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# Failure of nitric oxide donors to alter arrhythmias induced by acute myocardial ischaemia or reperfusion in anaesthetized rats

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**1** The aim of the present studies was to examine the effects of nitric oxide donors on arrhythmias induced by coronary artery occlusion and reperfusion, and on cardiac cyclic nucleotides. Experiments were performed in pentobarbitone-anaesthetized rats prepared for occlusion of the left coronary artery.

**2** Sodium nitroprusside (0.1, 0.3 and 1 $\mu g\,kg^{-1}\,min^{-1}$) had no significant effects on the incidence of ventricular tachycardia, total ventricular fibrillation or the mortality resulting from 25 min of acute myocardial ischaemia when compared with values in controls. In addition, there was no alteration in the number of ventricular premature beats that occurred in survivors.

**3** 3-Morpholinosydnonimine-N-ethylcarbamide (SIN-1, 10, 20 and 40 $\mu g\,kg^{-1}\,min^{-1}$) caused marked hypotension but did not alter the incidence or severity of ischaemia-induced arrhythmias. In rats subject to abrupt reperfusion after 5 min of myocardial ischaemia, lower doses of SIN-1 (1, 3 and 10 $\mu g\,kg^{-1}\,min^{-1}$) still caused significant reductions in systolic and diastolic blood pressure but were devoid of antiarrhythmic activity.

**4** In separate experiments in sham-operated rats, sodium nitroprusside (1 $\mu g\,kg^{-1}\,min^{-1}$), isosorbide dinitrate (30 and 60 $\mu g\,kg^{-1}\,min^{-1}$) and SIN-1 (20 and 40 $\mu g\,kg^{-1}\,min^{-1}$) had no significant effects on cardiac cyclic GMP content.

**5** These results indicate that nitric oxide donors do not alter arrhythmias induced by acute coronary artery occlusion or reperfusion in anaesthetized rats. Although increases in total *cardiac* cyclic GMP could not be detected, the results suggest that, at least in the rat, cyclic GMP does not influence these arrhythmias.

**Keywords:** Nitric oxide; SIN-1; sodium nitroprusside; arrhythmias; myocardial ischaemia; coronary artery occlusion; reperfusion; cyclic AMP; cyclic GMP

## Introduction

Nitric oxide has two effects which may be of particular importance in the setting of acute myocardial ischaemia. Firstly, it is a potent vasodilator, an activity that has been recognised since the discovery of endothelium-derived relaxing factor (Furchgott & Zawadzki, 1980) and its subsequent identification as nitric oxide (Palmer *et al.*, 1987). Secondly, nitric oxide prevents platelet adhesion and aggregation (Furlong *et al.*, 1987; Radomski *et al.*, 1987). Both antiplatelet actions and vasodilatation may reduce the severity of myocardial ischaemia and could therefore reduce the number or severity of arrhythmias associated with myocardial ischaemia. Prior to the recognition of the importance of endothelium-derived relaxing factor, there were reports that glyceryl trinitrate had antiarrhythmic activity in anaesthetized dogs subject to acute myocardial ischaemia (Borer *et al.*, 1974; Kent *et al.*, 1974; Stockman *et al.*, 1975). More recently, evidence has emerged which suggests that at least part of the antiarrhythmic benefit derived from ischaemic preconditioning is dependent on the release of endogenous nitric oxide (Vegh *et al.*, 1992). However, the studies with glyceryl trinitrate examined only single doses under particular experimental conditions. Little information is available about the effects of other nitric oxide donors or on dose-dependency in relation to effects on ischaemia-induced or reperfusion-induced arrhythmias.

Another reason for our interest in the possible effects of nitric oxide donors on arrhythmias associated with myocardial ischaemia is because these drugs increase guanosine 3':5'-cyclic monophosphate (cyclic GMP) (Kukovetz *et al.*, 1979). Previously, one conclusion that was drawn from studies with certain phosphodiesterase inhibitors was that increasing cardiac cyclic GMP may be antiarrhythmic (Holbrook & Coker, 1989). Although we have performed further studies with phosphodiesterase inhibitors, no firm conclusions about the possible influence of cyclic GMP on arrhythmias could be drawn from these experiments because we could not detect selective increases in cardiac cyclic GMP without concomitant increases in adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Barnes, 1993). Thus, instead of using drugs to prevent the metabolism of cyclic GMP we decided to investigate the effects of alternative means of increasing cyclic GMP. One other pharmacological way to elevate cyclic GMP is to use nitrovasodilator drugs which release nitric oxide, which in turn stimulates soluble guanylyl cyclase to increase cyclic GMP production (Katsuki *et al.*, 1977). Thus, the aim of the present work was to examine the effects of nitric oxide donors on ischaemia-induced and reperfusion-induced arrhythmias and to measure the effects of nitric oxide donors on cardiac cyclic nucleotides. Studies on arrhythmias and cardiac cyclic nucleotides were performed with sodium nitroprusside and 3-morpholinosydnonimine-N-ethylcarbamide (SIN-1), a metabolite of molsidomine (Noack & Feelisch, 1989) and in addition the effects of isosorbide dinitrate on cyclic nucleotides were also determined. Some of these results have been published in abstract form after presentation to the British Pharmacological Society (Coker & Barnes, 1993).

## Methods

### *Animal preparation*

Experiments were performed on male Wistar rats (250 to 355 g) supplied by the departmental animal unit. These

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animals had been housed in small groups in a room maintained at 20°C on a 12 h light/dark cycle with food (Labsure CRM diet) and water available *ad libitum*. Rats were anaesthetized with sodium pentobarbitone, 60 mg $kg^{-1}$, i.p., and prepared for coronary artery occlusion by the methods of Clark *et al.* (1980) using equipment described in detail previously (Coker & Ellis, 1987).

The trachea, a carotid artery and a femoral vein were cannulated, to allow artificial respiration, measurement of arterial blood pressure and drug administration respectively. A left thoracotomy was performed as follows. An incision was made in the skin approximately 3 mm to the left of the midline, from the level of the xiphisternum up to the level of the clavicle. The underlying muscle layer (fibres running diagonally from the sternum up towards the foreleg) was cut along the same line as the skin incision, with any bleeding being controlled by gentle pressure with a gauze swab. The connective tissue between the next major muscle layer (fibres running longitudinally) and the ribs was cleared and the muscle layer eased back to reveal the ribs. An initial incision in the thoracic wall was made in the sixth intercostal space and extended along between the sixth and seventh ribs. Ribs six, five and four were then sectioned approximately 2 mm from the sternum, taking care to avoid the sternal arteries. These ribs were held back while the pericardium was incised and cleared from the heart. The heart was then exteriorised briefly while a fine silk ligature attached to a reverse cutting needle (Mersilk, W812; Ethicon) was placed around the left coronary artery close to its origin. Whilst the heart was exteriorised the left atrial appendage was allowed to fall back away from the ventricular surface. The needle was inserted into the ventricular wall at a point which normally lies just under the middle of the tip of the left atrial appendage. The needle was then manoeuvred so that it exited on the other side of the left coronary artery (which runs alongside the visible vein) level with the edge of the left atrial appendage alongside the pulmonary cone.

As soon as the initial incision in the thoracic wall was made, the rats were ventilated with room air at a rate of 54 strokes $min^{-1}$, a stroke volume of 1 to 1.5 ml 100 $g^{-1}$ (adjusted to maintain an arterial $Po_2$ above 70 mmHg) and a positive end-expiratory pressure of 0.5 to 1 $cmH_2O$. Care was taken to ensure that the lungs were reinflated after the heart had been exteriorised briefly for placement of the ligature around the coronary artery. Rectal temperature was monitored with a thermometer and the anaesthetized rats were maintained at a temperature of 37 to 38°C by means of a heated table. A Lead I ECG was monitored along with arterial blood pressure, and drug or vehicle was infused at a rate of 0.05 ml $min^{-1}$ (Harvard Apparatus Syringe Infusion Pump 22, Edenbridge, Kent) via the venous cannula.

### *Ischaemia-induced arrhythmias*

After preparation and a 10 min stabilization period, drug or vehicle administration commenced and 10 min later the coronary artery was occluded to induce myocardial ischaemia. The arrhythmias that occurred during the first 25 min of myocardial ischaemia were identified in accordance with the definitions described in the Lambeth Conventions (Walker *et al.*, 1988). In survivors the total number of ventricular premature beats, including singles, bigeminy, salvos and ventricular tachycardia (defined as four or more consecutive ventricular premature beats) was counted. The incidence and duration of ventricular tachycardia and ventricular fibrillation were also recorded along with the mortality (due to sustained ventricular fibrillation, defined as continuous ventricular fibrillation persisting for at least 3 min).

Rats were excluded from the final analysis if any of the following occurred: arrhythmias prior to coronary artery occlusion; cardiac failure (defined as a profound reduction in arterial pressure, approaching zero within the first 5 min following coronary occlusion, usually accompanied by A–V block, which is probably due to the ligature being placed too deeply such that the septal branch of the left coronary artery is also occluded); no evidence of ischaemia after tying the ligature (changes in ST-segment or R wave amplitude, arrhythmias); mean blood pressure <60 mmHg prior to drug or vehicle administration. Any rats which were excluded were replaced immediately.

### *Reperfusion-induced arrhythmias*

Reperfusion-induced arrhythmias were examined in separate groups of rats. Animals were prepared for coronary artery occlusion as described above. Instead of tying the ligature around the coronary artery permanently, both ends of the ligature were passed through a small plastic button and coronary artery occlusion was induced by applying tension to the ligature and clamping it with a small pair of rubber-sheathed Dieffenbach forceps. After 5 min of myocardial ischaemia the tension on the ligature was released to allow reperfusion. The resulting reperfusion-induced arrhythmias were monitored for 10 min. The exclusion criteria were the same as those defined above for the experiments on ischaemia-induced arrhythmias. In addition, rats were also excluded if severe arrhythmias (ventricular tachycardia or ventricular fibrillation) were occurring at the time (+ 10 s) when reperfusion should have occurred, or if there was no evidence that release of the ligature had resulted in reperfusion (i.e. no prevention or reversal of ischaemia-induced ST-segment changes).

### *Protocols for arrhythmia experiments*

Two studies on ischaemia-induced arrhythmias and one on reperfusion-induced arrhythmias were performed. In each arrhythmia study there were 4 groups; three doses of drug and a contemporary vehicle control group. The first study on ischaemia-induced arrhythmias examined the effects of sodium nitroprusside (0.1, 0.3 and 1 $\mu g\,kg^{-1}\,min^{-1}$) and the second study looked at the effects of SIN-1 (10, 20 and 40 $\mu g\,kg^{-1}\,min^{-1}$). The effects of SIN-1 on reperfusion-induced arrhythmias were also investigated but in this study the doses of SIN-1 were 1, 3 and 10 $\mu g\,kg^{-1}\,min^{-1}$. Within each study, rats were allocated to individual drug or control groups in a random manner, with 12 rats in each group. Intravenous drug or vehicle infusion commenced 10 min before coronary artery occlusion and was maintained for the duration of the experiment.

### *Cyclic nucleotide experiments*

Separate groups of sham-operated rats ($n = 5$ per group) were used to determine the effects of sodium nitroprusside (1 $\mu g\,kg^{-1}\,min^{-1}$), isosorbide dinitrate (30 and 60 $\mu g\,kg^{-1}\,min^{-1}$) and SIN-1 (20 and 40 $\mu g\,kg\,min^{-1}$) on cardiac cyclic nucleotide contents. These rats were prepared in exactly the same manner as those used for the arrhythmia experiments but the ligature placed around the coronary artery was not tied. At 15 min after starting drug or vehicle administration (equivalent to 5 min post-occlusion in the arrhythmia experiments, the time at which ectopic activity most often starts to occur) the heart was exteriorized, by pulling on the ligature around the coronary artery, and the heart was freeze-clamped in Wollenberger tongs (Biomedix, Pinner, Middlesex), which had been pre-cooled in liquid nitrogen. The frozen tissue was then stored at −80°C until assayed for cyclic nucleotides.

Frozen hearts were broken up under liquid nitrogen with a stainless steel PM3 pestle and mortar (Biomedix, Pinner, Middlesex) and pieces of ventricular tissue were weighed quickly. Two samples of approximately 100 mg each were taken for cyclic GMP determination and four samples, approximately 25 mg each, were taken for cyclic AMP determination. Each frozen sample was placed in a plastic vial containing 1 ml of ice-cold 6% w/v trichloroacetic acid

(TCA) and homogenized for 90 s (9 × 10 s bursts) in an Ultra Turrax homogenizer fitted with an 8N shaft. The homogenizer shaft was then rinsed with a further 0.5 ml TCA. The samples were centrifuged at 8000 *g* and 4°C for 15 min in an MSE 18 centrifuge after which the supernatant of each sample was removed and placed in a 10 ml glass test tube and capped. The TCA was extracted from each sample 4 times with 5 volumes of water-saturated diethyl ether. After the final extraction the samples were dried at 40 to 50°C for 5 min to evaporate any residual traces of ether and then stored at −20°C until assay.

Prior to assaying for cyclic nucleotides, the samples were concentrated by being freeze-dried overnight then reconstituted in 400 µl of assay buffer. Aliquots (100 µl for cyclic GMP and 50 µl for cyclic AMP) were assayed in duplicate with commercially available kits (TRK 500 and TRK 432 respectively, Amersham, UK). The detection limits, arbitrarily defined as the amount of unlabelled nucleotide necessary to reduce the binding of tritiated nucleotide by 15% compared with the zero standard were 0.16 pmol/tube for cyclic GMP and 0.24 pmol/tube for cyclic AMP. Taking into account the amount of tissue homogenized and the dilution factors, these values are equivalent to 6.4 and 76.8 pmol $g^{-1}$ of heart for cyclic GMP and cyclic AMP respectively. For samples falling within the linear portions of the standard curves the intra-assay coefficient of variation for cyclic GMP was approximately 8% and that for cyclic AMP was <5%.

### *Drugs*

SIN-1 (3-morpholinosydnonimine-N-ethylcarbamide) was a generous gift from Cassella AG, Frankfurt am Main, Germany, isosorbide dinitrate solution (Isoket 0.1%) was purchased from the Royal Liverpool University Hospital Pharmacy and sodium nitroprusside was purchased from Sigma, Poole. SIN-1 and sodium nitroprusside were made up as stock solutions of 1 mg $ml^{-1}$ in normal saline, divided into aliquots and stored at −20°C until required. Further dilutions of all the nitric oxide donors were made in saline immediately prior to use and care was taken to ensure that all solutions of nitric oxide donors were protected from light. Diethyl ether and trichloroacetic acid (TCA) were obtained from FSA Laboratory Supplies, Loughborough, and sodium pentobarbitone from RMB Animal Health Ltd, Dagenham.

### *Statistics*

Where appropriate, values have been expressed as the mean ± s.e.mean or the geometric mean (with 95% confidence limits) of *n* experiments. Haemodynamic data were subject to analysis of variance with significance levels determined by modified *t* tests with Bonferroni correction. Cyclic nucleotide data and the numbers of ventricular premature beats were compared with Mann-Whitney U tests and the incidence of arrhythmias subject to $\chi^2$ analysis (Fisher's exact test). A probability value of $P<0.05$ was considered to be significant.

## Results

### *Effects of sodium nitroprusside on ischaemia-induced arrhythmias*

In control rats the induction of acute myocardial ischaemia by coronary artery occlusion caused arrhythmias which varied in severity from single ventricular premature beats to terminal ventricular fibrillation. The majority of the arrhythmias occurred between 5 and 15 min post-occlusion. Ventricular tachycardia occurred in all the rats in this study, except one which received the middle dose of sodium nitroprusside (this animal had a few ectopic beats then went straight into terminal ventricular fibrillation). All of the rats in the control group for this particular study had ventricular fibrillation but this was only sustained, resulting in death, in 6 of the 12 rats. At least 75% of the rats in each group receiving sodium nitroprusside (0.1, 0.3 or 1 µg $kg^{-1}min^{-1}$) also had ventricular fibrillation and in at least half the animals in each of these drug groups this ventricular fibrillation was sustained. Thus administration of these doses of sodium nitroprusside did not significantly alter the total incidence of ventricular fibrillation or the mortality (Figure 1). There was also no significant difference in the total number of ventricular premature beats (including those occurring as ventricular tachycardia) that occurred in rats which survived the 25 min period of ischaemia between any of the groups receiving sodium nitroprusside and the controls (Figure 2).

Heart rate was not altered by infusion of sodium nitroprusside. Before and during drug infusion there were no significant differences between the values in the control group and the values at corresponding time points in any of the groups receiving sodium nitroprusside (Figure 3). In contrast, sodium nitroprusside did cause some reduction in systolic and diastolic arterial blood pressure. The values measured 5 and 10 min after commencing infusion of the highest dose of sodium nitroprusside (1 µg $kg^{-1}$ $min^{-1}$) were significantly lower than those measured at the same time points in the control group (Figure 3).

### *Effects of SIN-1 on ischaemia-induced arrhythmias*

Similar experiments were performed with another nitric oxide donor, SIN-1. Infusion of 10, 20 or 40 µg $kg^{-1}$ $min^{-1}$ of SIN-1, commencing 10 min before coronary artery occlusion did not alter the incidence of ventricular tachycardia (100% in each group), ischaemia-induced ventricular fibrillation or the mortality (Figure 4). Similarly, the number of ventricular premature beats that occurred in survivors was not changed by administration of SIN-1 (Figure 5). Although SIN-1 had no significant effects on the number or severity of the ischaemia-induced arrhythmias the doses used here did have profound haemodynamic actions. Figure 6 illustrates clearly that SIN-1 caused marked reductions in systolic and diastolic arterial pressure. In addition a significant bradycardia was also observed with all three doses of SIN-1 (Figure 6).

The severity of ischaemia-induced arrhythmias did not seem to be related to the degree of hypotension resulting from infusion of SIN-1. The haemodynamic values, measured 10 min after starting drug infusion, in all the rats which

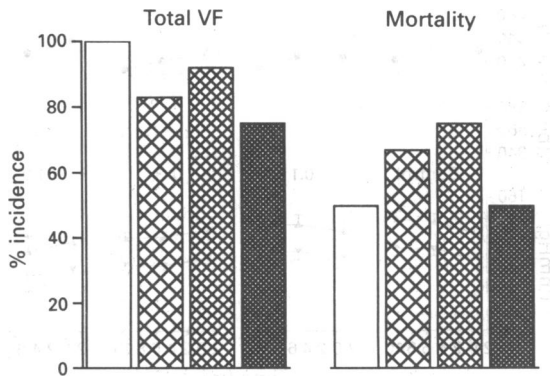


**Figure 1** The effects of sodium nitroprusside 0.1 µg $kg^{-1}$ $min^{-1}$ (light cross-hatched columns), 0.3 µg $kg^{-1}$ $min^{-1}$ (medium cross-hatched columns) and 1 µg $kg^{-1}$ $min^{-1}$ (dense cross-hatched columns) compared with saline controls (open columns) on the total incidence of ventricular fibrillation (VF) and the mortality due to sustained VF that occurred during the first 25 min of acute myocardial ischaemia. $n = 12$ per group.

received SIN-1 were pooled and subdivided into survivors and non-survivors. Heart rate (370 ± 10 and 375 ± 11 beats $min^{-1}$), systolic blood pressure (65 ± 3 and 69 ± 3 mmHg) and diastolic blood pressure (43 ± 3 and 42 ± 2 mmHg) in survivors ($n = 23$) and non-survivors ($n = 13$) respectively, were not significantly different.

### *Effects of SIN-1 on reperfusion-induced arrhythmias*

Further experiments were performed to investigate whether SIN-1 could alter the incidence or severity of reperfusion-induced arrhythmias. To avoid the profound hypotension observed previously, lower doses of SIN-1 were used in these studies. As anticipated, SIN-1 caused dose-dependent reductions in arterial blood pressure but of lesser magnitude than observed with the higher dose-range used in the study on ischaemia-induced arrhythmias. With the lowest dose of SIN-1 (1 $\mu g\ kg^{-1}\ min^{-1}$) the hypotension reached statistical significance only after 10 min of drug infusion. With the two higher doses the reductions in systolic and diastolic arterial blood pressure were significant 5 min after starting drug infusion (Figure 7). The effects on heart rate in this study are

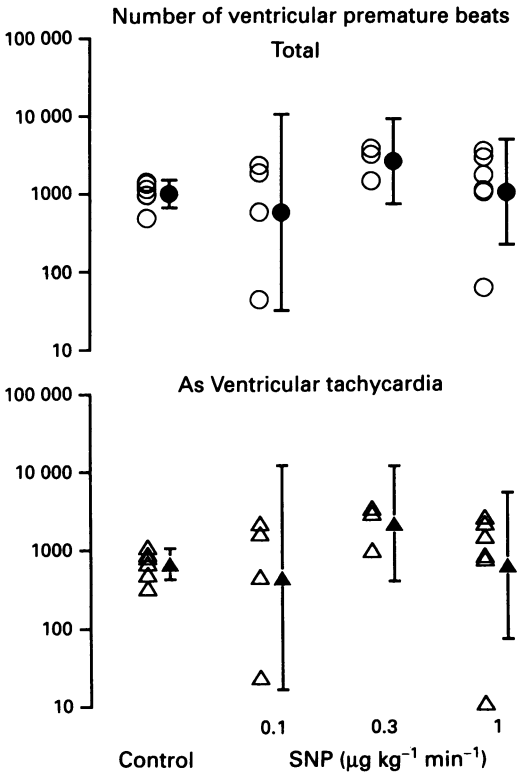


**Figure 2** The effects of sodium nitroprusside 0.1, 0.3 and 1 $\mu g\ kg^{-1}\ min^{-1}$ on the number of ventricular premature beats that occurred in anaesthetized rats which survived the first 25 min of acute myocardial ischaemia (expressed as the total and as those occurring as ventricular tachycardia). Open symbols indicate values in individual animals; closed symbols indicate the geometric mean with the vertical bars indicating the 95% confidence limits.

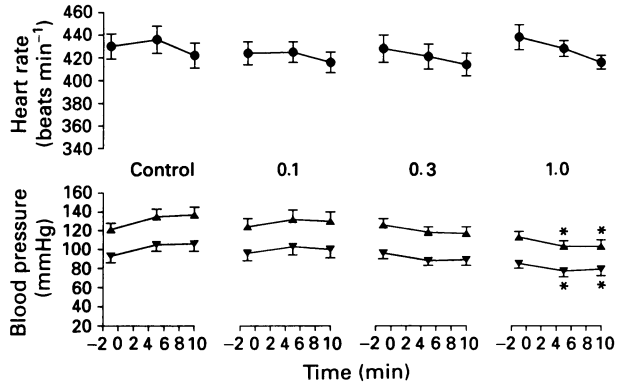


**Figure 3** Heart rate, systolic (▲) and diastolic (▼) blood pressure measured 1 min before and 5 and 10 min after commencing infusion of saline or sodium nitroprusside at 0.1, 0.3 or 1 $\mu g\ kg^{-1}\ min^{-1}$ in the anaesthetized rats used to study ischaemia-induced arrhythmias. Each value is the mean ± s.e. mean, $n = 12$ per group. $^*P<0.01$ compared with the same time point in the control group, analysis of variance and modified $t$ test (Bonferroni correction).

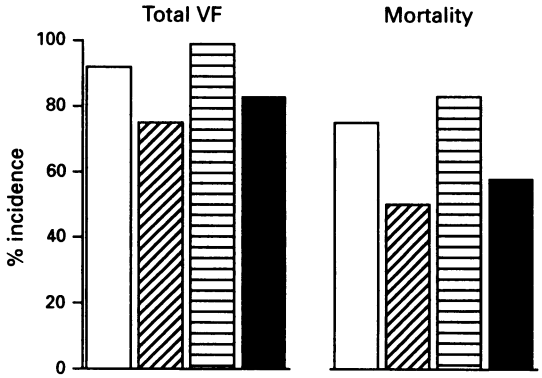


**Figure 4** The effects of SIN-1, 10 $\mu g\ kg^{-1}\ min^{-1}$ (diagonally hatched columns), 20 $\mu g\ kg^{-1}\ min^{-1}$ (horizontally hatched columns) and 40 $\mu g\ kg^{-1}\ min^{-1}$ (solid columns) compared with saline controls (open columns) on the total incidence of ventricular fibrillation (VF) and the mortality due to sustained VF that occurred during the first 25 min of acute myocardial ischaemia. $n = 12$ per group.

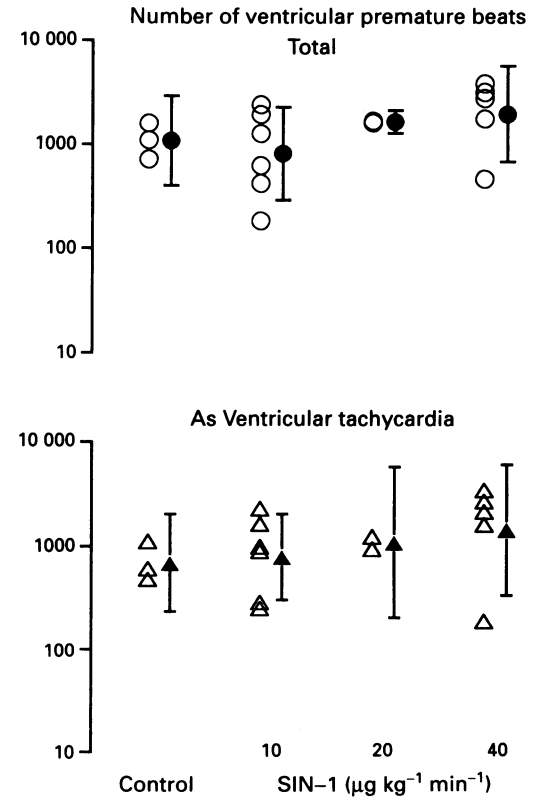


**Figure 5** The effects of SIN-1, 10, 20 and 40 $\mu g\ kg^{-1}\ min^{-1}$ on the number of ventricular premature beats that occurred in anaesthetized rats which survived the first 25 min of acute myocardial ischaemia (expressed as the total and as those occurring as ventricular tachycardia). Open symbols indicate values in individual animals; the solid symbols indicate the geometric mean with the vertical bars indicating the 95% confidence limits.

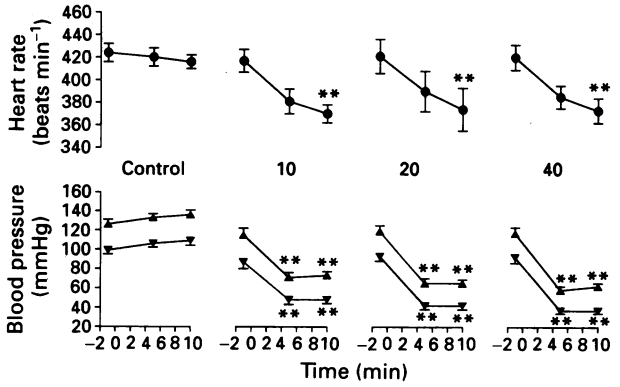


**Figure 6** Heart rate, systolic (▲) and diastolic (▼) blood pressure measured 1 min before and 5 and 10 min after commencing infusion of saline or SIN-1 10, 20 or 40 $\mu g\,kg^{-1}\,min^{-1}$ in the anaesthetized rats used to study ischaemia-induced arrhythmias. Each value is the mean ± s.e. mean, $n = 12$ per group. $^{**}P<0.001$ compared with the same time point in the control group, analysis of variance and modified $t$ test (Bonferroni correction).

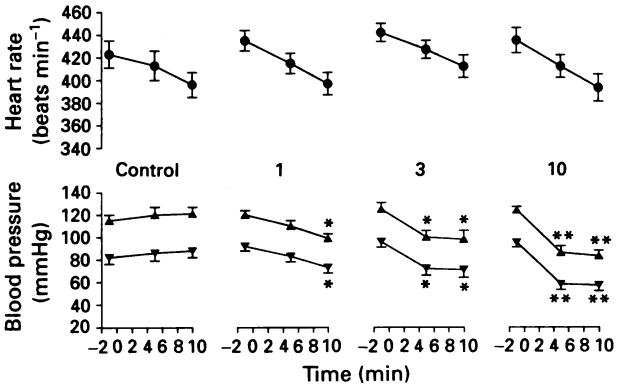


**Figure 7** Heart rate, systolic (▲) and diastolic (▼) blood pressure measured 1 min before and 5 and 10 min after commencing infusion of saline or SIN-1 1, 3 or 10 $\mu g\,kg^{-1}\,min^{-1}$ in the anaesthetized rats used to study reperfusion-induced arrhythmias. Each value is the mean ± s.e. mean, $n = 12$ per group. $^{*}P<0.01$, $^{**}P<0.001$ compared with the same time point in the control group, analysis of variance and modified $t$ test (Bonferroni correction).

more difficult to interpret. Figure 7 illustrates an apparent reduction in heart rate with SIN-1 but since heart rate also tended to decline in the control group no firm conclusion should be drawn from these data.

Abrupt reperfusion after 5 min of acute myocardial ischaemia usually causes severe arrhythmias which are rapid in onset but of brief duration compared to ischaemia-induced arrhythmias. In the control group for this particular study 8 out of 12 rats had reperfusion-induced ventricular fibrillation which was sustained resulting in death in all of these animals. None of the doses of SIN-1 significantly altered either the total incidence of reperfusion-induced ventricular fibrillation or the mortality (Figure 8). All of the rats had reperfusion-induced ventricular tachycardia. The arrhythmias started 7.3 ± 2.2 s after reperfusion in the controls and at 15.2 ± 3.7, 10.4 ± 2.9 and 7.6 ± 1.8 s in the rats which were receiving 1, 3 and 10 $\mu g\,kg^{-1}\,min^{-1}$ SIN-1 respectively. None of the latter values were significantly different from the value in the control group (Mann-Whitney U test). Similarly there were no significant differences in the duration of the reperfusion-induced arrhythmias in the survivors; the values were 179 ± 64, 152 ± 18, 190 ± 46 and 176 ± 42 s in the control, 1, 3 and 10 $\mu g\,kg^{-1}\,min^{-1}$ SIN-1 groups respectively. Thus, infusion of these doses of SIN-1 did not cause any significant

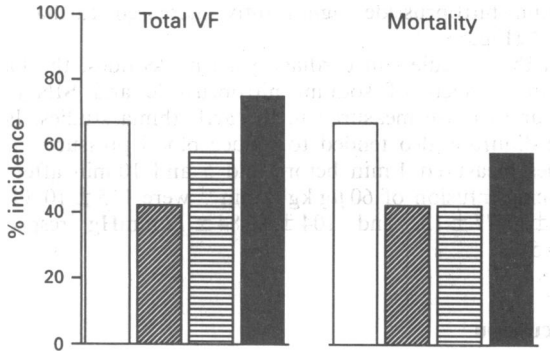


**Figure 8** The effects of SIN-1, 1 $\mu g\,kg^{-1}\,min^{-1}$ (diagonally hatched columns), 3 $\mu g\,kg^{-1}\,min^{-1}$ (horizontally hatched columns) and 10 $\mu g\,kg^{-1}\,min^{-1}$ (solid columns) compared with saline controls (open columns) on the total incidence of ventricular fibrillation (VF) and the mortality due to sustained VF induced by reperfusion following 5 min of coronary artery occlusion. $n = 12$ per group.

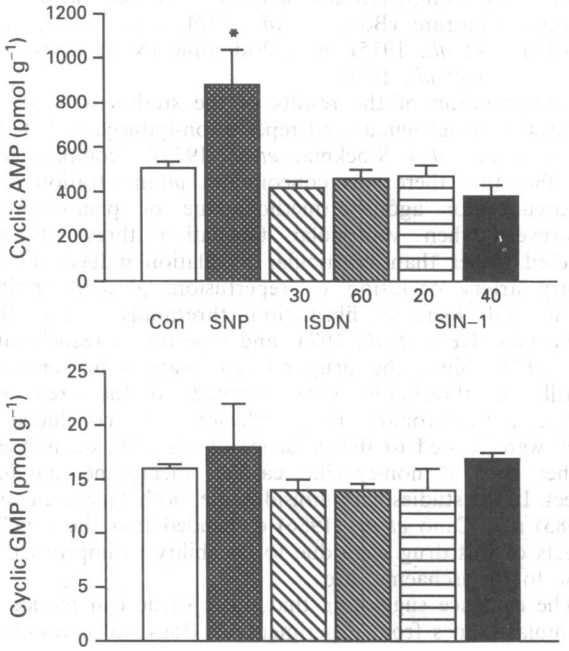


**Figure 9** Cardiac cyclic nucleotide levels in anaesthetized rats which had received sodium nitroprusside (SNP), 1 $\mu g\,kg^{-1}\,min^{-1}$, isosorbide dinitrate (ISDN), 30 or 60 $\mu g\,kg^{-1}\,min^{-1}$, or SIN-1, 20 or 40 $\mu g\,kg^{-1}\,min^{-1}$ for 15 min prior to freeze-clamping of the heart. $n = 5$ per group. $^{*}P<0.05$ compared with control group, Mann-Whitney U test.

alterations in the incidence or severity of reperfusion-induced arrhythmias.

### *Effects of nitric oxide donors on cardiac cyclic nucleotides*

Additional experiments were carried out to determine the effects of the nitric oxide donors, sodium nitroprusside, isosorbide dinitrate and SIN-1 on cardiac cyclic AMP and cyclic GMP contents. None of these nitric oxide donors caused any significant alteration in the value for cardiac cyclic GMP measured 15 min after starting drug infusion in anaesthetized rats prepared for coronary artery occlusion (Figure 9). The values for cardiac cyclic AMP in rats which had been receiving isosorbide dinitrate or SIN-1 were not different from the value in the control group. However,

sodium nitroprusside significantly increased cardiac cyclic AMP (Figure 9).

In these studies on cardiac cyclic nucleotides, the haemodynamic effects of sodium nitroprusside and SIN-1 were similar to those measured in the arrhythmia studies. Isosorbide dinitrate also tended to reduce blood pressure; e.g. the values measured 1 min before and 5 and 10 min after commencing infusion of 60 $\mu g\ kg^{-1}\ min^{-1}$ were $115 \pm 10/89 \pm 11$, $101 \pm 11/77 \pm 13$, and $104 \pm 11/84 \pm 12$ mmHg respectively ($n = 5$).

## Discussion

The data presented above indicate clearly that in the anaesthetized rat, nitric oxide donors do not alter arrhythmias associated with acute myocardial ischaemia. Despite using two different nitric oxide donors and a wide range of doses giving haemodynamic responses varying from very mild to marked hypotension, there were no changes in the incidence or severity of either ischaemia-induced or reperfusion-induced arrhythmias. These results contradict previous reports of antiarrhythmic activity observed with either glyceryl trinitrate (Borer *et al.*, 1974; Kent *et al.*, 1974; Stockman *et al.*, 1975) or molsidomine (Martorana *et al.*, 1983; Cano *et al.*, 1986).

Interpretation of the results of the studies with glyceryl trinitrate on ischaemia- and reperfusion-induced arrhythmias (Borer *et al.*, 1974; Stockman *et al.*, 1979) is complicated by the fact that there was concomitant administration of an α-adrenoceptor agonist (methoxamine or phenylephrine). However, when ventricular fibrillation thresholds were studied rather than ventricular fibrillation induced by coronary artery occlusion or reperfusion, glyceryl trinitrate alone did increase fibrillation thresholds during both ischaemia (Kent *et al.*, 1974) and reperfusion (Stockman *et al.*, 1979). Since the drug-induced changes in ventricular fibrillation thresholds were confined to the area made ischaemic by coronary artery occlusion it was concluded that they were related to the influence of the drug on ischaemia rather than a non-specific cardiac membrane stabilizing effect. In the studies with molsidomine, both Martorana *et al.* (1983) and Cano *et al.* (1986) concluded that the beneficial effects of this drug were due to its ability to improve blood flow to the ischaemic area.

The evidence suggesting that nitric oxide can reduce arrhythmias comes from experiments in dogs. In anaesthetized rats, a single dose of glyceryl trinitrate (15 $\mu g\ kg^{-1}\ min^{-1}$) did not alter reperfusion-induced ventricular fibrillation or mortality (Kane *et al.*, 1984). Coronary collateral flow is relatively high in dogs but low in rats (Maxwell *et al.*, 1987). It is possible that nitric oxide may only be able to reduce arrhythmias in species where it can increase flow through collateral vessels to the ischaemic area, i.e. the antiarrhythmic effect is a consequence of a reduction in the severity of myocardial ischaemia. In contrast, however, it has been shown recently that pirsidomine reduced the number of ischaemia-induced ventricular premature beats in anaesthetized pigs (Wainwright & Martorana, 1993), a species with virtually no collateral flow (Maxwell *et al.*, 1987). Although ventricular premature beats were reduced in pigs, pirsidomine had no effect on the incidence of ventricular fibrillation (Wainwright & Martorana, 1993). Thus it seems that nitric oxide donors may reduce ventricular fibrillation only in species with good collateral flow.

A recent abstract provides further evidence which supports our present findings that nitric oxide donors do not influence ischaemia-induced arrhythmias in anaesthetized rats. In a similar model, C87-3754, an active metabolite of a novel sydnonimine (Bohn *et al.*, 1991), did not alter arrhythmias and the nitric oxide synthase inhibitor $N^G$nitro-L-arginine methyl ester (L-NAME 100 mg $kg^{-1}$) also had no significant effects (Sun *et al.*, 1994). In addition, a recent study in our laboratory has also shown that administration of L-NAME (3, 10 or 30 mg $kg^{-1}$, i.v.), which caused significant dose-dependent increases in arterial blood pressure, had no effect on arrhythmias in anaesthetized rats subject to acute myocardial ischaemia (Aitchison & Coker, 1994). It is interesting to note that Sun *et al.* (1994) also found that L-NAME did not alter the marked antiarrhythmic effect of preconditioning in anaesthetized rats, whereas nitric oxide does appear to be involved in the antiarrhythmic effect of preconditioning in dogs (Vegh *et al.*, 1992). Thus it appears that neither endogenous nor exogenous nitric oxide influences arrhythmias associated with acute myocardial ischaemia in anaesthetized rats. Whether the failure of nitric oxide to have any antiarrhythmic activity in our experiments is related to the low collateral flow in the rat or some other feature unique to this particular species remains to be determined.

The results of the cyclic nucleotide assays indicate that administration of nitric oxide donors does not cause detectable increases in *cardiac* cyclic GMP. With the higher doses of SIN-1 marked reductions in arterial blood pressure were observed, which suggests that increases in vascular cyclic GMP did occur. The cardiac content of cyclic GMP is considerably lower than that of cyclic AMP. Our data indicate that there is approximately 25 fold less cardiac cyclic GMP than cyclic AMP and ratios indicating differences of up to 20 fold have been reported for rat heart by others (Kane *et al.*, 1985; Jones *et al.*, 1993). In vascular tissue there is much less difference between the levels of cyclic GMP and cyclic AMP. For example, in rat isolated aorta with endothelium, there is only 3 to 5 fold more cyclic AMP than cyclic GMP (Martin *et al.*, 1986). In addition, the amount of cyclic GMP in blood vessels is greater than in the whole heart. It is likely, therefore, that in the heart most of the cyclic GMP is in blood vessels (in endothelial cells, smooth muscle cells or platelets trapped in the coronary circulation as the heart was freeze-clamped) rather than in cardiac myocytes. Since the coronary vasculature represents a relatively small proportion of the heart, increases in coronary vascular cyclic GMP may have to be many times higher to be detected as increases in total *cardiac* cyclic GMP. We have confirmed that we could detect increases in cyclic GMP in rat isolated aortae incubated *in vitro* with sodium nitroprusside or SIN-1 (Barnes, 1993).

It is interesting to note that in the latter experiment, sodium nitroprusside increased both cyclic GMP and cyclic AMP (Barnes, 1993). Sodium nitroprusside was chosen for the first experiments on ischaemia-induced arrhythmias because it is inexpensive and readily available. At first we were puzzled by the results of the assays for cardiac cyclic nucleotides which showed that the highest dose of sodium nitroprusside used in the arrhythmia experiments did not alter cardiac cyclic GMP but did increase cyclic AMP significantly. However, it has been reported that there is a poor correlation between the vasodilator actions of sodium nitroprusside and its ability to increase vascular cyclic GMP and it has been suggested that sodium nitroprusside may have additional vasodilator activity (Feelisch, 1991). The elevation in cardiac cyclic AMP which we report here, supports the suggestion that sodium nitroprusside may indeed have additional actions. Whether these are a consequence of nitric oxide release from sodium nitroprusside or due to an independent mechanism cannot be determined from the present results. However, our data do suggest that sodium nitroprusside is not an ideal nitric oxide donor.

Having obtained the above results with sodium nitroprusside, we then decided to use an alternative nitric oxide donor and chose isosorbide dinitrate. The effects of this drug on cardiac cyclic nucleotides were examined first. Although this compound did not increase cyclic AMP it also failed to increase cardiac cyclic GMP and so was not used for arrhythmia experiments. Some recent reports contain evidence supporting our finding that isosorbide dinitrate did not increase cardiac cyclic GMP. No increases in plasma cyclic

GMP were detected in patients who had received isosorbide dinitrate orally, although significant reductions in blood pressure were observed (Shotan *et al.*, 1993). Similarly, neither oral nor i.v. administration of isosorbide dinitrate altered plasma cyclic GMP in rats (Kita *et al.*, 1994a,b).

We then examined the effects of SIN-1 since this compound does not need to interact with thiols to release nitric oxide (Feelisch, 1991). Again, and in this case, despite causing marked hypotension, cardiac cyclic GMP was not elevated. It was at this point that we reached the conclusion detailed above, that the drugs had not necessarily failed to increase cyclic GMP, but that any increase in vascular cyclic GMP which may have occurred could not be detected by measuring total *cardiac* cyclic GMP. SIN-1 was then used for the further arrhythmia studies.

The depressor responses to SIN-1 were greater than had been anticipated from preliminary pilot studies in closed chest rats. Comparison of the effects of SIN-1 on blood pressure in the ischaemia-induced and reperfusion-induced arrhythmia studies suggests that the responses obtained in the former experiments, although still dose-dependent were approaching maximum. It could be argued therefore that the degree of hypotension resulting from administration of these doses may have compromised myocardial perfusion. This could be expected to exacerbate arrhythmias, but SIN-1 also reduced heart rate, an effect which could be antiarrhythmic. There does not seem to be any obvious explanation for the bradycardia induced by SIN-1 in our experiments. In other species, SIN-1, or other sydnonimines, either caused tachycardia or had no effect on heart rate (Cano *et al.*, 1986; Groves *et al.*, 1993; Wainwright & Martorana, 1993). However, in the present study, the severity of arrhythmias did not appear to be related to the dose of SIN-1. In addition, subdivision of the rats into those which survived and those which died during the ischaemic period did not reveal any differences in blood pressure or heart rate. Thus it is unlikely that the haemodynamic effects of SIN-1 had a major influence on arrhythmias. Assuming that the hypotensive responses to SIN-1 indicate that elevations in cyclic GMP did occur in some areas (e.g. blood vessels), the lack of effect of SIN-1 on ischaemia-induced or reperfusion-induced arrhythmias suggests that cyclic GMP is not an important modulator of these events.

Thus to summarise, these studies have shown that nitric oxide donors do not alter arrhythmias induced by acute coronary artery occlusion or reperfusion in anaesthetized rats and suggest that, at least in the rat, cyclic GMP does not influence these arrhythmias. Further experiments are required to determine whether these results were obtained because of a particular feature found in the rat, such as low coronary collateral flow, or because nitric oxide is just not important in the rat coronary circulation.

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## References

AITCHISON, K.A. & COKER, S.J. (1994). Does endogenous nitric oxide influence ischaemia-induced arrhythmias? *J. Mol. Cell. Cardiol.*, **26**, (No. 6) LXVII.

BARNES, C.S. (1993). The potential influence of cyclic GMP on arrhythmias associated with myocardial ischaemia. *PhD Thesis, University of Liverpool.*

BOHN, H., BEYERLE, R., MARTORANA, P.A. & SCHONAFINGER, K. (1991). CAS 936, a novel sydnonimine with direct vasodilating and nitric oxide-donating properties: effects on isolated blood vessels. *J. Cardiovasc. Pharmacol.*, **19**, 522–527.

BORER, J.S., KENT, K.M., GOLDSTEIN, R.E. & EPSTEIN, S.E. (1974). Nitroglycerin-induced reduction in the incidence of spontaneous ventricular fibrillation during coronary artery occlusion in dogs. *Am. J. Cardiol.*, **33**, 517–520.

CANO, J-P., GUILLEN, J-C., JOUVE, R., LANGLET, F., PUDDU, P-E., ROLLAND, P-H. & SERRADIMIGNI, A. (1986). Molsidomine prevents post-ischaemic ventricular fibrillation in dogs. *Br. J. Pharmacol.*, **88**, 779–789.

CLARK, C., FOREMAN, M.I., KANE, K.A., McDONALD, F.M. & PARRATT, J.R. (1980). Coronary artery ligation in anesthetized rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. *J. Pharmacol. Methods*, **3**, 357–368.

COKER, S.J. & BARNES, C.S. (1993). Effects of the nitric oxide donor SIN-1 on haemodynamics, ischaemia-induced arrhythmias and cardiac cyclic nucleotides. *Br. J. Pharmacol.*, **109**, 43P.

COKER, S.J. & ELLIS, A.M. (1987). Ketanserin and ritanserin can reduce reperfusion-induced but not ischaemia-induced arrhythmias in anaesthetised rats. *J. Cardiovasc. Pharmacol.*, **10**, 479–484.

FEELISCH, M. (1991). The biochemical pathways of nitric oxide formation from nitrovasodilators: appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. *J. Cardiovasc. Pharmacol.*, **17** (Suppl. 3), S25–S33.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

FURLONG, B., HENDERSON, A.H., LEWIS, J.A. & SMITH, J.A. (1987). Endothelium-derived relaxing factor inhibits *in vitro* platelet aggregation. *Br. J. Pharmacol.*, **90**, 687–692.

GROVES, P.H., LEWIS, M.J., CHEADLE, H.A. & PENNY, W.J. (1993). SIN-1 reduces platelet adhesion and platelet thrombus formation in a porcine model of balloon angioplasty. *Circulation*, **87**, 590–597.

HOLBROOK, M. & COKER, S.J. (1989). Comparison of the effects of isobutylmethylxanthine and milrinone on ischaemia-induced arrhythmias and platelet aggregation in anaesthetized rabbits. *Br. J. Pharmacol.*, **98**, 318–324.

JONES, R.B., FRODSHAM, G., DICKENSON, K. & FOSTER, G.A. (1993). Effect of flosequinan on ischaemia-induced arrhythmias and on ventricular cyclic nucleotide content in the anaesthetized rat. *Br. J. Pharmacol.*, **108**, 1111–1116.

KANE, K.A., PARRATT, J.R. & WILLIAMS, F.M. (1984). An investigation into the characteristics of reperfusion-induced arrhythmias in the anaesthetized rat and their susceptibility to antiarrhythmic agents. *Br. J. Pharmacol.*, **82**, 349–357.

KANE, K.A., MORCILLO-SANCHEZ, E.J., PARRATT, J.R., RODGER, I.W. & SHAHID, M. (1985). The relationship between coronary artery occlusion-induced arrhythmias and myocardial cyclic nucleotide levels in the anaesthetized rat. *Br. J. Pharmacol.*, **84**, 139–145.

KATSUKI, S., ARNOLD, W., MITTAL, C. & MURAD, F. (1988). Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin, and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J. Cyclic Nucl. Res.*, **3**, 23–35.

KENT, K.M., SMITH, E.R., REDWOOD, D.R. & EPSTEIN, S.E. (1974). Beneficial electrophysiological effects of nitroglycerin during acute myocardial infarction. *Am. J. Cardiol.*, **33**, 516–517.

KITA, Y., HIRASAWA, Y., MAEDA, K., NISHIO, M. & YOSHIDA, K. (1994a). Spontaneous nitric oxide release accounts for the potent pharmacological actions of FK409. *Eur. J. Pharmacol.*, **257**, 123–130.

KITA, Y., SUGIMOTO, T., HIRASAWA, Y., YOSHIDA, K. & MAEDA, K. (1994b). Close correlation of the cardioprotective effect of FK409, a spontaneous NO releaser, with an increase in plasma cyclic GMP level. *Br. J. Pharmacol.*, **113**, 5–6.

KUKOVETZ, W.R., HOLZMANN, S., WURM, A. & POCH, G. (1979). Evidence for cyclic GMP-mediated relaxant effects of nitrocompounds in coronary smooth muscle. *Naunyn Schmied. Arch. Pharmacol.*, **310**, 129–138.

MARTIN, W., FURCHGOTT, R.F., VILLANI, G.M. & JOTHIANANDAN, D. (1986). Phosphodiesterase inhibitors induce endothelium-dependent relaxation of rat and rabbit aorta by potentiating the effects of spontaneously released endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther.*, **237**, 539–547.

MARTORANA, P.A., MOGILEV, A.M., KETTENBACH, B. & NITZ, R.-E. (1983). Effect of molsidomine on spontaneous ventricular fibrillation following myocardial ischemia and reperfusion in the dog. *Adv. Myocardiol.*, **4**, 605–613.

MAXWELL, M.P., HEARSE, D.J. & YELLON, D.M. (1987). Species variation in the coronary collateral circulation during regional myocardial ischaemia: a critical determinant of the rate of evolution and extent of myocardial infarction. *Cardiovasc. Res.*, **21**, 737–746.

NOACK, E. & FEELISCH, M. (1989). Molecular aspects underlying the vasodilator action of molsidomine. *J. Cardiovasc. Pharmacol.*, **14**, (Suppl. 11), S2–S5.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1987). Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *Br. J. Pharmacol.*, **92**, 181–187.

SHOTON, A., MEHRA, A., OSTRZEGA, E., HSUEH, W., DO, Y.S., FISHER, D.A., HURST, A., JOHNSON, J.V. & ELKAYAM, U. (1993). Plasma cyclic guanosine monophosphate in chronic heart failure: Hemodynamic and neurohumoral correlations and response to nitrate therapy. *Clin. Pharmacol. Ther.*, **54**, 638–644.

STOCKMAN, M.B., VERRIER, R.L. & LOWN, B. (1979). Effect of nitroglycerin on vulnerability to ventricular fibrillation during myocardial ischaemia and reperfusion. *Am. J. Cardiol.*, **43**, 233–238.

SUN, W., WAINWRIGHT, C.L. & PARRATT, J.R. (1994). The antiarrhythmic effect of ischaemic preconditioning is not prevented by inhibition of endothelium-derived nitric oxide in anaesthetized rats. *Br. J. Pharmacol.*, **112**, 380P.

VEGH, A., SZEKERES, L. & PARRATT, J.R. (1992). Preconditioning of the ischaemic myocardium; involvement of the L-arginine nitric oxide pathway. *Br. J. Pharmacol.*, **107**, 648–652.

WAINWRIGHT, C.L. & MARTORANA, P.A. (1993). Pirsidomine, a novel nitric oxide donor, suppresses ischemic arrhythmias in anaesthetized pigs. *J. Cardiovasc. Pharmacol.*, **22** (Suppl. 7) S44–S50.

WALKER, M.J.A., CURTIS, M.J., HEARSE, D.J., CAMPBELL, R.W.F., JANSE, M.J., YELLON, D.M., COBBE, S.M., COKER, S.J., HARNESS, J.B., HARRON, D.W.G., HIGGINS, A.J., JULIAN, D.J., LAB, M.J., MANNING, A.S., NORTHOVER, B.J., PARRATT, J.R., RIEMERSMA, R.A., RIVA, E., RUSSELL, D.C., SHERIDAN, D.J., WINSLOW, E. & WOODWARD, B. (1988). The Lambeth Conventions: Guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. *Cardiovasc Res.*, **22**, 447–455.

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