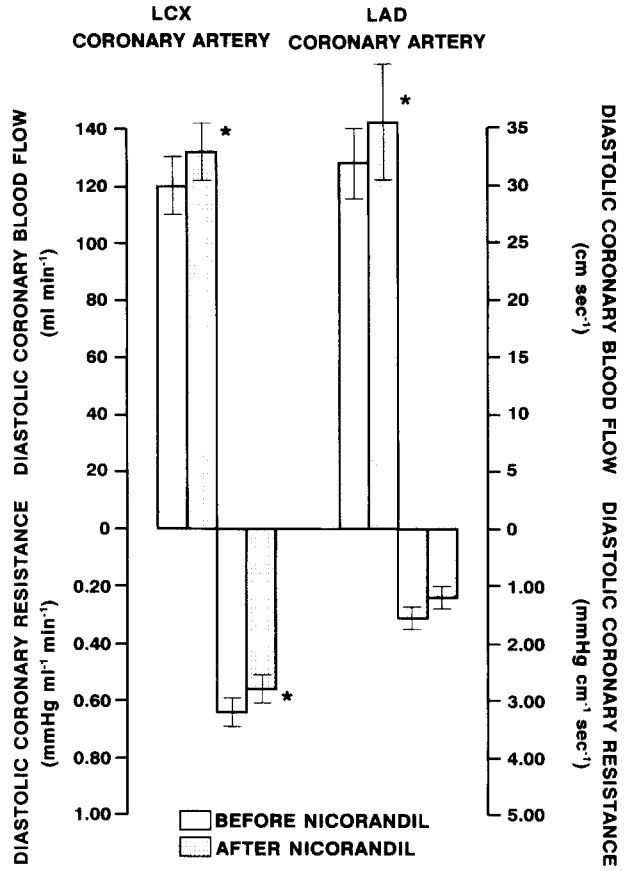

Fig. 1. Changes in coronary blood flow and coronary vascular resistance resulting from the local intracoronary administration of nicorandil. There is an increase in blood flow (and a decrease in calculated diastolic vascular resistance) in both the circumflex and anterior descending coronary arteries 20 min after commencement of the nicorandil infusion.

3.2. Haemodynamic effects of coronary artery occlusion in control dogs and in dogs administered nicorandil

The haemodynamic effects of coronary artery occlusion in this model have been documented elsewhere (e.g. Vegh et al., 1992a,b). There was a reduction in mean arterial blood pressure (from 99 ± 5 to 84 ± 4 mm Hg; $P < 0.05$), an increase in LVEDP (from 5.6 ± 0.4 to 22.2 ± 1.9 mm Hg; $P < 0.01$) and reductions in $LVdP/dt_{max}$ (positive; 3027 ± 355 to 1809 ± 319 mm Hg s^{-1}; $P < 0.05$ and

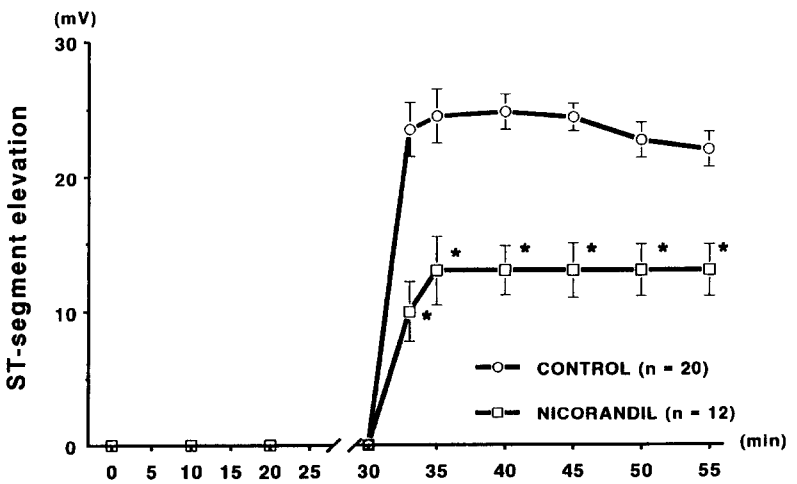

Fig. 2. Changes in ST-segment elevation, obtained from epicardial electrodes, during the infusion of nicorandil (□) and in control dogs (○) following a 25 min coronary artery occlusion. The ST-segment changes are significantly less in those dogs infused with nicorandil.

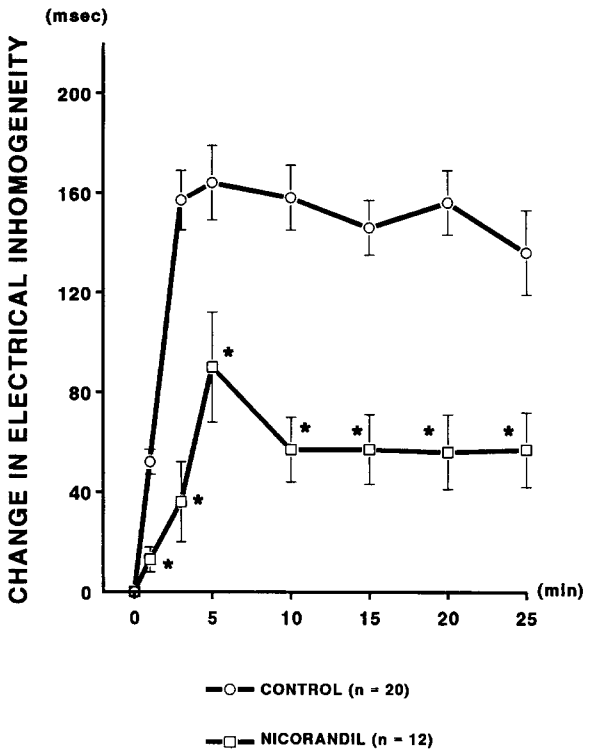

Fig. 3. Changes in electrical inhomogeneity within the ischaemic area in control dogs (○) and in dogs administered nicorandil into a side branch of the anterior descending coronary artery. There is a marked reduction in inhomogeneity of activation within the ischaemic area in those dogs given nicorandil.

negative; 2269 ± 271 to 1689 ± 266 $P < 0.05$). There was no change in heart rate when the coronary artery was occluded (146 ± 5 to 147 ± 5 beats min^{-1}). These effects of coronary artery occlusion were somewhat similar in the dogs infused with nicorandil (Table 1) although the increase in LVEDP (from 5.0 ± 0.3 to 17.0 ± 1.3 mm Hg was significantly ($P < 0.05$) less than in the controls, probably indicating a nitrate-like effect on capacitance vessels.

3.3. Effects of coronary artery occlusion, and nicorandil, on epicardial ST-segment elevation and the degree of inhomogeneity of electrical activation within the ischaemic area

These changes are shown in Figs. 2 and 3. In the control dogs there was a marked and significant increase in ST-segment elevation, to an extent similar to that previously described (e.g. Vegh et al., 1992a). This was significantly less in those animals in which nicorandil was infused (Fig. 2). Similarly, coronary artery occlusion leads to a marked increase in the degree of inhomogeneity within the area supplied by the occluded vessel and this was markedly reduced in those animals infused with nicorandil (Fig. 3).

3.4. Effect of nicorandil on ischaemia and reperfusion-induced ventricular arrhythmias

Ventricular ectopic activity is marked in this model and the arrhythmias in the control group were of a similar

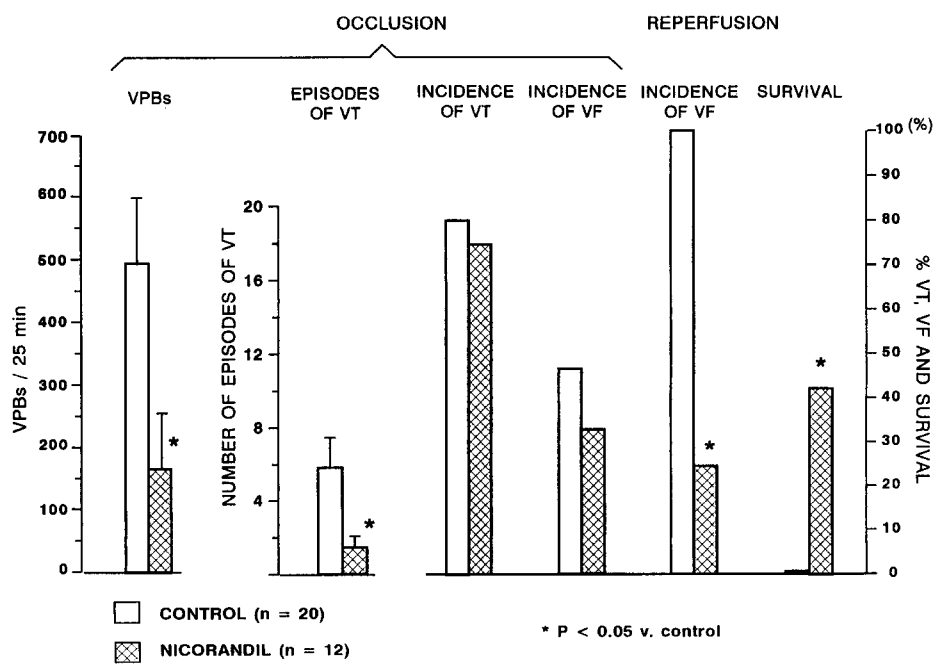

Fig. 4. Reduction in arrhythmia severity by the local, intracoronary administration of nicorandil in anaesthetised dogs subjected to a 25 min occlusion of the left anterior descending coronary artery followed by reperfusion. VPBs = number of ventricular beats; VT = ventricular tachycardia; VF = ventricular fibrillation. * $P < 0.05$.

severity to those previously described using this technique (Vegh et al., 1992a,b). There was a mean of 497 ± 109 VPBs in the control dogs during the 25 min coronary artery occlusion, with an incidence of VT of 80%, a mean of 9 ± 1.7 episodes of VT per dog and an incidence of ventricular fibrillation during occlusion of 47% (Fig. 4). The severity of these arrhythmias was markedly reduced in those dogs infused with nicorandil, with only a mean of 163 ± 87 VPBs ($P < 0.05$), only 1.6 ± 0.6 VT episodes per dog ($P < 0.05$ compared to the control group) and an incidence of VF of 33% (ns). There was a marked suppression of reperfusion-induced arrhythmias. All of the control dogs that survived the occlusion fibrillated when the myocardium was reperfused; in contrast, nicorandil markedly reduced the incidence of ventricular fibrillation on reperfusion to 25% and, therefore, the number of survivors from the combined ischaemia-reperfusion insult was significantly increased from 0% in the controls to 42% in the nicorandil-treated dogs $P < 0.05$).

4. Discussion

These results show, for the first time, that the local administration of nicorandil reduces the severity of those ventricular arrhythmias that occur when a coronary artery is occluded and, especially, when the ischaemic myocardium is reperfused. In this particular study we have avoided the confounding effect of systemic hypotension on ischaemia-induced arrhythmias by local intracoronary administration. The only significant haemodynamic effect was an increase in coronary blood flow, which occurred in both the circumflex and anterior descending branches of the left coronary artery, with a resultant decrease in coronary vascular resistance (Fig. 1). Despite the absence of marked haemodynamic changes nicorandil markedly reduced the severity of the arrhythmias that occurred both when the artery was occluded (ischaemia) and reopened (reperfusion). This probably results from the less severe ischaemia in those dogs administered nicorandil, as shown by less marked changes in ST-segment elevation recorded from epicardial electrodes and in the degree of inhomogeneity of electrical activation within the ischaemic area.

There are a number of possible mechanisms of this protection. These include:

1. the opening of K_{ATP}-dependent K^+ channels.
2. a reduction in ischaemia as a consequence of improved coronary perfusion, or
3. the ability of nicorandil to 'donate' nitric oxide to the ischaemic myocardium.

4.1. Cardioprotection by ATP channel openers

Although there is little doubt (see Introduction) that the opening of K_{ATP} channels is cardioprotective, as demonstrated by reduced myocardial necrosis following coronary artery occlusion and enhanced recovery of contractile function following a period of ischaemia and reperfusion (reviewed by Gross, 1993 and by Grover, 1994), the effect of such agents on early ischaemia-induced arrhythmias is more controversial. Although some studies with drugs that block these channels have shown a reduction in arrhythmia severity (Vegh et al., 1993; Billman, 1994), the effects of agonists on such arrhythmias are much less clear. The proarrhythmic effect of pinacidil (Chi et al., 1990) has been suggested to be peculiar to this drug, rather than a class effect, and to be due to systemic hypotension and catecholamine release (D'Alonzo and Grover, 1994). Similar mechanisms could explain the results of Coussaye et al. (1993) with levcromakalim in anaesthetised dogs. There have also been some studies which demonstrate an antiarrhythmic effect of K^+ channel openers (e.g. Fish et al., 1990). Our own studies (Kaszala et al., 1995) in anaesthetised dogs showed that cromakalim failed to modify significantly early ischaemia-induced ventricular arrhythmias in a dose which slightly lowered mean arterial blood pressure (from 87 ± 5 to 76 ± 5 mm Hg).

4.2. Cardioprotection by an increase in coronary blood flow

A reasonable explanation for the beneficial effects of nicorandil would be the increase in coronary perfusion, although this was not marked; a mean increase of 16% in blood velocity in the anterior descending branch and a flow increase of 10% in the circumflex branch. Whether such a moderate flow increase is sufficient to account for the reduction in arrhythmia severity is unclear and, indeed, where this flow increase occurred (predominant effect on coronary arterioles or pre-existing coronary collaterals) cannot be evaluated using the techniques employed. However, previous experience with levcromakalim (Kaszala et al., 1995) would argue against coronary vasodilatation per se as a major factor responsible for the reduction in arrhythmia severity. In this study, levcromakalim, also administered by the intracoronary route, increased blood flow to the same extent as did nicorandil but failed to modify either changes in epicardial ST-segment mapping, the degree of inhomogeneity of activation within the ischaemic area or ventricular arrhythmias. It could be argued, with good reason, that such studies are not truly comparative; levcromakalim could have a predominant effect on coronary arterioles yet nicorandil might, as with other nitric oxide donors, have a preferential effect on larger conductive channels in those dogs with a pre-existing collateral circulation.

4.3. Cardioprotection by the ability of nicorandil to 'donate' nitric oxide to the ischaemic myocardium

This is certainly one possible explanation for the beneficial effects we have shown, although we have no direct

evidence that this occurs under our experimental conditions, nor have we measured cGMP levels to substantiate this. A possible role for nitric oxide is based on evidence that its generation benefits the ischaemic myocardium (Maulik et al., 1995; Pabla and Curtis, 1995) and on our own experience with ischaemic preconditioning. Thus marked antiarrhythmic effects of preconditioning, in a similar model to that used in the present study (Vegh et al., 1992a), is probably due to the generation of nitric oxide from endothelial cells and the subsequent elevation of cGMP within the ischaemic myocardium. The evidence for this hypothesis has been recently reviewed (Parratt, 1994; Parratt and Vegh, 1996). Increasing cGMP under these conditions would reduce calcium influx, stimulate a cGMP dependent cAMP phosphodiesterase and reduce myocardial oxygen by depressing myocardial contractility (Parratt and Vegh, 1996). There is direct evidence that stable analogues of cGMP reduce arrhythmia severity in a conscious dog model of ischaemia and increased sympathetic activity (Billman, 1994).

An additional mechanism might be the less pronounced increase in left ventricular filling pressure that occurred in those dogs given nicorandil, with a resultant reduction in myocardial oxygen consumption. Whatever the mechanisms we demonstrate that the local administration of nicorandil reduces the severity of both myocardial ischaemia, and also the resultant life-threatening ventricular arrhythmias that are the consequences of an abrupt reduction in coronary blood flow.

Acknowledgements

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