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The dopamine receptor agonist Z1046 reduces ischaemia severity in a canine model of coronary artery occlusion

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Abstract

Z1046, (*S*)-6[[6-[[2-(2-methoxyphenoxy)ethyl]amino]propyl]amino]-5,6,7,8-tetra-hydro-1,2-naphtalenediol dihydrochloride, is an agonist at both dopamine D_1 and D_2 receptors. Since stimulation of dopamine D_2 receptors inhibits noradrenaline release, and because cardiac noradrenaline release has been implicated in the genesis of early ischaemia-induced, life-threatening ventricular arrhythmias, the effect of Z1046 has been examined for its effects on coronary artery occlusion in chloralose–urethane anaesthetised mongrel dogs. Z1046 (10 μg kg^{-1} intravenously or 1 μg kg^{-1} by local intracoronary injection) decreased heart rate and reduced arterial blood pressure and coronary blood flow, effects prevented by the prior administration of domperidone (40 μg kg^{-1} i.v.). The ischaemic changes induced by a 25-min occlusion of the left anterior descending coronary artery (including ST-segment elevation and ventricular ectopic activity) were much less marked in those dogs administered Z1046 and survival from the combined ischaemia–reperfusion insult was increased from 7% to 36% ($P < 0.05$) These effects of Z1046 were partly attenuated by domperidone. We conclude that the anti-ischaemic effects of Z1046 are due to inhibition of cardiac sympathetic responses. Studies using rat isolated perfused mesenteric vascular bed preparations subjected to sympathetic nerve stimulation confirmed that Z1046 inhibits synaptic transmission without modifying vascular responses to noradrenaline. © 1998 Elsevier Science B.V.

Keywords: Z1046; Dopamine receptor; Ventricular arrhythmia; Myocardial ischaemia; Coronary artery occlusion

1. Introduction

Many factors influence the severity of ventricular arrhythmias that occur when a coronary artery is occluded. These factors, which have been extensively reviewed (Parratt, 1982; Wit and Janse, 1993) include the extent of the potentially ischaemic area, the degree of the preexisting coronary collateral circulation in the area distal to the occlusion, haemodynamic factors such as changes in heart rate and arterial (perfusion) pressure, anaesthesia, blood electrolytes, blood gases, the balance between sympathetic and parasympathetic tone and numerous metabolic factors including arrhythmogenic (Curtis et al., 1993) and protective (antiarrhythmic) substances (Parratt, 1993) released from the ischaemic myocardium.

Much emphasis has been placed on the importance, in the generation of early (phase I) ischaemia-induced ventricular arrhythmias, of sympathetic activation and local cardiac noradrenaline release (Verrier and Lown, 1978; Meesmann, 1982; Schwartz, 1984; Vanoli and Schwartz, 1991). The evidence for such a mechanism includes the effects of sympathetic denervation, of drugs that block α- and β-adrenoceptors, of cardiac noradrenaline depletion with drugs such as 6-hydroxydopamine and, in some studies, evidence derived from the measurement of noradrenaline release under conditions of ischaemia (e.g., Marshall and Parratt, 1980). Many factors are involved in modulating sympathetic nervous activity in the heart, including a variety of presynaptic receptors either facilitating or inhibiting noradrenaline release (reviewed by Francis, 1995). These include dopamine (D_2) receptors, stimulation of which inhibits noradrenaline release from nerve terminals. Z1046, (*S*)-6[[6-[[2-(2-methoxyphenoxy)ethyl]amino]pro-

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pyl]amino]-5,6,7,8-tetra-hydro-1,2-naphtalenediol dihydrochloride, is a new dopamine receptor agonist which has both D_1-like and D_2-like activities, and is orally active yet has almost no activity on α_1 or α_2-adrenoceptors (Pocchiari et al., 1994). When administered to pentobarbitone-anaesthetised beagle dogs (Pocchiari et al., 1994) or to awake pigs (Duncker et al., 1997), it lowered blood pressure by peripheral vasodilatation and tended to reduce left ventricular work. Flow was increased in the renal, femoral (Pocchiari et al., 1994) cerebral and mesenteric (Duncker et al., 1997) vascular beds. It has been suggested that Z1046 might be of value in the treatment of hypertension and congestive heart failure (Pradella et al., 1994).

In the present studies we show that Z1046 markedly reduces the severity of ischaemia- and reperfusion-induced arrhythmias in a canine model and that this protection is partially reversed by domperidone. Some of these results were presented, in brief, to the Strathclyde meeting of the British Pharmacological Society (Parratt et al., 1995) and to the Versailles meeting of the International Society of Heart Research (Végh et al., 1997a,b).

2. Methods

2.1. Studies in anaesthetised dogs

Mongrel dogs, mainly Hungarian Alsatians or sheep dogs, of either sex and with a mean body weight in excess of 17 kg (24.2 ± 1.4 kg) were anaesthetised with a mixture of chloralose and urethane (60 and 200 mg kg^{-1}, respectively, given intravenously) and ventilated with room air using a Harvard respirator at a rate and volume sufficient to maintain arterial blood gases and pH within normal limits (Végh et al., 1992a). The temperature was measured from the oesophagus and maintained by a heating pad between 36.8 and 37.5°C.

A thoracotomy was performed at the fifth intercostal space and the anterior descending branch of the left coronary artery prepared for occlusion just proximal to the first main diagonal branch. Epicardial ST-segment changes and the degree of inhomogeneity of activation were measured from the left ventricular wall distal to the proposed coronary artery occlusion with unipolar electrodes and a composite electrode previously described (Végh 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 almost simultaneously, resulting in a single large spike. However, following occlusion widening and fractionation of the summarised R-wave occurs indicating that adjacent fibres are not simultaneously activated because of inhomogeneity of conduction. We expressed inhomogeneity of conduction as the greatest delay in activation (in milliseconds) within the ischaemic area.

Blood flow in the left circumflex coronary artery was measured in some of the experiments with a 2.0 mm electromagnetic flow probe and a Statham SP2202 flow meter. These parameters, together with a limb lead electrocardiogram, systemic arterial pressure and left ventricular (LV) pressure (Statham p23Dp transducers) and LV$dP/dt$ were recorded on an eight channel Medicor R81 recorder.

In some of the experiments a side branch of the left anterior descending coronary artery was catheterised, as outlined previously (Végh et al., 1992b); this branch was immediately proximal to the proposed occlusion site. This was used for the local intracoronary administration of Z1046.

Ventricular arrhythmias during ischaemia and reperfusion were analysed as outlined previously (Végh et al., 1992a). This analysis is based on the suggestions made at the 'Lambeth Conventions' (Walker et al., 1988). No distinction was made between couplets and salvos, which were included as single ventricular ectopic (premature) beats, and we defined ventricular tachycardia (VT) as a run of four or more ectopics at a rate faster than the resting sinus rate. We also estimated the number of episodes of ventricular tachycardia during coronary artery occlusion and the incidences of VT and ventricular fibrillation (VF) both during occlusion and on reperfusion at the end of the 25 min occlusion period. Survival indicates those dogs that were in sinus rhythm 5 min following reperfusion after a 25-min occlusion period.

At the end of the experiment the area at risk was assessed by infusing patent blue V dye into the occluded artery at the end of the experiment and at a pressure equivalent to that of mean arterial blood pressure. It was expressed as % age of the left ventricular wall and septum. The data was analysed statistically as previously described (Végh et al., 1992a), i.e., data are expressed as means ($\pm$S.E.M.) and differences between means were compared by the Student's *t*-test, corrected for multiple comparisons, using a two-way analysis of variance (ANOVA) or, for arrhythmias, by the Mann–Whitney *U*-test. For comparison of the incidences of VT and VF and survival from the combined ischaemia–reperfusion insult the χ^2 test for independence in a 2×2 table was used. Differences between groups were considered significant when $P < 0.05$.

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

Four groups of animals were used.

(i) Group 1 (controls). These dogs served as controls and were allowed to stabilise after surgery for 1 h; the left anterior descending coronary artery was then occluded for 25 min after which the ischaemic area was reperfused.

(ii) Group 2 (Z1046). Eleven dogs were given Z1046 in a dose of 10 μg kg^{-1}, administered intravenously into the left femoral vein slowly over a 2-min period. This dose of Z1046 was similar to that used by Pradella et al. (1994) in their measurements of regional blood flow in dogs. Thirty

minutes after the administration of Z1046, the coronary artery was occluded for 25 min and, at the end of this period, the myocardium was reperfused by rapidly reopening the occluded artery.

(iii) Group 3 (Z1046 in the presence of domperidone). In order to ascertain whether the effects of Z1046 were mediated through dopamine D_2 receptors, domperidone, a selective antagonist of dopamine at D_2 receptors (Mannelli et al., 1988), was given intravenously in a dose of 40 μg kg^{-1} to eight dogs 15 min before an intravenous dose of Z1046 (10 μg kg^{-1}). The dose of domperidone was chosen on the basis that, in pentobarbital-anaesthetised beagle dogs, it almost completely abolished the reduction in blood pressure resulting from Z1046 administration (Pradella et al., 1994).

(iv) Group 4 (Z1046). Ten dogs were given Z1046 in a dose of 1 μg kg^{-1} administered slowly into the catheterised side branch of the coronary artery. Then 30 min later, the coronary artery was occluded for 25 min and the myocardium rapidly reperfused. This dose of Z1046 was chosen on the assumption that approximately 10% of the above intravenous dose would reach the ventricular wall supplied by the left coronary artery. We have previously shown that the infusion of an equivalent volume of saline into this small coronary artery does not modify ischaemia-induced ventricular arrhythmias (Végh et al., 1992b).

2.2. Studies on the isolated perfused mesenteric bed of rats

Male Sprague–Dawley rats weighing between 250 and 300 g were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} by i.p. injection) and the abdominal cavity opened by a midline incision through the linea alba and the mesenteric bed excised as described by Fatehi-Hassanabad et al. (1995). In brief, the mesenteric bed was perfused through a cannula inserted into the superior mesenteric artery with Krebs–Henseleit solution of the following composition (nM); NaCl 118.4, KCl 4.7, $MgSO_4 \cdot H_2O$ 1.2, $KH_2PO_4 \cdot 2H_2O$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, and glucose 11.1 in distilled water. This solution was maintained at 37°C, bubbled with 5% CO_2 and 95% O_2 and perfused at a constant rate (2 ml min^{-1}; Gilson Minipuls 2, Anachem). The isolated, perfused preparations were placed on a Petri dish, which was supported in a heated water bath (37°C). The perfusate, which flowed from the cut ends of the vessels at the intestinal margin of the mesentery, was removed at a rate of 2 ml min^{-1} to prevent accumulation in the bath. The tissue was allowed to equilibrate for 30 min before the experiments were started.

The preparations were subjected to sympathetic nerve stimulation (SNS) through a ring electrode around the superior mesenteric artery by a Grass stimulator with supramaximal rectangular pulses (50 V) and a pulse duration of 3 ms. Changes in perfusion pressure were measured, using a Statham pressure transducer and a Grass recorder, before and during sympathetic nerve stimulation and following the administration of noradrenaline. In order to determine the effect of Z1046 on sympathetic transmission the drug was administered in concentrations ranging from 5×10^{-9} M to 2×10^{-6} M in the absence and presence of domperidone (1×10^{-8} M).

3. Results

3.1. Haemodynamic effects of Z1046; modification by domperidone

The detailed results are given in Table 1 and in Fig. 1. As expected, Z1046 lowered systemic arterial blood pressure; this decrease in pressure was almost immediate (mean arterial blood pressure was reduced from 94 ± 6 mmHg to 78 ± 4 mmHg within 1 min of administration), stabilised after 3–5 min and remained about 20 mmHg below the preadministration level over the entire recording period before the coronary artery was occluded 0.5 h later (Fig. 1). Heart rate was also reduced by Z1046 (Table 1), stabilising about 17 beats min^{-1} below the resting rate (Fig. 1). Left ventricular dP/dt decreased in parallel with the reduction in arterial pressure (Fig. 1). When the changes

Table 1
Haemodynamic effects of Z1046 (10 μg kg^{-1} i.v.) and of subsequent coronary artery occlusion

	Preinjection	0.5 h postinjection	5 min postocclusion
Arterial blood pressure			
Systolic (mmHg)	118 ± 8	92 ± 7[a]	86 ± 8
Diastolic (mmHg)	82 ± 6	62 ± 5	58 ± 6
Mean (mmHg)	94 ± 6	72 ± 6[a]	67 ± 7
Left ventricular end-diastolic pressure (mmHg)	5.0 ± 0.1	9 ± 0.9[a]	23 ± 1[b]
LV$dP/dt_{max}$ (+ve; mmHg s^{-1})	2780 ± 206	1956 ± 202[a]	1755 ± 195
LV$dP/dt_{max}$ (−ve; mmHg s^{-1})	2483 ± 168	2134 ± 139	1811 ± 169
Heart rate (beats min^{-1})	132 ± 7	115 ± 7[a]	115 ± 7

Values are means ± S.E.M. from 11 observations.
[a] $P < 0.05$ compared to preinjection values.
[b] $P < 0.05$ compared to preocclusion value.

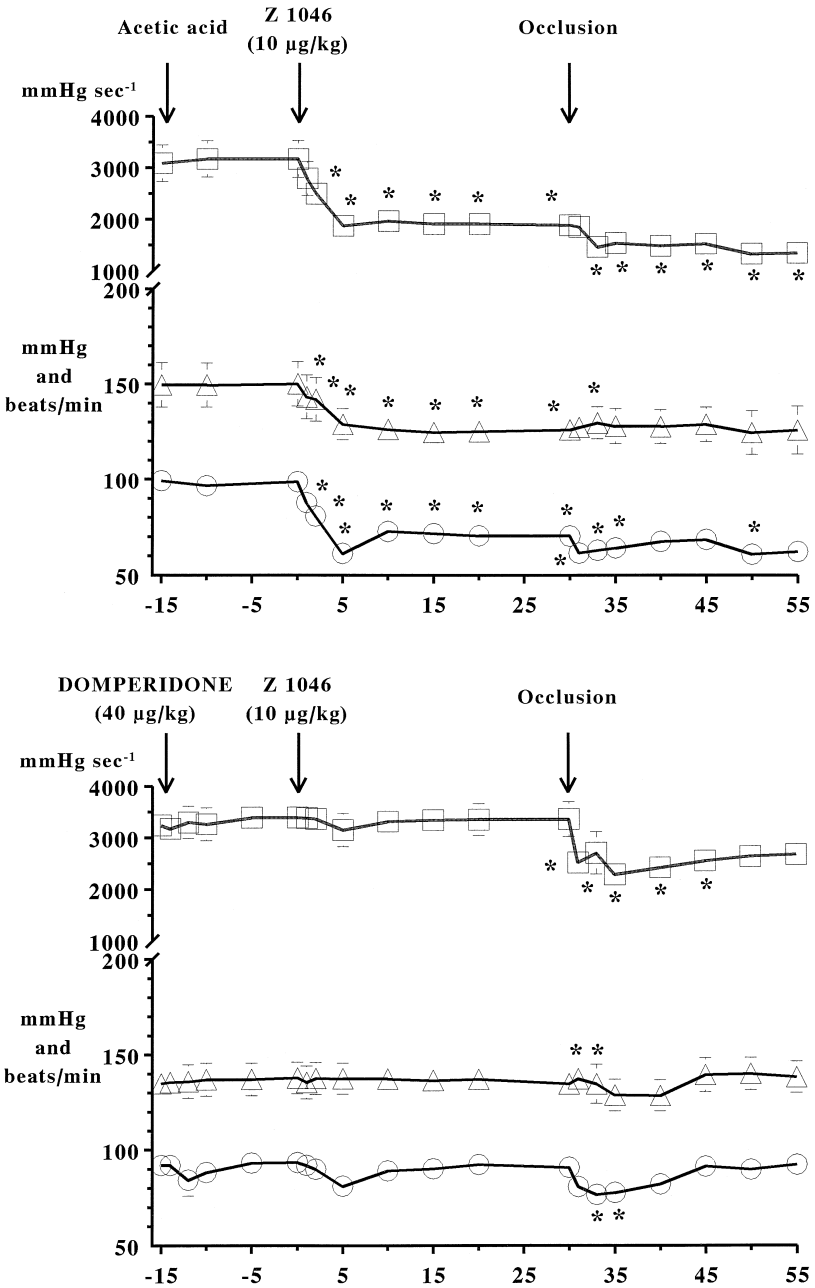

Fig. 1. Changes in, from above, left ventricular positive dP/dt_{max} (mmHg s^{-1}; open squares) heart rate (beats min^{-1}; open triangles) and mean arterial blood pressure (mmHg; open circles) in anaesthetised dogs after (above) the administration of Z1046 (10 μg kg^{-1} i.v., at time zero) followed 30 min later, by occlusion of the anterior branch of the left coronary artery). The haemodynamic effects of Z1046 are prevented (below) by the administration of domperidone (40 μg kg^{-1} i.v.) but not (above) by its solvent, acetic acid. Values given are as mean $\pm$ S.E.M. Where no standard error bars are given, the S.E.M. was less than the area occupied by the symbol itself. $* P < 0.05$ compared to the pre-Z1046 values.

in afterload were taken into account (by calculating $LVdP/dt/P$), the changes in $LVdP/dt_{max}$ could be accounted for by the alteration in perfusion pressure, indicating that there was no marked change in myocardial contractility which was perhaps in part maintained by an increase in left ventricular end-diastolic pressure (from 5 ± 0.1 mmHg to 9 ± 0.9 mmHg; Table 1).

The effects of Z1046 on coronary blood flow are shown in Fig. 2. Calculations of coronary vascular resistance revealed that there was no change in diastolic resistance after administration of Z1046 (0.79 ± 0.07 units prior to administration and 0.79 ± 0.10 units immediately prior to coronary artery occlusion). The coronary blood flow change thus appears to reflect changes in coronary perfusion pressure.

The haemodynamic changes induced by Z1046 were abolished by the prior administration of domperidone (40 μg kg^{-1}; Table 2 and Fig. 1), implying that these effects

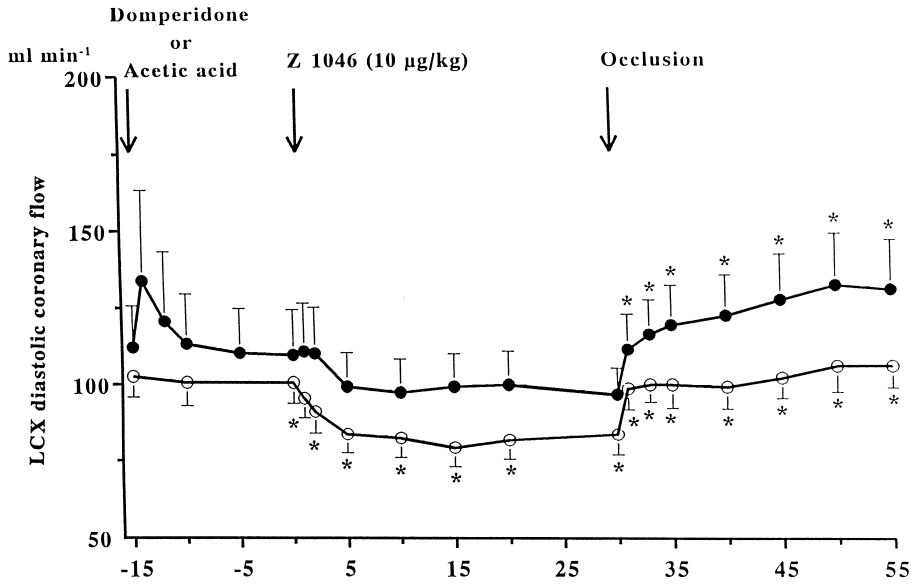

Fig. 2. Changes in diastolic blood flow (ml min^{-1}) in the left circumflex (LCX) coronary artery (open circles) in dogs following the administration of Z1046 (given at time zero) and of the domperidone solvent (acetic acid). Thirty minutes later the left anterior descending coronary artery was occluded. Z1046 reduced coronary blood flow but the compensatory coronary vasodilatation that occurs in the left circumflex vascular bed when the left anterior descending coronary artery is occluded was still present. Domperidone (40 μg kg^{-1}, given 15 min prior to the administration of Z1046) markedly attenuated the reduction in flow resulting from Z1046 administration (closed circles) and the compensatory vasodilatation in response to coronary occlusion was still present. Values are given as mean ± S.E.M. $*$ $P < 0.05$ compared to the pre-Z1046 values and, after 30 min, to the preocclusion values.

are mediated by the interaction of Z1046 with dopamine (D_2) receptors. Neither domperidone, nor its vehicle (acetic acid), had any significant haemodynamic effects. The reduction in coronary blood flow that also resulted from Z1046 administration was attenuated, but not abolished, by the same dose of domperidone (Fig. 2), perhaps implying the partial involvement of another dopamine receptor.

The administration of Z1046 increased both P–Q and Q–T intervals (from 112 ± 3 to 124 ± 3 ms and from 252 ± 11 to 268 ± 10 ms, respectively; $P < 0.05$) as recorded from the limb lead electrocardiogram. It had no effect on the inhomogeneity of electrical activation (51 ± 0.9 ms prior to administration and 52 ± 1.2 ms 30 min after administration and immediately prior to coronary artery occlusion).

The effects of the intracoronary administration of Z1046, in a dose 10 times lower than that used for the intravenous study, led to rather similar haemodynamic effects. There was a decrease in mean arterial blood pressure of around 14 mmHg (from 94 ± 5 to 80 ± 6 mmHg 0.5 h after the commencement of the injection), an increase in left ventricular end-diastolic pressure (from 5 ± 0.3 to 8 ± 0.7 mmHg; $P < 0.05$), reductions in LV$dP/dt$ (from 2642 ± 130 to 2393 ± 165 for positive dP/dt (ns) and from 2663 ± 104 to 2267 ± 146 for negative dP/dt, $P < 0.05$). Coronary blood flow in the left circumflex artery was

Table 2
Haemodynamic effects of Z1046 (10 μg kg^{-1} i.v.) in the presence of domperidone (40 μg kg^{-1})

	Pre-domperidone	Post-domperidone[a]	Post Z1046[b]
Arterial blood pressure			
Systolic (mmHg)	119 ± 6	120 ± 8	115 ± 6
Diastolic (mmHg)	79 ± 5	68 ± 11	79 ± 7
Mean (mmHg)	93 ± 5	85 ± 9	91 ± 6
Left ventricular end-diastolic pressure (mmHg)	6.9 ± 1.3	6.9 ± 1.4	8.1 ± 1.6
LV$dP/dt_{max}$ (+ve; mmHg s^{-1})	3273 ± 208	3431 ± 262	2514 ± 176
LV$dP/dt_{max}$ (−ve; mmHg s^{-1})	2713 ± 99	2753 ± 153	2514 ± 176
Heart rate (beats min^{-1})	133 ± 7	135 ± 8	133 ± 7
Coronary blood flow (diastolic; ml min^{-1})	93 ± 8	90 ± 11	88 ± 8

The values are given as means ± S.E.M. The changes should be compared with those in Table 1.
[a]15 min after injection.
[b]30 min after injection; these values are all significantly different ($P < 0.05$) from those for Z1046 in the absence of domperidone.

reduced from 97 ± 9 to 77 ± 6 ml min^{-1} (diastolic; $P < 0.05$) but diastolic coronary artery resistance was again unchanged (0.94 ± 0.1 to 0.94 ± 0.09 units). There were no changes in heart rate in the ST-segment recorded from the epicardial electrodes or in the inhomogeneity of activation within the potentially ischaemic area (48 ± 1 ms to 50 ± 0.1 ms; ns) following the local administration of Z1046.

3.2. Changes induced by coronary artery occlusion in control dogs and in dogs administered Z1046

The haemodynamic changes following left anterior descending coronary artery occlusion are also shown in Table 1 and in Fig. 1. Occlusion-induced changes in coronary blood flow, in epicardial ST-segment elevation and in the degree of inhomogeneity of activation within the ischaemic area) are illustrated in Figs. 2–4, respectively.

In control dogs, coronary artery occlusion resulted in a reduction in arterial pressure (mean of −15 ± 1 mmHg, maximal after 3–5 min), a marked increase in left ventricular end-diastolic pressure (from 5.6 ± 0.4 mmHg to 22.2 ± 1.9 mmHg) and reductions in both positive and negative $LVdP/dt_{max}$. The changes were similar, but somewhat less marked, in those dogs given Z1046 (Table 1). Apart from a somewhat lower systemic arterial pressure (mean 72 ± 6 vs. 84 ± 4 mmHg in the controls) and heart rate (115 ± 7 vs. 147 ± 5 beats min^{-1}), the haemodynamic parameters in the two groups immediately after occlusion were rather similar (e.g., positive $LVdP/dt_{max}$ 1755 ± 195 vs. 1809 ± 316 mmHg s^{-1}; negative $LVdP/dt_{max}$ 1811 ± 169 vs. 1868 ± 266 mmHg s^{-1}; LVEDP 23 ± 1 vs. 22.2 ± 1.9 mmHg 5 min after coronary artery occlusion) in the Z1046 group and controls, respectively.

The pronounced compensatory (metabolic) vasodilatation that normally occurs in the areas of the left ventricular wall not predominantly supplied by the occluded, anterior descending branch of the left coronary artery (Végh et al., 1994) was still observed in the presence of Z1046, and of Z1046 given after domperidone (Fig. 2).

Z1046 markedly reduced two important indices of myocardial ischaemia, epicardial ST-segment elevation (Fig. 3) and inhomogeneity of activation within the ischaemic area (Fig. 4). Z1046, when given intravenously, also profoundly reduced the severity of the ventricular arrhythmias (Fig. 5). There were only 40 ± 23 ventricular premature beats during the occlusion period (compared to 439 ± 72 in the controls; $P < 0.01$) and very few episodes of ventricular tachycardia (considerably less than one episode per dog compared with 7.8 ± 2.4 episodes per dog in the controls; $P < 0.01$). There was also a lower incidence of ventricular fibrillation both during ischaemia (18% vs. 43%; $P < 0.05$) and also on reperfusion (56% vs. 86%). This resulted in a somewhat higher survival rate from the combined ischaemia–reperfusion insult (36% compared to only 7% in the controls). There was no significant difference in the occluded zone between the control and the

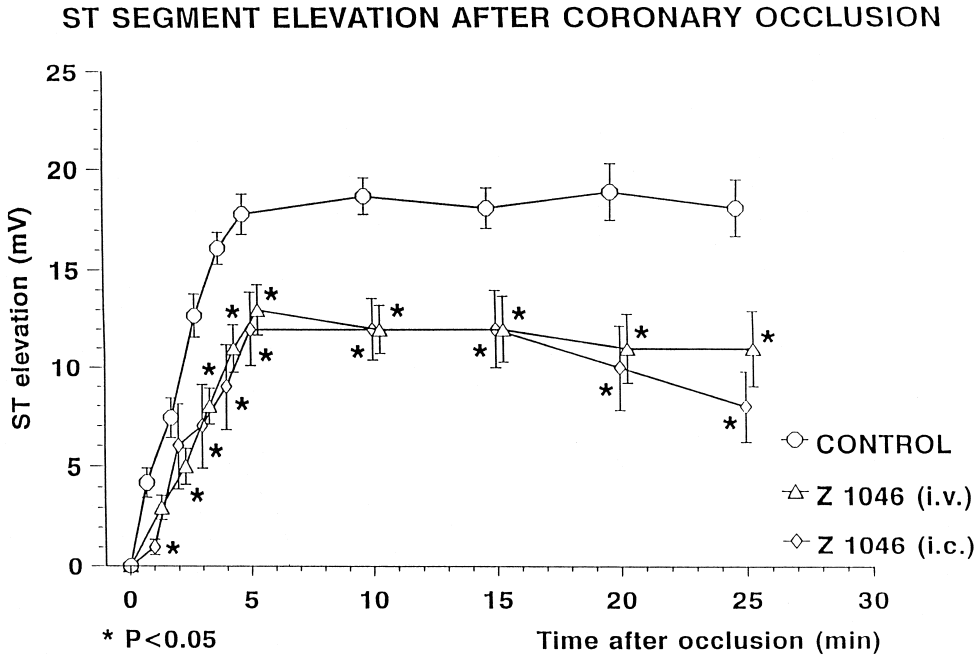

Fig. 3. Changes in ST-segment elevation (mV), recorded from epicardial electrodes, after coronary occlusion in control dogs (open circles) and in dogs pretreated with Z1046 10 $\mu g\ kg^{-1}$ i.v. (open triangles) or 1 $\mu g\ kg^{-1}$ by intracoronary administration (open diamonds). Z1046 markedly reduced the extent of ST-segment elevation following coronary artery occlusion. $* P < 0.05$ in comparison with value in untreated control dogs. Values are mean ± S.E.M. from 8–12 observations.

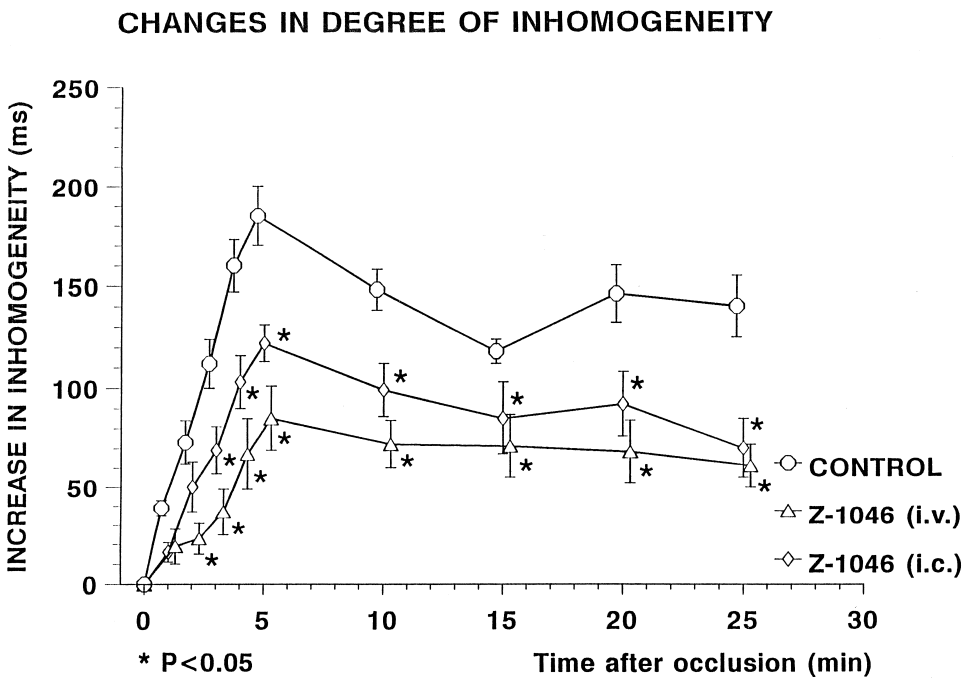

Fig. 4. Changes in the inhomogeneity of electrical activation (in ms) within the area supplied by the left anterior descending coronary artery when this artery is occluded (at time zero). The marked increase in inhomogeneity that occurs in control dogs is reduced by Z1046 either when administered intravenously (10 μg kg^{-1}; open triangles), or when given by local intracoronary injection (1 μg kg^{-1}; open diamonds). Values are means $\pm$ S.E.M. $* P < 0.05$ compared to values in untreated control dogs.

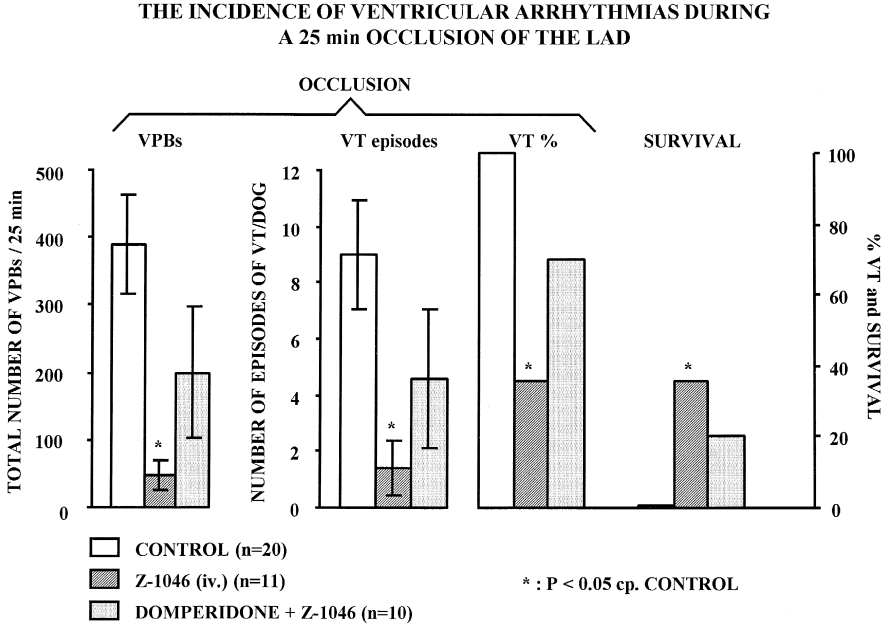

Fig. 5. The incidence of ventricular arrhythmias during a 25-min occlusion of the left anterior descending coronary artery in anaesthetised dogs. Shown are the number of ventricular premature beats (VPBs) the number of episodes and the incidence of ventricular tachycardia (VT) and survival from the combined ischaemia–reperfusion insult in control dogs (open histograms), in dogs given Z1046 intravenously (shaded histograms) and in dogs given domperidone prior to the administration of Z1046 (stippled histograms). Z1046 reduces arrhythmia severity and increases survival from the ischaemia–reperfusion insult and this antiarrhythmic effect is attenuated by domperidone signifying a predominant effect of Z1046 on dopamine (D_2) receptors.

Z1046 groups (41.7 ± 2.2% in the controls vs. 38.5 ± 2%). Z1046, when given by the intracoronary route, also reduced the number of ventricular premature beats (to 180 ± 70; $P < 0.01$ compared to controls) and the number of episodes of ventricular tachycardia (to 2.0 ± 1.0; $P < 0.01$) but failed to modify the incidence of ventricular fibrillation during occlusion (40%) or to increase survival (10%).

3.3. Modification of the anti-ischaemic effects of Z1046 by domperidone

As outlined above, the haemodynamic effects of Z1046 were completely prevented by the prior administration of domperidone in a dose of 40 μg kg^{-1} (Fig. 1). The effects of Z1046 on the responses to coronary artery occlusion were also, in the main, markedly attenuated by domperidone. This was certainly true of changes in the degree of inhomogeneity of activation within the ischaemic area (Fig. 6) although, somewhat surprisingly, the reduction in epicardial ST-segment elevation during ischaemia by Z1046 was quite unaffected by the prior administration of domperidone (Fig. 6). The number of ventricular premature beats and the incidence and number of episodes of ventricular tachycardia during occlusion were also greater in those dogs given domperidone (Fig. 5) and the survival from the combined ischaemia–reperfusion insult was also less in these dogs. The marked suppression of phase 1a arrhythmias which resulted from Z1046 administration was also reversed by domperidone although there was no significant effect on phase 1B arrhythmias (Fig. 7).

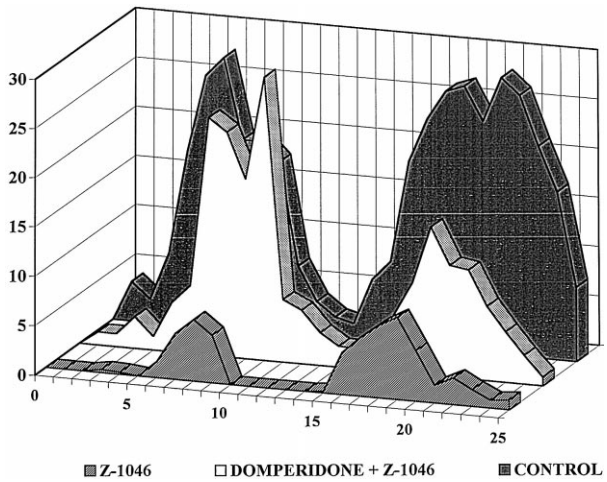

Fig. 7. The distribution of ventricular premature beats (at 1 min intervals) following occlusion of the left anterior descending coronary artery in control dogs (filled areas), in dogs administered Z1046 (shaded areas) and in dogs given domperidone prior to a 25 min occlusion of the administration of intravenous Z1046 (clear areas). There are two distinct phases of arrhythmia severity, an early phase (1a) peaking around 7 min and a second phase (1b) peaking around 18–22 min. Both phases of arrhythmia severity are markedly reduced by Z1046 and these are attenuated, particularly during phase 1a, by the prior administration of domperidone.

3.4. Evidence for impairment of sympathetic transmission by Z1046 and its antagonism by domperidone

The effects of sympathetic nerve stimulation on perfusion pressure in the rat isolated perfused mesenteric bed are illustrated in Fig. 8. There was a frequency-dependent

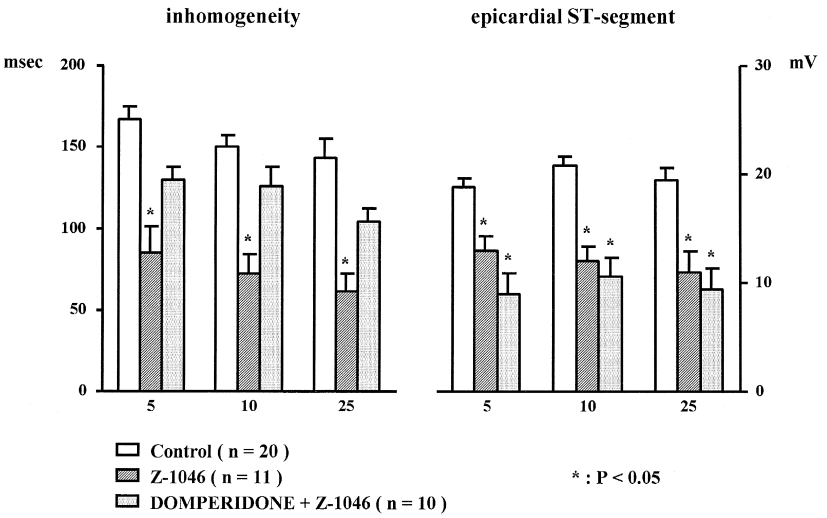

Fig. 6. Changes in the inhomogeneity of electrical activation within the ischaemic region (ms) and in epicardial ST-segment elevation (mV) at various times (5, 10, 25 min) after coronary artery occlusion in anaesthetised dogs. Changes in inhomogeneity and ST-segment elevation are marked in control dogs following coronary occlusion (open histograms) and these changes are markedly reduced by Z1046 (shaded histograms). The effects on inhomogeneity of activation, but not effects of Z1046 on ST-segment elevation, are attenuated by the prior administration of domperidone (stippled histograms). Values given are mean ± S.E.M. of from 10 to 20 observations. * $P < 0.05$.

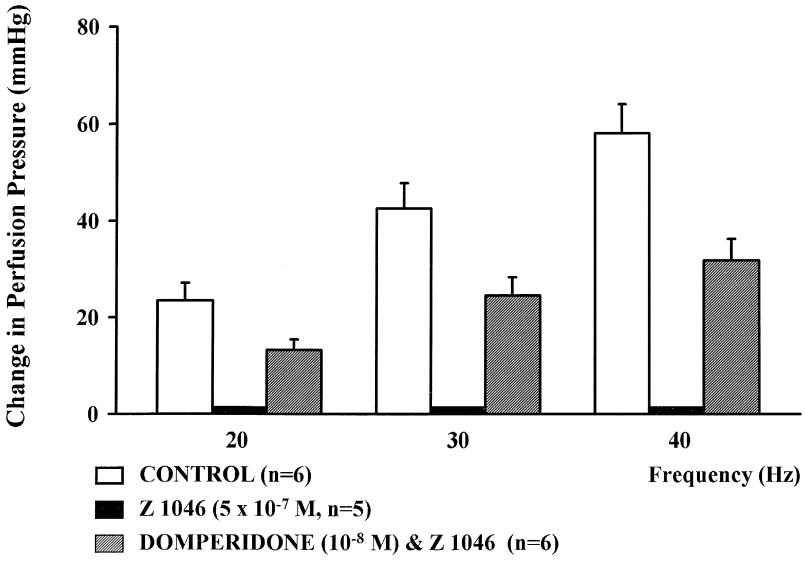

Fig. 8. Changes in perfusion pressure in the mesenteric vascular bed of rats perfused at constant flow (2 ml min^{-1}) subjected to sympathetic nerve stimulation. The frequency-dependent increase in perfusion pressure is prevented by Z1046 in a concentration of 5×10^{-7} M. The results are the means, $\pm$S.E.M. of five preparations (with Z1046) and 13 preparations (controls). Also shown is the partial reversal of the inhibition of sympathetic transmission by domperidone (1×10^{-8} M; $n = 6$).

increase in perfusion pressure from the basal perfusion pressure of 24 ± 2.4 mmHg. Z1046 had no effect on these pressor responses in concentrations of 5×10^{-9} and 5×10^{-8} M but in concentrations of 2×10^{-7} M the response was inhibited in three out of five experiments with a maximum inhibition 31 ± 6 min after Z1046 administration. In two of these experiments, the response was still significantly reduced at 1 h despite repeated washing. At a concentration of 5×10^{-7} M, the response to sympathetic nerve stimulation was completely abolished within 10–15 min and was also completely abolished within 5 min at a concentration of 2×10^{-6} M. This inhibition of sympathetic responses by Z1046 was difficult to reverse by repeated washing, e.g., at the highest concentration the response was only 10–15% of that in the controls (at a stimulation frequency of 40 Hz) despite repeated washing of tissues for up to 1 h. At a concentration of 5×10^{-7} M, the increase in perfusion pressure resulting from noradrenaline administration was unaffected (Fig. 9).

The effect of Z1046 on sympathetic nerve stimulation was partially reversed by domperidone 10^{-8} M (Fig. 8), which by itself had no effect on basal perfusion pressure (22 ± 2 mmHg). We did not attempt to determine whether higher concentrations of domperidone would completely reverse the effect of Z1046.

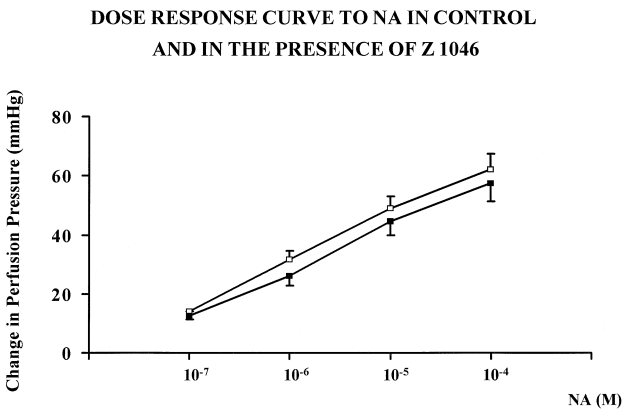

Fig. 9. Noradrenaline (NA) dose–response curves for changes in perfusion pressure in isolated perfused mesenteric vascular beds from rats in the absence (open squares) and presence (closed squares) of Z1046 in a concentration of 5×10^{-7} M. The results are the means $\pm$ S.E.M. of six to eight preparations.

4. Discussion

What stimulated the present experiments was our interest over the years in the marked antiarrhythmic effect of ischaemic preconditioning (Végh et al., 1992a and reviewed by Parratt and Vegh, 1994). The mechanisms of this marked protection are unclear but the possibilities include a reduction in the rate of utilisation of high energy phosphates, myocardial stunning, enhanced potassium uptake, the opening of ATP-dependent K^+ channels, mast cell stabilisation and inhibition of cardiac responsiveness to sympathetic neurotransmitters. The evidence for these, which is not entirely convincing, has been recently reviewed (Parratt, 1994). One unifying hypothesis that would explain both the marked reduction in infarct size, which

also results from ischaemic preconditioning, and the profound antiarrhythmic effect is that preconditioning might reduce the release of noradrenaline from cardiac sympathetic nerves which normally occurs following myocardial ischaemia. One of the consequences of noradrenaline release would be the generation of ventricular arrhythmias and an increase in myocardial oxygen demands at a time when myocardial perfusion is impaired. It was this possibility that prompted the present study since one might expect that if, through stimulation of dopamine D_2 receptors, cardiac noradrenaline release was impaired then there would be a reduction in myocardial oxygen demand resulting from the reduction in afterload and heart rate as a consequence of Z1046 administration. Although, in this particular study, we did not assess myocardial contractility by, for example, pressure/volume loop analysis, calculations of $LVdP/dt/P$ (a relatively crude index of myocardial contractility) showed that it was not altered by Z1046. It is possible that myocardial contractility was maintained, despite a reduction in sympathetic drive, by the increase in left ventricular end-diastolic pressure (Table 1). In a recent study in conscious pigs, Duncker et al. (1997) showed that, in a similar dose, Z1046 also reduced $LVdP/dt$ in parallel with the reduction in afterload. This is not to imply that Z1046 has no direct effect on cardiac muscle, although we know of no studies showing the presence of these particular dopamine receptors on canine cardiomyocytes. Certainly, in the present study the local administration of Z1046 also resulted in a slight decrease in $LVdP/dt$, and in coronary blood flow although, as with intravenous administration, these appeared to parallel the decrease in arterial blood pressure.

In the present study we provide evidence that the effects of Z1046 are mediated through dopamine D_2 receptors, presumably at the sympathetic nerve terminal. Certainly, the haemodynamic effects of Z1046 are prevented by domperidone. Although we did not measure noradrenaline release in this study, evidence for the impairment of sympathetic drive by Z1046 comes from the reductions in arterial blood pressure, heart rate and $LVdP/dt$, as well as the reduced severity of ischaemia. This, despite a rather larger occluded risk zone, is suggested by the less pronounced effect of coronary artery occlusion on epicardial ST-segment elevation and on the degree of inhomogeneity of activation in those dogs given the dopamine D_2 receptor agonist Z1046 (Figs. 3 and 4). More direct evidence that Z1046 results in reduced sympathetic transmission comes from the studies in the perfused mesenteric bed. Z1046 reduced responses to nerve stimulation in a dose-dependent fashion yet, as also demonstrated in the study by Duncker et al. (1997), without modifying the postsynaptic α-adrenoceptor mediated effects of noradrenaline itself. If this also occurred in vivo then the neuronal noradrenaline release which occurs under conditions of coronary artery occlusion, and which contributes to arrhythmia severity early in ischaemia (for references see Section 1), would be reduced after the administration of Z1046. This would be a reasonable explanation for the marked antiarrhythmic effect observed during ischaemia in the presence of the drug.

There are, of course, many other factors that modify arrhythmia severity in ischaemia (see Section 1). These include haemodynamic alterations (e.g., in blood pressure and heart rate) and in coronary blood flow. A marked difference in coronary blood flow between control dogs and dogs administered Z1046 is however unlikely. Measurements of peripheral (retrograde) coronary pressure (as a reasonable functional index of collateral flow; Schaper, 1979) during occlusion in a similar group of mongrel dogs to those used in the present study showed that resting coronary collateral flow in this canine population is around 20% and was unchanged during a 25 min coronary artery occlusion. Further, the difference in peripheral coronary pressure between individual dogs was small in this particular series (mean peripheral coronary pressure 20 ± 3 mmHg, Vegh et al., 1997c). A significant change in coronary collateral flow after Z1046 is unlikely since resting coronary blood flow was reduced rather than increased by the drug (Fig. 2 and Duncker et al., 1997).

There was an interesting haemodynamic difference between the effects of the intravenous and local coronary administration of Z1046 on ischaemia and reperfusion-induced ventricular arrhythmias. The antiarrhythmic effect was marked when Z1046 was given intravenously but less pronounced following local, intracoronary administration. The only haemodynamic difference between intravenous and intracoronary administered Z1046 was that there was a marked reduction in heart rate following systemic administration, but hardly any change in heart rate when the dopamine agonist was given locally. Presumably, this reflects access to the sinus node. This result suggests that one of the major factors influencing the severity of arrhythmias following coronary artery occlusion is the change in heart rate. There is other evidence for this. For example, a reduction in heart rate reduces the incidence of sudden cardiac death in a conscious canine model of myocardial ischaemia (Vanoli and Schwartz, 1991) and ventricular fibrillation on reperfusion is less likely to occur at lower heart rates (Coker and Parratt, 1985; Tosaki et al., 1987). However, others (Verrier et al., 1974) have shown, in a similar model to that used in the present study, that although there is an increase in ventricular vulnerability during sympathetic stimulation, this in uninfluenced by either changes in blood pressure or heart rate.

The relationship between cardiac sympathetic activity and arrhythmogenesis in the early stages of myocardial ischaemia is well-documented (Corr et al., 1986; Schömig and Richardt, 1991) and the evidence is generally strong that inhibition of noradrenaline release leads to suppression of early, ischaemia-induced ventricular arrhythmias. This is the most likely explanation for the pronounced antiarrhythmic effects demonstrated in the present studies with the dopamine agonist Z1046.

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