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The effects of Z13752A, a combined ACE/NEP inhibitor, on responses to coronary artery occlusion; a primary protective role for bradykinin

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1 The effects on the responses to coronary artery occlusion of a combined ACE/NEP inhibitor (Z13752A) were examined in anaesthetized dogs.

2 A 1 h infusion of Z13752A (128 $\mu g\ kg^{-1}\ min^{-1}$ intravenously) decreased arterial blood pressure (by 11±3%; $P<0.05$) and increased coronary blood flow (by 12±4%, $P<0.05$). There were no other significant haemodynamic changes.

3 Z13752A inhibited both NEP and ACE enzymes both in dog plasma and in tissue (lung ACE; kidney NEP). Pressor responses to angiotensin I *in vivo* were inhibited and systemic vasodilator responses to bradykinin were potentiated.

4 When the left anterior descending coronary artery was occluded for 25 min, Z13752A markedly reduced the severity of the resultant ventricular arrhythmias. No ventricular fibrillation (VF) occurred (compared to 7/16 in the controls: $P<0.05$), and ventricular tachycardia (VT) was reduced (VT in 2/9 dogs treated with Z13752A cp. 16/16 of controls; episodes of VT 0.2±0.1 c.p. 10.7±3.3; $P<0.05$).

5 Reperfusion of the ischaemic myocardium led to VF in all control dogs but occurred less frequently in dogs given Z13752A (survival from the combined ischaemia-reperfusion insult 67% c.p. 0% in controls: $P<0.05$).

6 Z13752A reduced two other indices of ischaemia severity; epicardial ST-segment elevation and inhomogeneity of electrical activation.

7 These protective effects of Z13752A during ischaemia and reperfusion were abolished by the administration of icatibant (0.3 $mg\ kg^{-1}$, i.v.) a selective antagonist of bradykinin at B_2 receptors: the ischaemic changes in dogs given both icatibant and Z13752A were similar to those in the controls.

8 We conclude that this ACE/NEP inhibitor is effective at reducing the consequences of coronary artery occlusion in this canine model and that this protection is primarily due to potentiation of released bradykinin.

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Keywords: Myocardial ischaemia; ventricular arrhythmias; Z13752A; bradykinin; combined ACE/NEP inhibition; anaesthetized dogs

Abbreviations: A1, angiotensin I; A2, Angiotensin II; ACE, angiotensin converting enzyme; B_2 Bradykinin 2 receptor; DABP, diastolic arterial blood pressure; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; LV left ventricle; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; MABP, mean arterial blood pressure; NEP, neutral endopeptidase; SABP, systolic arterial blood pressure; VBP, ventricular premature beats; VF, ventricular fibrillation; VT, ventricular tachycardia

Introduction

The cardioprotective effects of ACE inhibition in myocardial ischaemia are well documented in both experimental animal models (Parratt, 1994; Linz *et al.*, 1995; Liu *et al.*, 1996) and in clinical situations (e.g. Lonn *et al.*, 1994; Ikram, 1996), and a clear role for bradykinin in mediating these protective effects has been demonstrated based mainly on studies in which its effects on bradykinin (B_2) receptors were antagonized (Schölkens *et al.*, 1989; Linz *et al.*, 1990; Martorana *et al.*, 1990; 1991; Ehring *et al.*, 1994; Liu *et al.*, 1996; Shimada & Avkiran, 1996).

There have been relatively few studies with inhibitors of the other major enzyme responsible for kinin degradation, neutral endopeptidase 24.11 (NEP; Erdös & Skidgel, 1989). This is a membrane bound metallopeptidase present in endothelial cells (Graf *et al.*, 1995) and in (rat) cardiomyocytes (Piedimonte *et al.*, 1994) which has a high affinity for a variety of vasoactive peptides including substance P, bradykinin, atrial natriuretic peptide and endothelin (for references see Graf *et al.*, 1995). In the heart this is a particularly important enzyme responsible for kinin degradation (Piedimonte *et al.*, 1994; Yang *et al.*, 1997; Kokkonen *et al.*, 1999) but there are few studies dealing with the effects of inhibition of this enzyme, especially under conditions of ischaemia. In rat hearts inhibition of neutral endopeptidase increases myocardial blood flow (Maxwell *et al.*, 1995), reduces infarct size (Yang *et al.*, 1997) and prevents isoprenaline-induced myocardial hypoperfusion (Piedimonte *et al.*, 1994), effects mediated primarily through inhibition of kinin breakdown.

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The paucity of studies involving NEP inhibition, especially in large animal models, is surprising in view of the considerable evidence for the cardioprotective effects of both administered and endogenously produced kinins. Kinins reduce ischaemia-induced cell necrosis (Daniell *et al.*, 1984; Noda *et al.*, 1993; Richard *et al.*, 1993), enhance recovery of contractile function after a period of ischaemia and reperfusion (Grocott-Mason *et al.*, 1993; Ehring *et al.*, 1994) and have a particularly pronounced effect in reducing the severity of ischaemia and reperfusion-induced ventricular arrhythmias (Tobe *et al.*, 1991; Végh *et al.*, 1991a). Further there are studies suggesting a role for bradykinin release in the protection of the heart afforded by ischaemic preconditioning (Végh *et al.*, 1994; Wall *et al.*, 1994; Goto *et al.*, 1995; Parratt *et al.*, 1995; 1997; Bouchard *et al.*, 1998) and by cardiac pacing (Kaszala *et al.*, 1997).

In the present study we have examined the effects of a combined ACE/NEP inhibitor Z13752A (N-[(2S)-3-mercapto-2-phenylmethylpropionyl]-4-(2-thiazolyl)-L-phenylalanine; Pradella *et al.*, 1998; Morazzoni *et al.*, 1998a) on the responses of anaesthetized dogs to acute coronary artery occlusion, with particular reference to ischaemia and reperfusion-induced ventricular arrhythmias. Z13752A is a newly developed ACE/NEP inhibitor with an IC_{50} of 3.2 nM on ACE and of 1.8 nM on NEP (Morazzoni *et al.*, 1998a). Z13752A has been found to potently inhibit plasma and tissue ACE, as well as tissue NEP activity in various *in vitro* and *in vivo* experiments. Z13752A resulted in a long lasting antihypertensive effect in both SHR and DOCA-salt hypertensive rats after intravenous or oral administration (Morazzoni *et al.*, 1998b; Pradella *et al.*, 1998). A preliminary account of these results was presented at the Fourth European Congress of Pharmaceutical Sciences in Milan (Morazzoni *et al.*, 1998a, b; Pradella *et al.*, 1998) and at the World Congress of the International Society for Heart Research (Rastegar *et al.*, 1998).

Methods

Evaluation of the effects of Z13752A on plasma and tissue ACE and NEP activities

These experiments were performed at the Zambon Group in Milan, Italy, using beagle dogs of either sex and weighing between 8–12 kg, anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.) Following experimental preparation for the measurement of arterial blood pressure and heart rate and also collecting blood, Z13752A was infused for 3 h at a flow rate of 0.5 ml min^{-1} in six dogs in a dose of 0.3 mol kg^{-1} min^{-1} (i.e. 128 $\mu g\ kg^{-1}\ min^{-1}$) and in eight dogs in a dose of 1.0 $\mu mol\ kg^{-1}\ min^{-1}$ (426 $\mu g\ kg^{-1}\ min^{-1}$). In a further six and eight dogs respectively, captopril 0.3 $\mu mol\ kg^{-1}\ min^{-1}$ (65 $\mu g\ kg^{-1}\ min^{-1}$) or vehicle (saline plus NaOH 0.1 N) was infused for 3 h at a flow rate of 0.5 ml min^{-1}. The haemodynamic effects were measured at various times over the 3 h observation period and blood samples (3 ml) for the determination of plasma ACE were collected every 30 min. At the end of the experiments, and before sacrificing the animals, the left kidney was removed for the measurement of tissue NEP activity and an apical portion of the lung for the determination of tissue ACE activity. These tissues were immediately frozen in liquid nitrogen. ACE activity, both in the plasma and in the lung homogenates in the presence of 300 mM NaCl and using [H^3]-hippuryl-glycyl-glycine as the substrate, was determined by a radiochemical method according to Ryan and colleagues (1977). NEP activity was determined in kidney homogenates at pH 7.6 containing 0.1% Triton X-100 by using spectrophotometric kinetic determination in which glutaryl-ala-ala-phe-2naphtylamide was the reaction substrate (Orlowsky & Wilk, 1981).

In vivo *studies in anaesthetized dogs*

Mongrel dogs with a body weight in excess of 17 kg (see Végh *et al.*, 1991a) were anaesthetized with a mixture of chloralose and urethane (60 and 200 mg kg^{-1}, i.v. respectively), and ventilated with room air using a Harvard Respirator at a rate and volume sufficient to maintain arterial blood gases and pH within normal limits (Végh *et al.*, 1992). The animals were thoracotomized at the fifth intercostal space and the anterior descending branch of the left coronary artery (LAD) prepared for occlusion just proximal to the first main diagonal branch. This gives an area at risk, as assessed by infusing blue V dye into the occluded artery at the end of the experiment, of around 35–42% (see Results).

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

All these parameters, together with a limb lead electrocardiogram, systemic arterial and left ventricular (LV) systolic (S) and end-diastolic (ED) pressures (Statham P23XL transducers) and LVdP/dt were recorded on an eight channel Medicor R81 recorder.

Ventricular arrhythmias during a 25 min coronary artery occlusion (i.e. ischaemia) were assessed and analysed as outlined previously (Végh *et al.*, 1992) i.e. total ventricular premature beats (VPBs), the incidence and number of episodes of ventricular tachycardia (VT) and the incidence of ventricular fibrillation (VF). At the end of the period of ischaemia the area supplied by the occluded vessel was rapidly reperfused. The only reperfusion arrhythmia that was determined was VF. Survival (from the combined ischaemia-reperfusion insult) was defined in terms of those dogs which were predominantly in sinus rhythm 10 min after the commencement of reperfusion.

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

Experimental protocol

There were four groups of animals. Nine dogs were infused with Z13752A, in a dose of 128 $\mu g\ kg^{-1}\ min^{-1}$ intravenously over a 1 h period. At the end of this infusion time the left

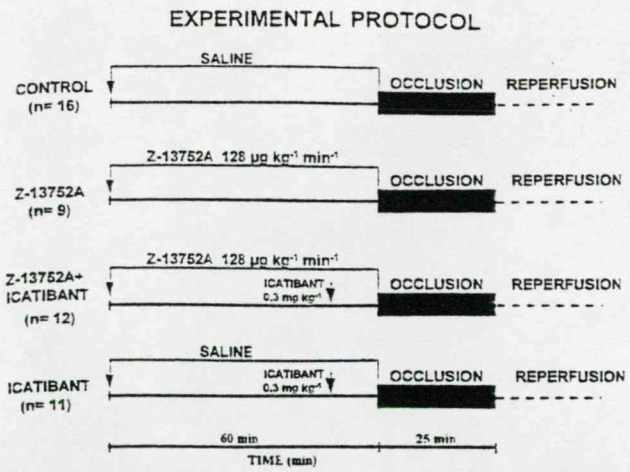

Figure 1 Experimental protocol for the studies involving Z13752A, and its modification by icatibant, an antagonist of bradykinin at B2 receptors. The duration of the Z13752A infusion was 1 h, the occlusion time was 25 min and icatibant was given 10 min prior to occlusion.

anterior descending coronary artery was occluded for 25 min, and the artery was then re-opened rapidly to allow reperfusion. A second group of 11 animals was given icatibant, an antagonist of bradykinin at B_2 receptors, in a dose of 0.3 mg kg^{-1} as an i.v. bolus, 10 min prior to coronary artery occlusion. This dose of icatibant was sufficient to abolish the protection against ventricular arrhythmias afforded by ischaemic preconditioning (Végh *et al*., 1994). A third group of 12 additional dogs were also infused with Z13752A, in the dose given above, and after 50 min (i.e. 10 min prior to coronary artery occlusion) were also given icatibant. The responses were compared with those of 16 control dogs which were infused with a similar volume (60 ml) of the vehicle for 1 h and then subjected to coronary artery occlusion followed by reperfusion. The protocol for these four groups is illustrated in Figure 1.

In order to determine the effect of Z13752A on the blood pressure responses to angiotensin I (A1), angiotensin II (A2) and bradykinin, separate groups of dogs were given either A1 and A2 (in doses of 5, 10, 15 and 20 ng kg^{-1} intravenously, $n=11$) or bradykinin (in doses of 0.1, 0.25,

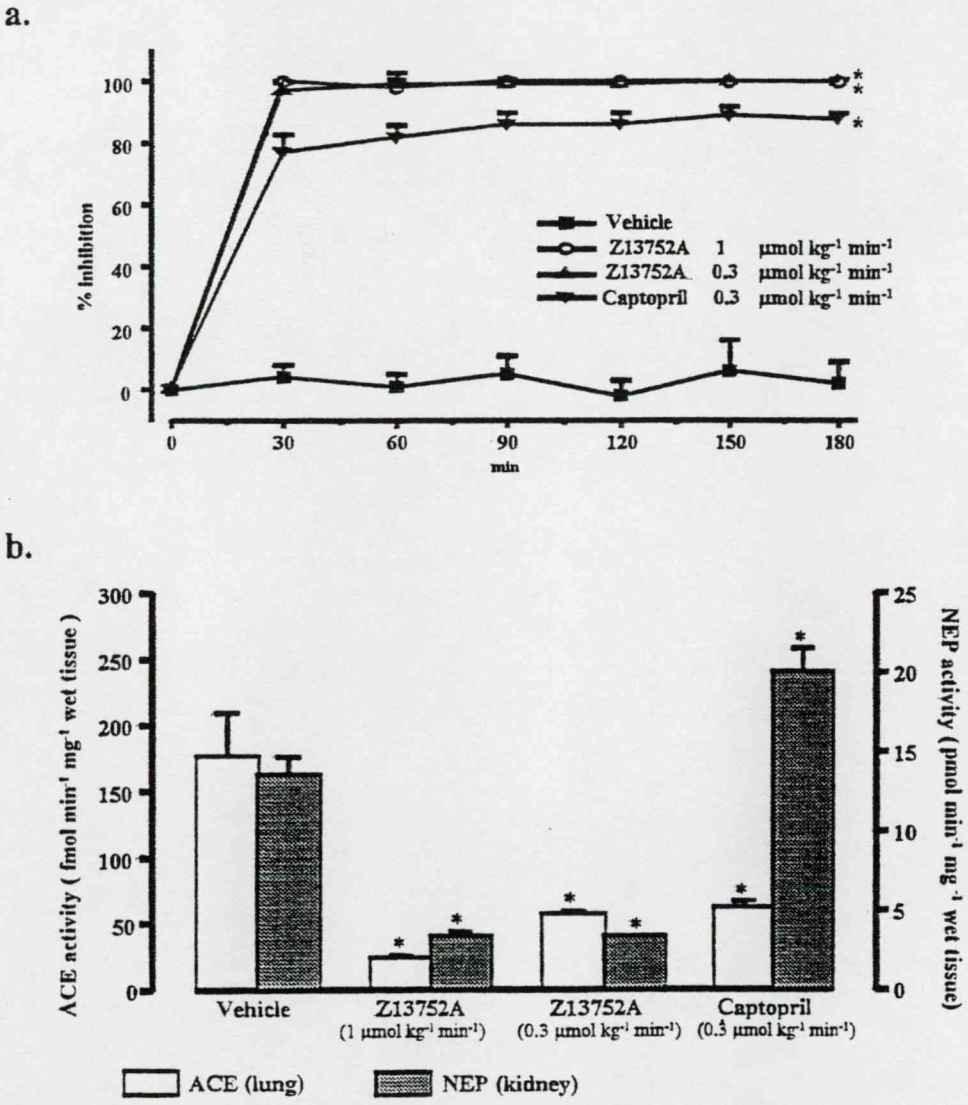

Figure 2 (a) Plasma ACE activity in anaesthetized dogs given intravenous infusion of vehicle (saline + NaOH 1N: $n=6$), Z13752A in doses of 0.3 µmol kg^{-1} ml^{-1} (128 µg kg^{-1} ml^{-1}: $n=6$) and 1.0 µmol kg^{-1} ml^{-1} (426 µg kg^{-1} ml^{-1}: $n=6$) and of captopril in a dose of 0.3 µmol kg^{-1} ml^{-1} (65 µg kg^{-1} ml^{-1}: $n=6$) over a period of 3 h. Values are means ± s.e.mean: $^*P<0.05$ compared to the vehicle controls. (b) *Ex vivo* determination of tissue ACE and NEP activities, measured in the lung and in the kidney, respectively, following intravenous infusion of vehicle (saline − NaOH 1N: $n=8$), Z13752A in doses of 0.3 µmol kg^{-1} ml^{-1} (128 µg kg^{-1} ml^{-1}; $n=6$) and 1.0 µmol kg^{-1} ml^{-1} (426 µg kg^{-1} ml^{-1}: $n=8$) and of captopril in a dose of 0.3 µmol kg^{-1} ml^{-1} (65 µg kg^{-1} ml^{-1}; $n=6$) over a period of 3 h. Values are means ± s.e.mean: $^*P<0.05$ compared to the vehicle controls.

0.5 and 1 μg kg^{-1}; $n=4$) both before and at the end of the 1 h infusion period of Z13752A. These responses were compared to those in control dogs given either A1 and A2 ($n=6$) or bradykinin ($n=4$), in the doses outlined above, but in which Z13752A was replaced by infusion of the vehicle.

Statistical evaluation

All the data were analysed statistically as previously described (Végh *et al.*, 1992) i.e. data were expressed as means (±s.e.mean) and the differences between means were compared by analysis of variance (ANOVA for repeated measures) or the Student's *t*-test as appropriate. A one-way ANOVA was undertaken to determine whether or not there were significant haemodynamic differences between the groups. Ventricular premature beats were compared by using the Mann-Whitney Rank Sum test, and the incidence of arrhythmias was compared using the Fisher Exact test. Differences between groups were considered significant when $P<0.05$.

Results

The effect of Z13752A on plasma and lung ACE and on kidney NEP activities

The effects of two doses of Z13752A, in comparison with captopril and vehicle controls, were examined on plasma ACE activity (Figure 2a) and on tissue ACE and NEP activities (Figure 2b). Both doses of Z13752A completely inhibited plasma ACE activity within the first 30 min of the infusion and this inhibition was maintained throughout the entire infusion period (Figure 2a). Following a 3 h infusion of the 0.3 and 1.0 μmol kg^{-1} min^{-1} doses of Z13752A, both lung ACE and kidney NEP activities were markedly reduced (Figure 2b) whereas captopril (0.3 μmol kg^{-1} min^{-1}) inhibited ACE activity in the lung but was without effect on renal NEP activity (Figure 2b). Infusion of the vehicle did not influence the activity of either of these enzymes (Figure 2b).

Haemodynamic effects of Z13752A

At the end of a 1 h infusion of Z13752A (total dose 7.68 mg kg^{-1}) the only significant haemodynamic changes, immediately prior to coronary artery occlusion, were reductions in arterial blood pressure (systolic 129±4 to 117±3 mmHg; diastolic 80±3 to 71±4 mmHg; mean 97±3 to 86±3 mmHg; $P<0.05$) and in negative LVdP/dt$_{max}$ (3242±150 to 2879±252 mmHg s^{-1}; $P<0.05$) and a slight increase in coronary blood flow (and a reduction in coronary vascular resistance; Figure 3). There were no significant changes in LVEDP (5.6±0.6 to 5.3±0.3 mmHg), in heart rate (142±6 to 138±8 beats min^{-1}) or in positive LVdP/dt$_{max}$ (3666±151 to 3533±225 mmHg s^{-1}). In control dogs a 1 h infusion of the vehicle resulted in no significant haemodynamic changes.

Angiotensin and bradykinin responses before and after Z13752A

In a separate group of dogs the effects of intravenous bolus injections of angiotensin and bradykinin were examined prior to, and at the end of, an infusion of Z13752A in the doses outlined above. These responses were compared to those obtained from control dogs in which the Z13752A was replaced by the vehicle. The results are illustrated in Figures 4 and 5. The vasodepressor response to bradykinin was significantly augmented at all dose levels (Figure 4),

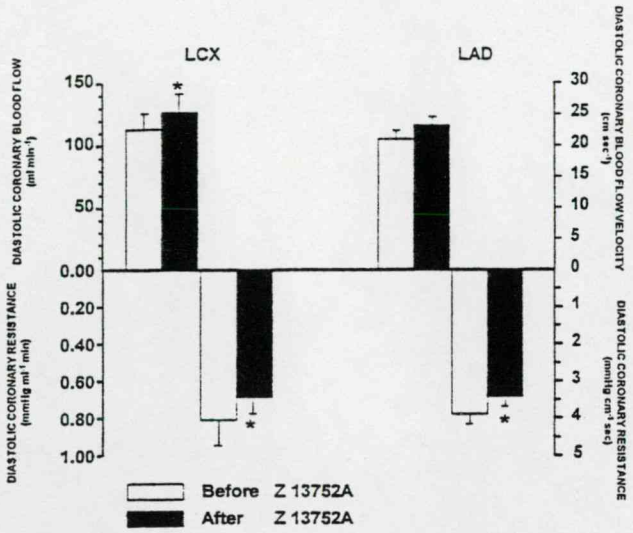

Figure 3 Changes in diastolic coronary blood flow and resistance induced at the end of a 1 h infusion of Z13752A in a dose of 128 μg kg^{-1} min^{-1}. There is an increase in blood flow in both the circumflex (LCX) and anterior descending (LAD) branches of the left coronary artery and a decrease in coronary vascular resistance. Values are means±s.e.mean: $^*P<0.05$ compared to the identical values before giving Z13752A.

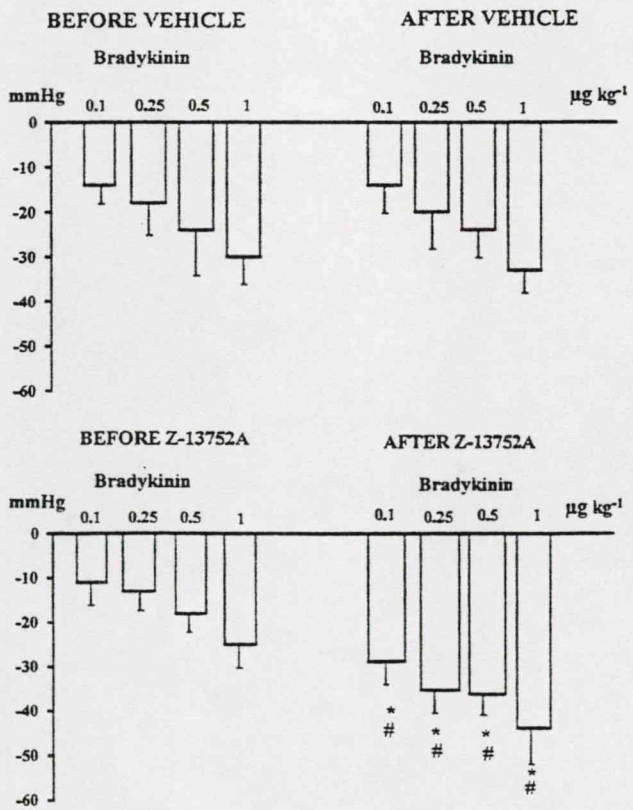

Figure 4 Changes in blood pressure induced by bolus injections of bradykinin in control, vehicle treated dogs (upper panels) and in dogs before and after the administration of Z13752A (lower panels). Values are means±s.e.mean: $^*P<0.05$ compared to the values before giving Z13752A. $^\#P<0.05$ compared to the values of the vehicle-treated controls.

particularly so with the lowest doses (100 and 250 ng kg^{-1}). In contrast, angiotensin I responses were significantly reduced and those to angiotensin II slightly potentiated (Figure 5).

Modification of the haemodynamic effects of Z13752A by icatibant

Icatibant was given, in a dose of 0.3 mg kg^{-1} i.v. in two series of experiments (see protocol Figure 1). The only significant haemodynamic effects of icatibant were a slight (4 ± 1 mmHg) increase in mean arterial pressure (95 ± 3 to 99 ± 3 mmHg) and a reduction in heart rate (of 4 ± 1 beats min^{-1} from 153 ± 7 beats min^{-1}). When given to dogs infused for 1 h with Z13752A there was a small, but significant ($P<0.05$), increase in mean arterial blood pressure (of 5 ± 1 mmHg) and a decrease in $LVdP/dt_{max}$ (positive of 336 ± 130 mmHg s^{-1}; negative of 80 ± 150 mmHg s^{-1}) and in heart rate (of 5 ± 2 beats min^{-1}). These icatibant-induced changes were somewhat more pronounced in dogs given Z13752A than in dogs not infused with this drug.

Haemodynamic changes induced by coronary artery occlusion in control dogs, and in dogs given icatibant, Z13752A or Z13752A together with icatibant

The results are shown in Table 1. In all dogs coronary artery occlusion resulted in decreases in arterial blood pressure and $LVdP/dt_{max}$. The marked increase in LVEDP was significantly ($P<0.05$) less marked in dogs given Z13752A (from 5.3 ± 0.3 to 13.7 ± 1.5 mmHg) than in either the controls (increase from 6.0 ± 0.3 to 18.4 ± 0.6 mmHg) or in dogs given icatibant, either alone (from 4.0 ± 0.4 to 20.1 ± 0.8 mmHg) or in the presence of Z13752A (from 5.3 ± 0.7 to 18.4 ± 1.1 mmHg). The less pronounced increase in LV filling pressure (and the less marked reduction in negative $LVdP/dt_{max}$) following occlusion in dogs given Z13752A was not apparent in dogs infused with the drug and then given icatibant (Table 1).

Occlusion of the anterior descending coronary artery led to an immediate and sustained increase in blood flow (maximal at 2 min) in the other major (circumflex) branch of the left coronary artery. This compensatory blood flow increase was unaffected by Z13752A, whether or not icatibant had been administered (Table 1).

Effects of Z13752A, of icatibant and of a combination of Z13752A and icatibant on ventricular arrhythmias following coronary artery occlusion and reperfusion

In this canine model occlusion of the left anterior descending coronary artery leads to pronounced ventricular ectopic

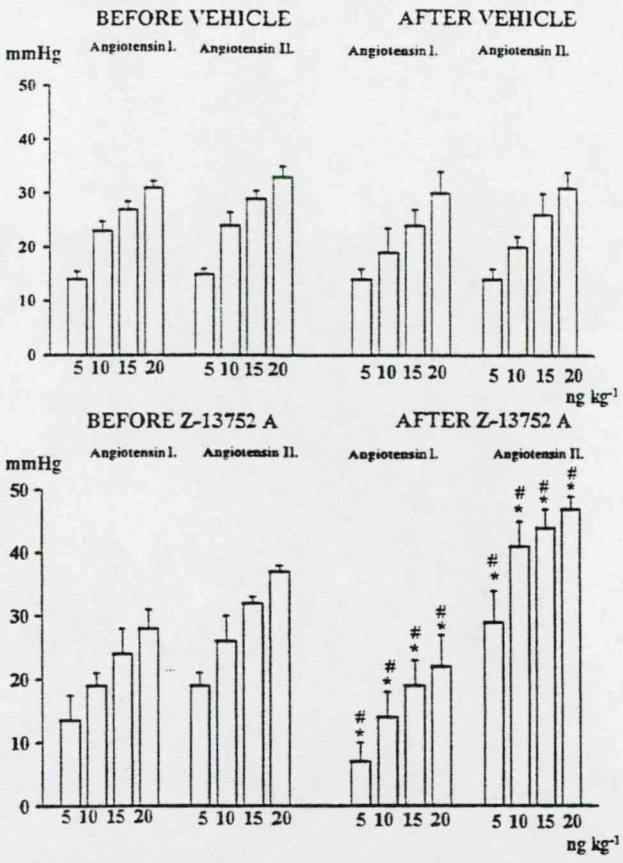

Figure 5 Changes in blood pressure induced by bolus injections of angiotensin I and angiotensin II in vehicle-treated control dogs (upper panels) and in dogs before and after the administration of Z13752A (lower panels). Values are means±s.e.mean; $*P<0.05$ compared to the values before giving Z13752A. $\#P<0.05$ compared to the values of the vehicle-treated controls.

Table 1 Effects of occlusion of the LAD in dogs, pretreated with either saline (controls), the ACE/NEP inhibitor Z13752A (128 μg kg^{-1} min^{-1} i.v.), the antagonist of bradykinin at B2 receptors (icatibant 0.3 mg kg^{-1}) or combination of both drugs. Values are means±s.e.mean of the maximum change (3–5 min) after occlusion

	Control (n = *16*)		*Z13752a* (n = *9*)		*Icatibant* (n = *11*)		*Z13752A + Icatibant* (n = *12*)	
	Initial value	*Change*	*Initial value*	*Change*	*Initial value*	*Change*	*Initial value*	*Change*
Arterial blood pressure								
systolic (mmHg)	125±5	−14±2*	117±3	−13±3*	114±4	−20±5*	131±4	−15±2*
diastolic (mmHg)	90±4	−13±1*	71±3	−7±2*#	81±4	−19±4*	92±3	−13±2*
mean (mmHg)	102±4	−13±3*	86±3	−9±2*	92±4	−19±1*	105±3	−14±2*
LVSP (mmHg)	128±7	−16±3*	123±5	−13±4*	108±4	−20±4*	117±3	−15±2*
LVEDP (mmHg)	6.0±0.3	12.7±0.6*	5.3±0.3	8.3±1.5*#	4.0±0.4	16.0±1.2*#	5.3±0.7	14.1±0.9*
LVdP/dtmax:								
(+ve:mmHg s^{-1})	2622±216	−644±99*	3666±252	−635±264*	3315±274	−624±139*	3275±213	−1134±160*
(−ve:mmHg s^{-1})	2914±242	−641±116*	2879±252	−165±131#	2748±278	−551±106	3126±320	−487±133*
Heart rate (beats min^{-1})	155±4	1±1	138±8	3±2	149±7	5±2	157±6	10±3*
Coronary (LCX) diastolic blood flow (ml min^{-1})	82±8	14±3*	123±18	21±3*	82±4	35±8*#	99±9	40±4*#
Coronary (LCX) diastolic resistance (mmHg ml^{-1} min^{-1})	1.13±0.12	−0.23±0.05*	0.70±0.12	−0.21±0.01*	1.04±0.03	0.30±0.66*	−0.98±0.08	−0.41±0.06*

$*P<0.05$ vs initial (value pre-occlusion): $\#P<0.05$ vs controls.

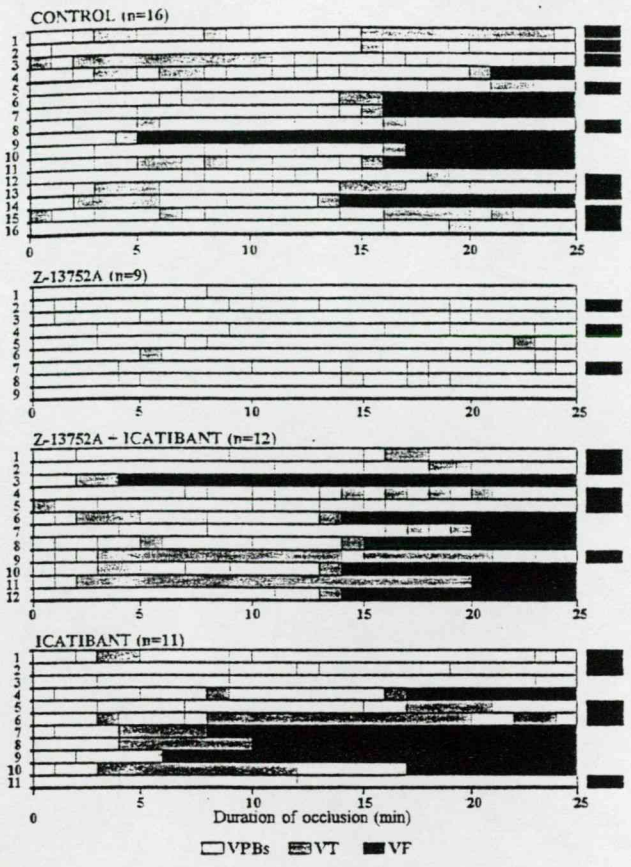

Figure 6 The distribution of ventricular arrhythmias in control dogs, and in dogs infused with Z13752A, with and without icatibant, during a 25 min coronary artery occlusion followed, at the end of this period, by reperfusion. The ACE/NEP inhibitor markedly reduced the severity of these arrhythmias and 6/9 dogs survived the combined ischaemia-reperfusion insult. This protective effect was reversed by icatibant suggesting a role, in the protection, for bradykinin.

activity. In the 16 control dogs in this study all had ventricular premature beats (with a mean of 353 ± 79 over the 25 min occlusion period) and all exhibited VT at some stages during the period of ischaemia. Seven of the dogs fibrillated, usually between 14 and 17 min of the commencement of the occlusion. The distribution of these arrhythmias is illustrated in Figure 6 and the results are summarized in Figure 7.

These ischaemia-induced arrhythmias were much less pronounced in dogs given Z13752A (Figures 6 and 7). Although all but one of these dogs had some ventricular ectopic activity (mean of 91 ± 41 premature beats: $P<0.05$ vs control), VT occurred in only two of these nine dogs ($P<0.05$ vs control) and in both these cases was extremely short-lived (Figure 6). There was no VF during occlusion ($P<0.05$ vs control).

This marked protection was not seen in dogs infused with Z13752A and then given icatibant (Figures 6 and 7). In these dogs there was again marked ventricular ectopic activity (number of VPBs: 541 ± 256; $P<0.05$ v Z13752A alone) and a high incidence of (100%) and large number of episodes of VT (32.2 ± 26.8; $P<0.05$ v Z13752A alone). Arrhythmia severity in these dogs during ischaemia was thus no different to that seen in the controls. Furthermore, seven out of these 12 dogs (58%; $P<0.05$ v Z13752A alone) fibrillated during the occlusion. Apart from an increase in the number of episodes of VT (to 22.5 ± 13.8), arrhythmia severity after icatibant alone was not significantly different to that in the controls (Figures 6 and 7).

VF occurred following reperfusion in all the control dogs that survived the ischaemic period (Figures 6 and 7) but was less in those dogs given Z13752A (3/9 vs 9/9, $P<0.05$; Figure 5). This protective effect of Z13752A was abolished by icatibant (reperfusion VF 5/5). Survival from the combined ischaemia-reperfusion insult was thus markedly increased by Z13752A compared to controls (67% vs 0%: $P<0.05$) and this increase in survival was abolished by icatibant (survival 0%, $P<0.05$ vs Z13752A alone).

Effect of Z13752A, of icatibant and of a combination of Z13752A and icatibant, on coronary artery occlusion-induced changes in epicardial ST-segment elevation and in the degree of inhomogeneity of electrical activation within the ischaemic area

In control dogs subjected to coronary artery occlusion, the ST-segment recorded from the epicardial electrocardiograms increased within 1 min of the onset of the ischaemia, peaked at 5 min (Figure 8a) and was sustained at this level throughout the 25 min occlusion period. There were similar changes in conduction delay as assessed by the degree of inhomogeneity of electrical activation within the area of ischaemia (Figure 8b). These changes were significantly less marked, and the onset slower, in dogs given Z13752A (Figure 8a,b). Icatibant reversed the protective effect of Z13752A on the degree of inhomogeneity, at least over the initial 15 min (Figure 8b) and, again initially, the ST-segment changes in this group of dogs were similar to that in the controls (Figure 8a).

Area at risk in dogs subjected to coronary artery occlusion

There was no significant difference between the four groups in the area at risk from necrosis (i.e. supplied by the occluded artery). These were $38.5 \pm 2.0\%$ in the controls, $41.4 \pm 2.6\%$ in the dogs infused with Z13752A, $40.8 \pm 2.5\%$ in the icatibant group and $45.7 \pm 0.8\%$ in the dogs given both Z13752A and icatibant. There were also no significant differences between groups in respect to the weight of the dogs, or in gender distribution. The body weights were 27.2 ± 1.4 kg in the controls, 28.8 ± 0.5 kg in the Z13752A group, 26 ± 1.3 kg in the icatibant alone group and 28.9 ± 1.1 kg in the dogs given both Z13752A and icatibant.

Discussion

The present studies demonstrate that the intravenous administration of the combined ACE/NEP inhibitor Z13752A markedly reduces the detrimental changes that result from coronary artery occlusion and reperfusion in a well documented canine model. Especially pronounced was the marked suppression in the severity of both ischaemia and reperfusion-induced arrhythmias; no dog given the drug fibrillated during the occlusion period and two-thirds of the dogs survived the combined ischaemia-reperfusion insult. This degree of protection against arrhythmias is similar to that previously shown in this model with ischaemic preconditioning (Végh *et al.*, 1990 and reviewed by Parratt & Végh, 1994; 1998), by cardiac pacing (Végh *et al.*, 1991b) and following the local intracoronary infusion of bradykinin (Végh *et al.*, 1991a).

There has been just one study, in Lewis inbred rats, that has examined the effects of an inhibitor of neutral endopeptidase

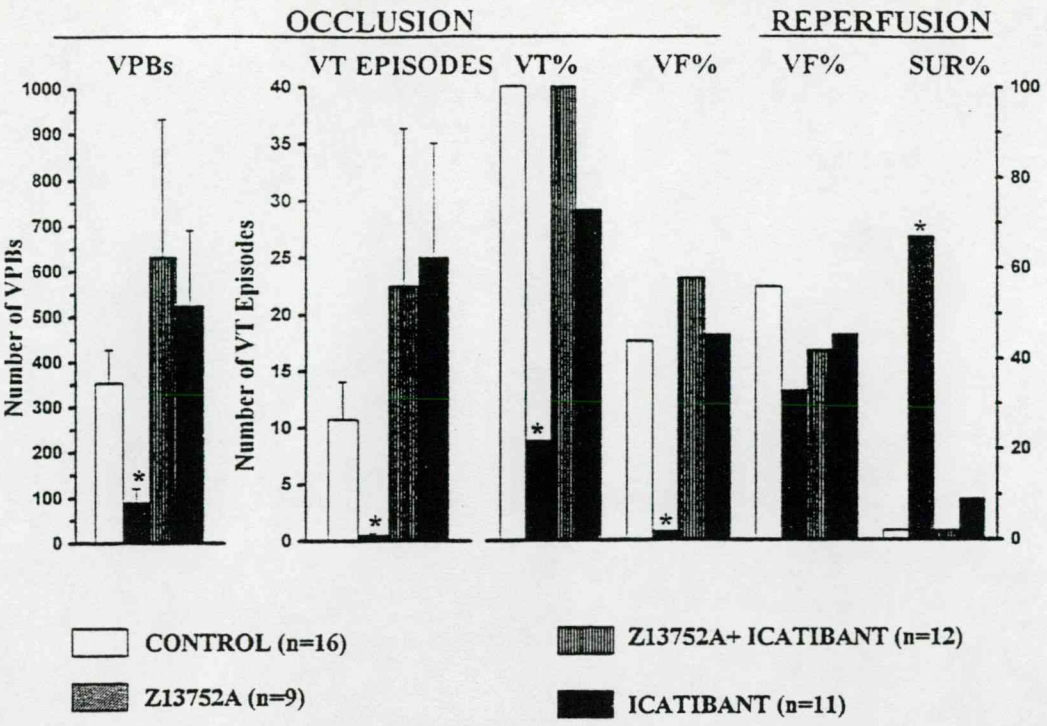

Figure 7 A summary of the effects of Z13752A, with or without icatibant, and of icatibant alone in comparison with control (saline infused) dogs, on ventricular arrhythmias resulting from coronary artery occlusion and subsequent reperfusion. VPBs = ventricular premature beats; VT = ventricular tachycardia; VF = ventricular fibrillation (during occlusion and reperfusion) and SUR = survival. The marked antiarrhythmic effect of Z13752A is abolished by icatibant. Values are means ± s.e.mean; $*P<0.05$ c.p. controls.

24.11 on myocardial reperfusion injury (Yang *et al.*, 1997). Using the Ciba-Geigy inhibitor CGS 24592 these authors showed a reduction in infarct size which was similar to that resulting from ramiprilat administration. This protection was abolished by icatibant but unaffected by the ANF receptor antagonist HS-142-1. Their conclusion was that the infarct size reduction following NEP inhibition was mediated by kinins. Yang and colleagues (1997) did not examine arrhythmia severity during coronary occlusion although they did attempt to examine whether reperfusion arrhythmias were modified by CGS 24592. However, the model used (30 min coronary artery occlusion and then reperfusion) is inappropriate to examine these arrhythmias since they are inconsequential after this particular period of ischaemia (Kane *et al.*, 1984). The fact that the ventricular premature complexes which did arise following reperfusion were reduced, albeit not significantly, by this NEP inhibitor is again suggestive of a role for kinins in cardioprotection. In contrast to the findings of Yang and colleagues (1997) and to our previous (Végh *et al.*, 1991a; 1994) and also to our present work, bradykinin has been found to facilitate, rather than alleviate reperfusion arrhythmias in guinea-pig and human myocardial ischaemia models (Hatta *et al.*, 1999). According to this study bradykinin released during myocardial ischaemia accumulates at sympathetic nerve endings and facilitates exocytotic and carrier mediated noradrenaline release which contribute to coronary vasoconstriction and to the generation of ventricular arrhythmias following reperfusion. This unfavourable effect of bradykinin was abolished by the bradykinin B_2 receptor antagonist, icatibant (Hatta *et al.*, 1999). However, icatibant was not able to inhibit noradrenaline release unless enalaprilate or a combined kininase I and kininase II inhibitor was present, indicating that under these conditions endogenous bradykinin levels at the nerve endings may not be high enough to facilitate ischaemic noradrenaline release. Although the explanation for this effect of bradykinin is still not known, most of the available evidence supports the idea that the potential beneficial (protective) effect of bradykinin depends on the site of the predominant bradykinin formation in the heart.

The cardioprotective effect of elevated levels of bradykinin resulting from inhibition of cardiac NEP activity has been recently demonstrated in isolated human cardiac membranes (Kokkonen *et al.*, 1999). In these preparations, in which there is a low enzymatic activity of ACE, bradykinin metabolism is mediated mostly by NEP. These results suggest that inhibition of cardiac NEP activity could be cardioprotective by elevating the local concentration of bradykinin in the heart.

As with the Yang and colleagues (1997) study, albeit in a quite different model of ischaemia-reperfusion injury, the most likely explanation for the protective effects of Z13752A in the present experiments is potentiation of the cardioprotective effects of bradykinin by inhibition of its breakdown. Although ACE inhibition presumably plays a role, since Z13752A inhibits both enzymes (Figure 3b), the evidence from the IC50 values (0.0032 μM against ACE; 0.0018 μM against NEP; Morazzoni *et al.*, 1998a) and from the present experiments showing a more marked potentiation of bradykinin vasodilator than of inhibition of angiotensin vasopressor responses in the presence of Z13752A (Figure 5), suggests a predominant effect on neutral endopeptidase 24.11. Indeed, responses to angiotensin II itself were potentiated by the drug (Figure 5), as in the human studies of Richards *et al.* (1992), an effect attributed by them to reduced angiotensin II clearance. The fact that the protection against arrhythmias was completely abolished by icatibant, a selective antagonist of bradykinin at B_2 receptors, and that this drug also abrogated the changes in ST-segment elevation and in the degree of inhomogeneity of electrical activation within the ischaemic area, both indices of ischaemia severity, again supports the view that

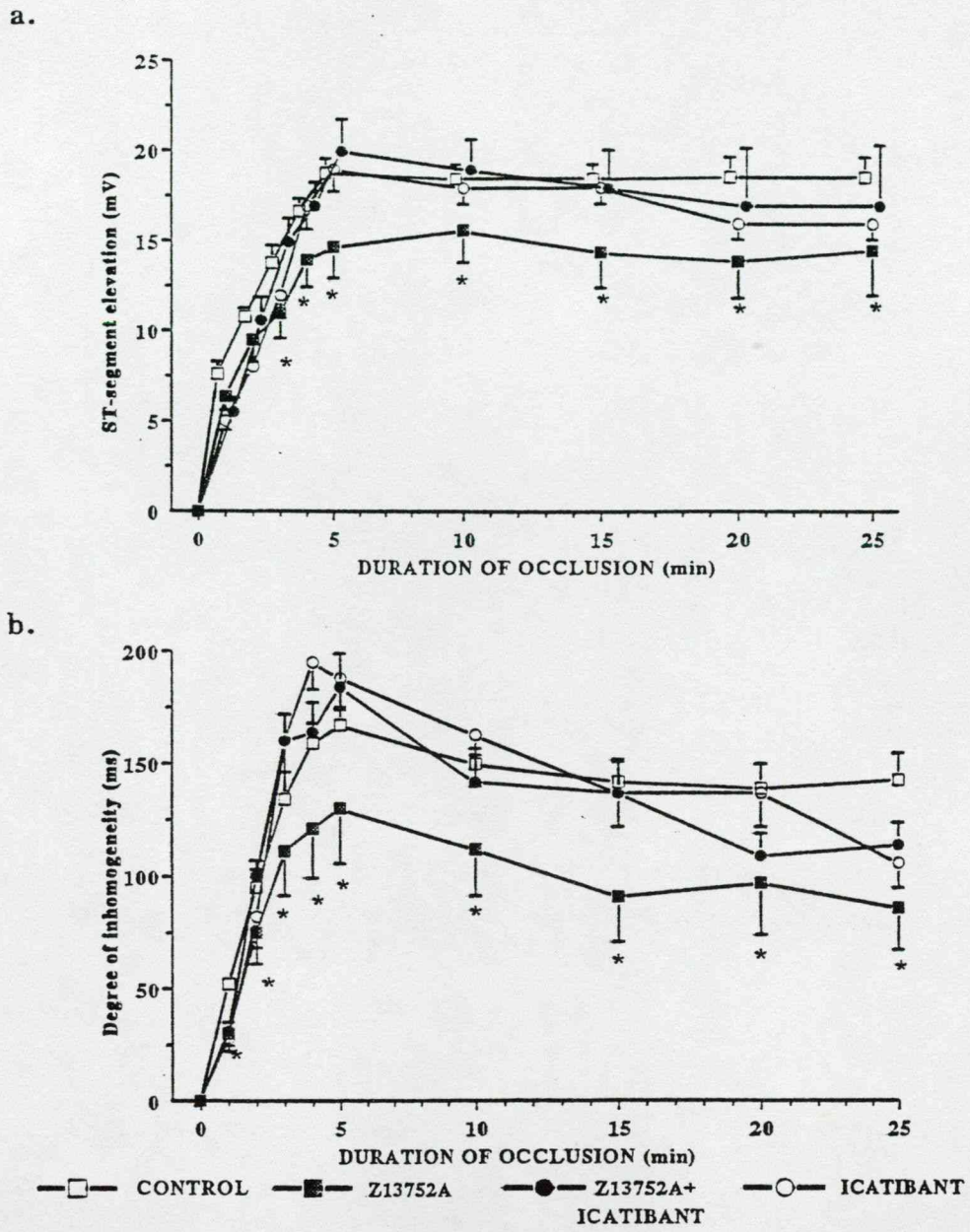

Figure 8 (a) Changes in epicardial ST segment during a 25 min occlusion of the left anterior descending coronary artery in anaesthetized dogs given saline, Z13752A, icatibant and Z13752A in the presence of icatibant. The administration of the ACE/NEP inhibitor leads to a reduction in the severity of the ischaemia, an effect reversed by icatibant. Values are means ± s.e.mean; $^*P<0.05$ c.p. control group. (b) Changes in the degree of inhomogeneity of electrical activation during a 25 min occlusion of the left anterior descending coronary artery in control dogs, in dogs given Z13752A, in dogs given icatibant and in dogs given Z13752A in the presence of icatibant. The reduction in this index of the severity of ischaemia is reduced by Z13752A and, again, this is reversed by icatibant. Values are means ± s.e.mean; $^*P<0.05$ c.p. control group.

the cardioprotection observed is largely kinin-mediated. We do not know if this protection, like that afforded by bradykinin itself (Végh *et al.*, 1993), is ultimately due to nitric oxide (NO) and prostacyclin generated and released as a result of an effect of bradykinin on endothelial B_2 receptors (Parratt *et al.*, 1997). However, it is known that NEP inhibition leads to an increase in NO production in canine isolated coronary microvessels, and that this is mediated by kinins (Zhang *et al.*, 1998).

Besides kinin breakdown, NEP is also concerned with the breakdown of other peptides such as endothelin (Sokolovsky *et al.*, 1990) and atrial natriuretic peptide (ANP). ANP when infused intravenously in the model we have used in the present study also reduces arrhythmia severity during occlusion and reperfusion (Végh *et al.*, 1998) and could conceivably play a role in the cardioprotective effects of Z13752A. Although selective ANP receptor antagonists are available we have no means of examining such a role for ANP in this particular large animal model. The finding that icatibant abolishes the cardioprotection resulting from Z13752A administration however would argue against this possibility, as does the study of Yang and colleagues (1977) showing that the protective effects of the NEP inhibitor CGS 24592 are unaffected by ANP receptor blockade.

We believe that these results add weight to the hypothesis (Parratt & Végh, 1996) that bradykinin acts as an endogenous myocardial protective substance (Parratt, 1994) and that it plays a role in the protection of the

myocardium afforded by ischaemic preconditioning. This hypothesis, the evidence for which has been recently reviewed (Parratt *et al.*, 1997), suggests that brief (preconditioning) periods of ischaemia result, like clinical coronary angioplasty, in the enhanced release of bradykinin from the heart. This then acts on endothelial B_2 receptors and stimulates the generation and release of other mediators which, like bradykinin itself, are able to protect the heart against the consequences of prolonged ischaemia. That NO is a particularly important mediator is borne out by the marked attenuation of the cardioprotective effects of bradykinin, given by intracoronary administration, by inhibitors of the L-arginine-NO pathway (Végh *et al.*, 1993). It would be interesting to determine if NEP inhibition potentiates and/or prolongs the antiarrhythmic effects of ischaemic preconditioning. Like similar previous studies involving adenosine potentiation this would require using a sub-threshold preconditioning stimulus and an analysis of how long the cardioprotective effects of Z13752A are maintained. The present study does not attempt to examine these possibilities.

In summary then, this particular ACE/NEP inhibitor protects the heart from the consequences of ischaemia and evidence is adduced to suggest that this protection is mediated by bradykinin.

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References

BOUCHARD, J.-F., CHOUINARD, J. & LAMONTAGNE, D. (1998). Role of kinins in the endothelial protective effect of ischaemic preconditioning. *Br. J. Pharmacol.*, **123**, 413–420.

DANIELL, H.B., CARSON, R.R., BALLARD, K.D., THOMAS, G.R. & PRIVITERA, P.J. (1984). Effects of captopril on limiting infarct size in conscious dogs. *J. Cardiovasc. Pharmacol.*, **6**, 1043–1047.

EHRING, T., BAUMGART, D., KRAJCAR, M., HÜMMELGEN, M., KOMPA, S. & HEUSCH, G. (1994). Attenuation of myocardial stunning by the ACE inhibitor ramiprilat through a signal cascade of bradykinin and prostaglandins but not nitric oxide. *Circulation*, **90**, 1368–1384.

ERDÖS, E.G. & SKIDGEL, R.A. (1989). Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. *FASEB J.*, **3**, 145–151.

GOTO, M., LIU, Y.G., YANG, X.M., ARDELL, J.L., COHEN, M.V. & DOWNEY, J.M. (1995). Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. *Circ. Res.*, **77**, 611–621.

GRAF, K., KOEHNE, P., GRÄFE, M., ZHANG, M., AUCH-SCHWELK, W. & FLECK, E. (1995). Regulation and differential expression of neutral endopeptidase 24.11 in human endothelial cells. *Hypertension*, **26**, 230–235.

GROCOTT-MASON, R.M., ANNING, P.B., LEWIS, M.J. & SHAH, A.M. (1993). Captopril modifies ventricular relaxation in intact hearts via endogenous bradykinin. *Endothelium*, **1** (supplement), S62.

HATTA, E., MARUYAMA, R., MARSHALL, S.J., IMAMURA, M. & LEVI, R. (1999). Bradykinin promotes ischemic norepinephrine release in guinea pig and human hearts. *J. Pharmacol. Exp. Ther.*, **288**, 919–927.

IKRAM, H. (1996). The renin-angiotensin-aldosterone system and cardiac ischaemia. *Heart* (Supp 3): **76**, 60–67.

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

KASZALA, K., VÉGH, Á., PAPP, J.G. & PARRATT, J.R. (1997). Modification by bradykinin B2 receptor blockade of protection by pacing against ischaemia-induced arrhythmias. *Eur. J. Pharmacol.*, **328**, 51–60.

KOKKONEN, J.O., KUOPPALA, A., SAARINEN, J., LINDSTEDT, K.A. & KOVANEN, P.T. (1999). Kallidin- and bradykinin-degrading pathways in human heart. Degradation of kallidin by aminopeptidase M-like activity and bradykinin by neutral endopeptidase. *Circulation*, **99**, 1984–1990.

LINZ, W., MARTORANA, P.A., GRÖTSCH, H., BEI-YIN, Q. & SCHÖLKENS, B.A. (1990). Antagonizing bradykinin (BK) obliterates the cardioprotective effects of bradykinin and angiotensin-converting enzyme (ACE) inhibitors in ischemic hearts. *Drug Devel. Res.*, **19**, 393–408.

LINZ, W., WIEMER, G., GOHLKE, P., UNGER, T. & SCHÖLKENS, B.A. (1995). Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacol. Rev.*, **47**, 25–49.

LIU, Y.-H., YANG, X.-P., SHAROV, V.G., SIGMON, D.H., SABBAH, H.N. & CARRETERO, O.A. (1996). Paracrine systems in the cardioprotective effect of angiotensin-converting enzyme inhibitors on myocardial ischemia/reperfusion injury in rats. *Hypertension*, **27**, 7–13.

LONN, E.M., YUSUF, S., JHA, P., MONTAGUE, T.J., TEO, K.K., BENEDICT, C.R. & PITT, B. (1994). Emerging role of angiotensin-converting enzyme inhibitors in cardiac and vascular protection. *Circulation*, **90**, 2056–2068.

MARTORANA, P.A., KETTENBACH, B., BREIPOHL, G., LINZ, W. & SCHÖLKENS, B.A. (1990). Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a bradykinin antagonist. *Eur. J. Pharmacol.*, **182**, 395–396.

MARTORANA, P.A., LINZ, W. & SCHÖLKENS, B.A. (1991). Does bradykinin play a role in the cardiac antiischemic effect of the ACE-inhibitors? *Basic Res. Cardiol.*, **86**, 293–296.

MAXWELL, A.J., HUSSEINI, W.K., PIEDIMONTE, G. & HOFFMAN, J.I.E. (1995). Effects of inhibiting neutral endopeptidase and kininase II on coronary and systemic hemodynamics in rats. *Am. J. Physiol.*, **269**, H1016–H1029.

MORAZZONI, G., ALLIEVI, L., BRANCA, E., DA ROS, B., FERLENGA, P., LEGNANI, G., MARCHINI, F., POCCHIARI, F. & SEMERARO, C. (1998a). In vitro and ex vivo characterization of Z13752A, a new dual-acting ACE/NEP inhibitor. *Fourth European Congress of Pharmaceutical Sciences*, Milan, September 11–13, 1998. EPSCED 6 (Suppl 1): S33 (abstract 139).

MORAZZONI, G., ALLIEVI, L., PAUSELLI, F., POCCHIARI, F. & SEMERARO, C. (1998b). Dual inhibition of ACE and NEP activities induced by i.v. and oral administration of Z13752A in spontaneously hypertensive rats. *Fourth European Congress of Pharmaceutical Sciences*, Milan, September 11–13, 1998. EPSCED 6 (Suppl 1): S33 (abstract 140).

NODA, K., SASAGURI, M., IDEISHI, M., IKEDA, M. & ARAKAWA, K. (1993). Role of locally formed angiotensin II and bradykinin in the reduction of myocardial infarct size in dogs. *Cardiovasc. Res.*, **27**, 334–340.

ORLOWSKY, M. & WILK, S. (1981). Purification and specificity of a membrane bound metalloendopeptidase from bovine pituitary. *Biochemistry*, **20**, 4924–4950.

PARRATT, J.R. (1994). Cardioprotection by angiotensin converting enzyme inhibitors–the experimental evidence. *Cardiovasc. Res.*, **28**, 183–189.

PARRATT, J.R. & VÉGH, Á. (1994). Pronounced antiarrhythmic effects of ischemic preconditioning. *Cardioscience*, **5**, 9–18.

PARRATT, J.R. & VÉGH, Á. (1996). Endothelial cells, nitric oxide and ischaemic preconditioning. *Basic Res. Cardiol.*, **91**, 27–30.

PARRATT, J.R. & VÉGH, Á. (1998). Preconditioning induces both immediate and delayed protection against arrhythmias resulting from ischaemia and reperfusion. In: (eds) Bittar, E.E. & Das, D.K. *Advances in Organ Biology. Myocardial Preservation and Cellular Adaptation*. JAI Press Inc. Stamford, Connecticut, pp. 1–20.

PARRATT, J.R., VÉGH, Á. & PAPP, J.G. (1995). Bradykinin as an endogenous myocardial protective substance with particular reference to ischemic preconditioning–a brief review of the evidence. *Can. J. Physiol. Pharmacol.*, 73, 837–842.

PARRATT, J.R., VÉGH, Á., ZEITLIN, I.J., AHMAD, M., OLDROYD, K., KASZALA, K. & PAPP, J.G. (1997). Bradykinin and endothelial-cardiac myocyte interactions in ischemic preconditioning. *Am. J. Cardiol.*, 80 (3A), 124A–131A.

PIEDIMONTE, G., NADEL, J.A., LONG, C.S. & HOFFMAN, J.I.E. (1994). Neutral endopeptidase in the heart: Neutral endopeptidase inhibition prevents isoproterenol-induced myocardial hypoperfusion in rats by reducing bradykinin degradation. *Circ. Res.*, 75, 770–779.

PRADELLA, L., BRAMBILLA, N., VEZZOLA, M., PALMA, S., MORAZZONI, G., ALLIEVI, L., MARCHINI, F., PAUSELLI, M., POCCHIARI, F. & SEMERARO, C. (1998). Z13752A, a new potent dual angiotensin converting enzyme and neutral endopeptidase inhibitor produces antihypertensive effect in SHR rats and DOCA salt hypertensive rats. *Fourth European Congress of Pharmaceutical Sciences*, Milan, September 11–13, 1998. EPSCED 6 (Suppl 1): S36 (abstract 141).

RASTEGAR, M.A., VÉGH, Á., PAPP, J.G.Y. & PARRATT, J.R. (1998). Does inhibition of bradykinin catabolism modify the severity of arrhythmias in myocardial ischaemia?. *J. Mol. Cell. Cardiol.*, 30, A6, P15 (abstract).

RICHARD, V., GHALEH, B., BERDEAUX, A. & GIUDICELLI, J.-F. (1993). Comparison of the effects of EXP2174, an angiotensin II antagonist and enalaprilate on myocardial infarct size in anaesthetised dogs. *Br. J. Pharmacol.*, 110, 969–974.

RICHARDS, A.M., WITTERT, G.A., ESPINER, E.A., YANDLE, T.G., IKRAM, H. & FRAMPTON, C. (1992). Effect of inhibition of endopeptidase 24.11 on responses to angiotensin II in human volunteers. *Circ. Res.*, 71, 1501–1507.

RYAN, J.W., CHUNG, A., AMMONS, C. & CARLTON, M.K. (1977). A simple radioassay for angiotensin-converting enzyme. *Biochem. J.*, 167, 501–504.

SCHÖLKENS, B.A., LINZ, W. & KÖNIG, W. (1989). Effects of the angiotensin converting enzyme inhibitor, ramipril, in isolated ischaemic rat heart are abolished by a bradykinin antagonist. *J. Hypertension*, 6, S25–S28.

SHIMADA, Y. & AVKIRAN, M. (1996). Attenuation of reperfusion arrhythmias by selective inhibition of angiotensin-converting enzyme/kininase II in the ischemic zone: Mediated by endogenous bradykinin? *J. Cardiovasc. Pharmacol.*, 27, 428–438.

SOKOLOVSKY, M., GALRON, R., KLOOG, Y., BDOLAH, A., INDIG, F.E., BLUMBERG, S. & FLEMINGER, G. (1990). Endothelins are more sensitive than sarafotoxins to neutral endopeptidase: Possible physiological significance. *Proc. Natl., Acad. Sci. U.S.A.*, 87, 4702–4706.

TOBE, T.J.M., LANGEN, C.D.J., DE TIO, R.A., BEL, K.J., MOOK, P.H. & WESSELING, H. (1991). Effects of bradykinin on inducible sustained ventricular tachycardia two weeks after myocardial infarction in pigs. *J. Cardiovasc. Pharmacol.*, 17, 701–706.

VÉGH, Á., KOMORI, S., SZEKERES, L. & PARRATT, J.R. (1992). Antiarrhythmic effects of preconditioning in anaesthetised dogs and rats. *Cardiovasc. Res.*, 26, 487–495.

VÉGH, Á., PAPP, J.G. & PARRATT, J.R. (1994). Attenuation of the antiarrhythmic effects of ischaemic preconditioning by blockade of bradykinin B2 receptors. *Br. J. Pharmacol.*, 113, 1167–1172.

VÉGH, Á., PAPP, J.G., SZEKERES, L. & PARRATT, J.R. (1993). Prevention by an inhibitor of the L-arginine-nitric oxide pathway of the antiarrhythmic effects of bradykinin in anaesthetized dogs. *Br. J. Pharmacol.*, 110, 18–19.

VÉGH, Á., RASTEGAR, M.A., PAPP, J.G.Y. & PARRATT, J.R. (1998). The antiarrhythmic effects of ANP in a canine model of ischaemia-reperfusion. *J. Mol. Cell. Cardiol.*, 30, A7, P20 (abstract).

VÉGH, Á., SZEKERES, L. & PARRATT, J.R. (1990). Protective effects of preconditioning of the ischaemic myocardium involve cyclo-oxygenase products. *Cardiovasc. Res.*, 24, 1020–1022.

VÉGH, Á., SZEKERES, L. & PARRATT, J.R. (1991a). Local coronary infusions of bradykinin profoundly reduce the severity of ischaemia-induced arrhythmias in anaesthetised dogs. *Br. J. Pharmacol.*, 104, 294–295.

VÉGH, Á., SZEKERES, L. & PARRATT, J.R. (1991b). Transient ischaemia induced by rapid cardiac pacing results in myocardial preconditioning. *Cardiovasc. Res.*, 25, 1051–1053.

VÉGH, Á., SZEKERES, L. & UDVARY, E. (1987). Effect of the blood supply to the normal noninfarcted myocardium on the incidence and severity of early post-occlusion arrhythmias in dogs. *Basic Res. Cardiol.*, 82, 159–171.

WALL, T.M., SHEEHY, R. & HARTMAN, J.C. (1994). Role of bradykinin in myocardial preconditioning. *J. Pharmacol. Exp. Ther.*, 270, 681–689.

WILLIAMS, D.O., SHERLAG, B.J., HOPE, R.R., EL-SHERIF, N. & LAZZARA, R. (1974). The pathophysiology of malignant ventricular arrhythmias during acute myocardial ischemia. *Circulation*, 50, 1163–1172.

YANG, X.-P., LIU, Y.-H., PETERSON, E. & CARRETERO, O.A. (1997). Effect of neutral endopeptidase 24.11 inhibition on myocardial ischemia/reperfusion injury: The role of kinins. *J. Cardiovasc. Pharmacol.*, 29, 250–256.

ZHANG, X., NASJLETTI, A., XU, X. & HINTZE, T.H. (1998). Neutral endopeptidase and angiotensin-converting enzyme inhibitors increase nitric oxide production in isolated canine coronary microvessels by a kinin-dependent mechanism. *J. Cardiovasc. Pharmacol.*, 31, 623–629.

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Atrial natriuretic peptide reduces the severe consequences of coronary artery occlusion in anaesthetised dogs

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Short running title: The antiarrhythmic effect of atrial natriuretic peptide

Abstract

The aim of the present study was to examine the effects of atrial natriuretic peptide (ANP) on the responses to coronary artery occlusion. In chloralose-urethane anaesthetised mongrel dogs either saline (controls) or human synthetic ANP was infused intravenously (10 μg kg^{-1} + 0.1 μg kg^{-1} min^{-1}), starting 30 min before and continuing 10 min during a 25 min occlusion of the left anterior descending coronary artery (LAD). ANP infusion resulted in a fall in mean arterial blood pressure (by 17 ± 2 mmHg, $P < 0.05$), a transient (max. at 5 min) increase in coronary blood flow (by 24 ± 5 ml min^{-1}, $P < 0.05$) and a reduction in coronary vascular resistance (by 0.27 ± 0.05 mmHg ml^{-1} min^{-1}, $P < 0.05$). When the LAD coronary artery was occluded there was a less marked elevation in left ventricular end-diastolic pressure (LVEDP) in the ANP-treated dogs than in the controls (9.0 ± 0.9 vs 12.2 ± 0.8 mmHg, $P < 0.05$). Compared to the controls, ANP reduced the number of ventricular premature beats (VPBs, 26 ± 12 vs 416 ± 87, $P < 0.05$), the number of episodes of ventricular tachycardia per dogs (VT, 0.7 ± 0.3 vs 12.4 ± 4.2, $P < 0.05$) and the incidences of VT (45% vs 100%, $P < 0.05$) and ventricular fibrillation (VF 18% vs 57%, $P < 0.05$) during occlusion. Reperfusion of the ischaemic myocardium at the end of the occlusion period led to VF in all the control dogs (survival from the combined ischaemia-reperfusion insult was therefore 0 %) but VF following reperfusion was much less in the dogs given ANP (survival 64%; $P < 0.05$). The severity of myocardial ischaemia, as assessed from changes in the epicardial ST-segment and the degree of inhomogeneity, was significantly less marked in dogs given ANP. We conclude that ANP protects the myocardium from the consequences of myocardial ischaemia, resulting from acute coronary artery occlusion and reperfusion in anaesthetised dogs.

Key Words: Atrial natriuretic peptide, arrhythmias, ischaemia, myocardial protection

1. Introduction

Several substances are released from the heart in the very early stages of myocardial ischaemia and these almost certainly contribute to the severity of the phase1 arrhythmias that arise soon (minutes) after the onset of the coronary artery occlusion. Some of these substances may be protective and reduce arrhythmia severity; these 'endogenous myocardial protective substances' include adenosine, prostacyclin, plasma kinins and nitric oxide [1]. One possible mechanism of the protective effects of some of these substances (e.g. nitric oxide) is mediated through elevation of cGMP; a hypothesis, first put forward by Opie in 1982 [2]. This hypothesis is supported by studies in which cGMP derivatives, with the ability to penetrarte cardiac cells, have also shown to be protective during ischaemia [3]. In addition, one of the proposed mechanisms for the antiarrhythmic effects of ischaemic preconditioning is elevation of cardiac myocyte cGMP levels as a result of bradykinin-triggered nitric oxide generation by endothelial cells, an example of endothelial-myocyte cross-talk [4].

Another possible means by which myocardial cGMP level can be increased, albeit by a different mechanism, is by elevating plasma levels of atrial natriuretic peptide (ANP). This can be achieved by cardiac pacing [5], by inhibiting ANP breakdown using angiotensin converting enzyme (ACE) or neutral endopeptidase enzyme (NEP) inhibitors [6] or by infusing exogenous synthetic ANP. This peptide hormone is synthesized, stored and released primarily from adult mammalian atrial myocytes [7], but is also found in normal ventricular tissue (when it co-exists with the related peptide BNP [8]) and in a variety of extracardial tissues, including aorta, lung and brain [9]. It is secreted into the circulation in response to a variety of stimuli, including atrial stretch (e.g. in response to volume loading [10]), acute hypoxia [11] and following administration of a variety of endogenous vasoactive substances such as acetylcholine,

adrenaline, dopamine [12] and endothelin [10]. Endogenous plasma ANP levels are also elevated in a number of pathological states, including congestive heart failure, ischaemic heart disease [13] and pulmonary hypertension [14].

ANP has a wide range of potent biological effects, including vasodilation, natriuresis and inhibition of the renin-angiotensin-aldesterone system [7]. In particular ANP, causes transient vasodilation of coronary resistance vessels with an increase in coronary blood flow [15-16]. Recently Takata and colleagues [17] found that the administration of ANP in dogs led to a significant elevation in plasma cGMP and prevented reperfusion arrhythmias. Ischaemia-induced ventricular arrhythmias, however, were not measured.

The aim of the present study was to determine in a well documented canine model of ischaemia and reperfusion [18-19] whether infusing exogenous synthetic ANP, presumably by elevating cGMP, protects against the severe consequences of myocardial ischaemia and particularly against those life-threatening ventricular arrhythmias that result from coronary artery occlusion.

2. Material and Methods

2.1. In vivo studies in anaesthetised dogs

These were similar to those already described in detail elsewhere [18]. In brief, mongrel dogs of both sexes with a mean body weight of 24.2 ± 1.4 kg were anaesthetised with a mixture of chloralose and urethane (60 and 200 $mg \cdot kg^{-1}$ i.v., respectively) and ventilated with room air using a Harvard Respirator at a rate and volume sufficient to maintain arterial blood gases and pH within normal limits [19].

The animals were thoracotomised at the fifth intercostal space and the anterior descending branch of the left coronary artery (LAD) prepared for occlusion just proximal to the first main diagonal branch. This gives an area at risk, as assessed by infusing blue V dye into the occluded coronary artery at the end of the experiment, of around 38-46 % (see results). The circumflex branch of the left coronary artery (LCX) was also dissected free for the measurement of coronary blood flow by means of a 2.0 mm electromagnetic flow probe attached to a Statham SP2202 flowmeter. 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 [20, 21]. 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 summarized R-waves occurs indicating that adjacent fibers are not simultaneously activated because of inhomogeneity of conduction. We expressed this as the greatest delay in activation (ms) within the ischaemic area. This reflects, in part, local changes in myocardial blood flow.

All these parameters, together with a limb lead electrocardiogram, systemic arterial and left ventricular systolic and end-diastolic pressures (Statham P23XL transducers) and LV dP/dt_{max} were recoreded on an eight channel Medicor R81 recorder.

Ventricular arrhythmias during a 25 min coronary artery occlusion (i.e. ischaemia) were assessed and analysed as outlined previously [19], i.e. total ventricular premature beats (VPBs), the incidence and number of episodes of ventricular tachycardia (VT) and the incidence of ventricular fibrillation (VF). At the end of this period of ischaemia the area supplied by the occluded vessel was rapidly reperfused. The only reperfusion arrhythmias that was determined was VF. Survival, from the combined ischaemia-reperfusion insult, was defined in terms of those dogs which were predominantly in sinus rhythm 10 min after the commencement of reperfusion.

2.2. Experimental protocol

The experimental protocol is illustrated in Figure 1. Two groups of dogs were used. In eleven dogs human synthetic ANP (Sigma) was given in a dose of 10 $\mu g\ kg^{-1}$ as an intravenous bolus injection followed by infusion of 0.1 $\mu g\ kg^{-1}\ min^{-1}$ over a period of 40 min, commencing 30 min prior to, and 10 min during, the 25 min occlusion of the LAD. The responses were compared with those of fourteen control dogs which were given a similar volume of saline and then subjected to coronary artery occlusion for 25 min. The artery was then re-opened rapidly to allow reperfusion. All heamodynamics were recorded continuously and measurements were made at one min intervals during the first 5 min of an intervention (i.e. infusion of ANP or

saline, or coronary artery occlusion) and then at five min intervals by recording changes on a higher paper speed (100 mm^{-1}).

2.3. Ethics

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

2.4. Statistical evaluation

All the data were expressed as means ± S.E.M., and differences between means were compared by a Student's t-test corrected for multiple comparisons using a two way ANOVA. Ventricular premature beats were compared by using the Mann-Whitney Rank Sum test, and the incidences of arrhythmias (such as of the VT, VF and survival from the combined ischaemia-reperfusion insult) were compared using the Fisher Exact test. Differences between groups were considered significant when $P < 0.05$.

3. Results

3.1 Haemodynamic effects of intravenous ANP

The maximum haemodynamic changes which occurred during the 30 min infusion of ANP are illustrated in Figure 2. Thus, ANP resulted in significant reductions in arterial blood pressure (systolic 119 ± 7 to 98 ± 7 mmHg; diastolic 77 ± 5 to 62 ± 5 mmHg, mean 91 ± 6 to 74 ± 6 mmHg, $P < 0.05$) and in positive and negative $LVdP/dt_{max}$ (3417 ± 117 to 3155 ± 177 mmHg s^{-1} and 2875 ± 258 to 2534 ± 217 mmHg s^{-1}, respectively, $P < 0.05$). There were no significant changes in heart rate (134 ± 5 to 135 ± 4 beats min^{-1}, Figure 2) or in LVEDP (5.6 ± 0.3 to 5.6 ± 0.3 mmHg). The most marked haemodynamic effect of ANP was a transient increase (of 24 ± 5 ml min^{-1}, from 110 ± 10 to 134 ± 13 ml min^{-1}) in left circumflex diastolic coronary blood flow and a decrease in the coronary resistance (of 0.27 ± 0.05 mmHg ml^{-1} min^{-1}, from 0.78 ± 0.11 to 0.51 ± 0.09 mmHg ml^{-1} min^{-1}) measured 5 min after the onset of the ANP infusion (Figure 3). All of these haemodynamic changes, however, returned to the initial values prior to the commencement of the coronary artery occlusion.

3.2. Haemodynamic changes induced by coronary artery occlusion in control dogs and in dogs given ANP

These results are summarised in Table 1. Occlusion of the LAD resulted in similar decreases in arterial blood pressure and in positive dP/dt_{max} in both the controls and the ANP treated dogs. However, the marked increase in LVEDP and the decrease in negative dP/dt_{max}, observed in control dogs during ischaemia were significantly less pronounced in dogs given ANP.

Occlusion of the LAD resulted in an immediate and sustained increase in blood flow through the other major (circumflex) branch of the left coronary artery. This compensatory increase in blood flow of this artery was significantly greater if the LAD was occluded in the presence of ANP (Table 1).

3.3. Effect of ANP on ventricular arrhythmias following coronary artery occlusion and reperfusion.

The distribution of ventricular arrhythmias resulting from coronary artery occlusion and reperfusion in control and ANP treated dogs is illustrated in Figure 4 and the total arrhythmia severity is summarised in Figure 5. In this canine model occlusion of the LAD coronary artery for 25 min leads to severe ventricular arrhythmias [18]. In this study, there was a mean of 416 ± 87 VBPs in the control dogs during coronary artery occlusion, and all dogs exhibited VT at some stages during the period of ischaemia with a mean of 12.4 ± 4.2 episodes of VT per dog. Eight of the 14 dogs (57 %) fibrillated during the occlusion period and the remaining six dogs (43 %) fibrillated following reperfusion of the ischaemic myocardium. Thus, no control dog survived the combined ischaemia-reperfusion insult.

These ischaemia and reperfusion-induced arrhythmias were much less pronounced in dogs given ANP (Figure 4). There was a mean of only 26 ± 12 VPBs ($P < 0.05$) during the occlusion and only 5 out of 11 dogs had VT with a mean of 0.7 ± 0.3 VT episodes ($P < 0.05$ compared to controls). Furthermore, only two dogs (18%), treated with ANP fibrillated during the occlusion period; two additional dogs fibrillated during reperfusion. Thus, survival

from the combined ischaemia-reperfusion insult in the ANP-treated dogs was 64% ($P < 0.05$ versus control; Figure 5).

3.4. Effect of ANP on coronary artery occlusion-induced changes in epicardial ST-segment elevation and in the degree of inhomogeneity of electrical activation within the ischaemic area.

In control dogs subjected to coronary artery occlusion, the ST-segment (Figure 6) and the degree of inhomogeneity of electrical activation (Figure 7), both recorded from the epicardial surface of the myocardium distal to the occlusion site, increased rapidly within the first 5 min of the onset of ischaemia. These changes were significantly less marked in dogs given ANP (Figures 6 and 7).

3.5. *Area at risk in dogs subjected to coronary artery occlusion*

There was no significant difference between the groups in the area at risk from necrosis (i.e. supplied by the occluded artery). These were 45.3 ± 2.6 % in the controls, 44.6 ± 2.3% in the dogs infused with ANP. There were also no significant differences between groups in respect to the weight of the dogs, or in gender distribution. The body weights were 24.2 ± 1.6 kg in the controls and 23.8 ± 0.8 kg in the ANP group.

4. Discussion

These results in anaesthetised dogs show that the intravenous administration of ANP resulted in marked cardioprotective effects. There was a significant reduction in the severity of both occlusion and reperfusion-induced ventricular arrhythmias and in the two measured indices of ischaemia severity; epicardial ST-segment elevation and the degree of inhomogeneity of electrical activation, a measure of conduction delay within the ischaemic area.

As shown previously [15, 16, 22] ANP in this species results in a slight increase in coronary blood flow and a somewhat more pronounced reduction in coronary vascular resistance [23, 24]. The question arises as to whether this coronary vascular effect is responsible for the reduced ischaemia severity as assessed, for example, by modifications of the electrocardiographic effects of coronary artery occlusion; i.e. the less marked delay in conduction within the ischaemic segment following ANP infusion. The effect of ANP on intramural vessels, [25] resulting via more favourable redistribution of flow to the subendocardium under conditions of ischaemia may well have contributed to the protection observed. We did not attempt to assess changes in blood flow within the ischaemic region itself because the haemodynamic effects of microsphere injection during this vulnerable, early (25 min) period following the onset of ischaemia might well have modified arrhythmia severity; studies involving blood flow measurements during ischaemia have usually been performed much later during the ischaemic period at a time when these early life-threatening (phase 1) ventricular arrhythmias have diasppeared and when sinus rhythm has been re-established.

We believe, especially in view of the rather moderate effect of ANP on coronary flow observed in the present study (Figure 3), that a more likely explanation for the profound

protection is an ANP-induced increase in myocardial cGMP. The present study did not attempt to measure tissue cGMP levels, because this would require myocardial biopsy samples to be taken before and during the occlusion, creating an additional ischaemic focus which would modify ischaemia-induced arrhythmia severity. There is a recent evidence, reported by Takata and colleagues [17] that ANP infusion increases plasma cGMP and this contributes to the suppression of reperfusion-induced ventricular arrhythmias. Increases in plasma and vascular cGMP levels following ANP administration have also been described [16, 26].

In view of Opie's [2] original suggestion regarding the relation between cardioprotection and cGMP levels, it is of interest that one proposal for the pronounced and acute antiarrhythmic effects of brief periods of coronary artery occlusion (ischaemic preconditioning [18, 19]), and also of the delayed protective effects of cardiac pacing [27], involves the release of substances that lead ultimately to an increase in myocardial levels of cGMP [28]. This hypothesis, the evidence for which has been recently reviewed [29, 30] proposes that the initial trigger for this protection is the early release, possibly from coronary vascular and endocardial endothelial cells, of bradykinin [4] which then stimulates the release of other endothelium-derived mediators such as prostacyclin and nitric oxide (NO). The latter, by diffusing to cardiac myocytes, stimulates soluble guanylate cyclase and elevates cGMP, leading to a reduced influx of calcium and to an increased activity of a cGMP stimulated phosphodiesterase and hence reduced levels of cAMP. It is proposed that this alteration in the balance of cGMP/cAMP in favour of cGMP leads to protection against phase1 arrhythmias. The evidence for this hypothesis [30] includes abolition of the protection by blockade of bradykinin B2 receptors, by inhibition of NO synthesis and by methylene blue, and also by the direct measurement, following preconditioning, of myocardial cGMP levels [31]. The present results with ANP

would seen to further support such a hypothesis; elevating cGMP, albeit by the activation of a different (particulate) guanylate cyclase enzyme is as protective as ischaemic preconditioning.

The precise mechanism of the protective effects of elevating cGMP in the myocardium, by whatever means, are unclear, but may involve a decrease in calcium influx [32] and reduction in cAMP level described above, and the opening of ATP sensitive K^+ channels, an effect that has been reported following elevated cGMP levels, at least in vascular smooth muscle [33]. There is a good deal of evidence [34, 35] that the opening of these channels, especially in mitochondria, is cardioprotective and may be involved in the protection afforded by ischaemic preconditioning [36, 37]. The process of this protection would thus involve mediator release (e.g. NO, ANP), elevation of cGMP levels and, by transduction mechanisms (PKC? tyrosine kinase?) at present ill-defined, would ultimately lead to the opening of mitochondrial K_{ATP} channels. This seems to be a valid working hypothesis for a number of procedures leading to protection of the myocardium against ischaemia.

Acknowledgments

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References

1. Parratt JR. Endogenous myocardial protective (antiarrhythmic) substances. *Cardiovasc Res* 1993; 27:693-702.

2. Opie LH. Role of cyclic nucleotides in heart metabolism. *Cardiovasc Res* 1982;16:483-507.

3. Billman GE. Effect of carbachol and cGMP on susceptibility to ventricular fibrilation. *FASEB J* 1990;4:1668-73.

4. Parratt JR, Végh Á, Zeitlin IJ, et al. Bradykinin and endothelial-cardial myocyte interactions in ischemic preconditioning. *Am J Cardiol* 1997;80:124A-131A.

5. Stevens TL, Rasmussen TE, Wei CM, Kinoshita M, Matsuda Y, Burnett JC. Renal role of the endogenous natriuretic peptide system in acute congestive heart failure. *J Cardiac Failure* 1996;2:119-125.

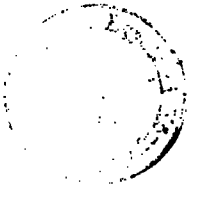
6. Graf K, Koehne P, Grafe M, Zhang M, Auch-Schwelk W, Feleck E. Regulation and differential expression of neutral endopeptidase 24.11 in human endothelial cells. *Hypertension* 1995;26,230-235.

7. de Bold AJ. Atrial natriuretic factor:a hormone produced by the heart. *Science* 1985; 230:767-770.

8: Ogawa, Y, Itoh, H., Nakao, K. Molecular biology and biochemistry of the natriuretic peptide family. *Clin Exp Pharmacol Physiol* 1995;22:49-53.

9. Matsuo H, Kangawa K, Miyata A. Atrial natriuretic polypeptide (ANP); molecular forms and distribution in mammalian tissues and plasma. In: Deber CM, Hruby VJ, Kopple KD, eds. *Peptide Structure and Function.* 1985; 932-952.

10. Fyhrquist F, Sirvo ML, Helin K, et al. Endothelin antiserum decreases volume-stimulated and basal plasma concentrations of atrial natriuretic peptide. *Circulation* 1993;88:1172-1176.



11. Baertschi AJ, Adams JM, Sullivan MP. Acute hypoxemia stimulates atrial natriuretic factor secretion in vivo. *Am J Physiol* 1988; 255:H 295-H300.

12. Chen YF, Jin H, Paul R, Nagahama S. Blunted pressor responsiveness to quinpirole, a specific dopamine D2 receptor agonist, in conscious DOCA/NACL hypertensive rats is related to ANP release. *J Pharmacol Exp Ther* 1998; 246:485-493.

13. Naruse M, Takeyama Y, Tanabe A, et al., Atrial and brain natriuretic peptides in cardiovascular diseases. *Hypertension* 1994; 23(SUPPL): I-231-I-234.

14. Hirata Y, Suzuki E, Hayakawa H, Matsuoka H, et al.. Role of endogenous ANP in sodium excretion in rats with experimental pulmonary hypertension. *Am J Physiol* 1992;262: H1684-H1689.

15. Chu A, Cobb FR. Effects of atrial natriuretic peptide on proximal epicardial coronary arteries and coronary blood flow in conscious dogs. *Circ Res* 1987;61:485-491.

16. Chu A, Morris K, Kuehl W, et al. Effects of atrial natriuretic peptide on the coronary arterial vasculature in humans. *Circulation* 1989;80:1627-1635.

17. Takata Y, Hirayama Y, Kiyomi S, et al. The beneficial effects of atrial natriuretic peptide on arrhythmias and myocardial high-energy phosphates after reperfusion. *Cardiovasc Res* 1996;32:286-293.

18. Végh Á, Komori S, Szekeres L, Parratt JR. Antiarrhythmic effects of preconditioning in anaesthetised dogs and rats.; *Cardiovasc Res* 1992;26:487-495.

19. Végh Á, Szekeres L, Parratt JR. Preconditioning of the ischaemic myocardium, involvement of the L-arginine nitric oxide pathway. *Br J Pharmacol* 1992;107:648-652.

20. Végh Á, Szekeres L, Udvary É. Effect of the blood supply to the normal noninfarcted myocardium on the incidence and severity of early post-occlusion arrhythmias in dogs. *Basic Res Cardiol* 1987;82,159-171.

21. William DO, Scherlag BJ, Hope RR, El-Sherif N, Lazzara R. The pathophysiology of malignant ventricular arrhythmias during acute myocardial ischemia. *Circulation* 1974; 50:1163-1172.

22. Bache RJ, Dai X, Schwartz JS, Chen DG: Effects of atrial natriuretic peptide in the coronary circulation. *Circ Res* 1988;62:178-183.

23. Laxson DD, Dai XZ, Schwartz JS, Bache RJ: Effects of atrial natriuretic peptide on coronary vascular resistance in the intact awake dog. *J Am Coll Cardiol* 1988;11:624-629.

24. Bauman RP, Rembert JC, Himmelstein SI, Klotman PE, Greefield JC. Effect of atrial natriuretic factor on transmural myocardial blood flow distribution in dogs. Circulation 1987,76:705-709.

25. Chu A, Stakely A, Lin C-C, Cobb FR: Effects of atrial natriuretic peptide on transmural blood flow and reactive hyperemia in the presence of flow limiting coronary stenosis in the awake dog: Evidence for dilation of the intramural vasculature. *Circ Res* 1989;64: 600-606.

26. Rapoport RM, Waldman SA, Schwartz K, Winquist RJ. Effects of atrial natriuretic factor, sodium nitroprusside and acetylcholine on cyclic GMP levels and relaxation in rat aorta. *Eur J Pharmacol* 1985;115:219-229.

27. Kaszala K, Végh Á, Papp JGy, Parratt JR. Modification by bradykinin B2 receptor blockade of protection by pacing against ischaemia-induced arrhythmias. *Eur J Pharmacol* 1997;328:51-60.

28. Parratt JR. Protection of the heart by ischaemic preconditioning: Mechanisms and possibilities for pharmacological exploitation. *Trends Pharmacol Sci* 1994;15:19-25.

29. Parratt JR, Végh Á. Endothelial cells, nitric oxide and ischaemic preconditioning, *Basic Res Cardiol* 1996;91:27-30.

30. Parratt JR, Végh Á. Coronary vascular endothelium, preconditioning and arrhythmogenesis. In: Lewis MJ, Shah AM. eds. *Endothelial Modulation of Cardiac Function*, Harwood Academic Publishers, 1997, 237-255.

31. Parratt JR. Possibilities for the pharmacological exploitation of ischaemic preconditioning. *J Mol Cell Cardiol* 1995,27:991-1000.

32. Tohse B, Kanno M. Human atrial natriuretic peptide decreases cardiac calcium currents through activation of cGMP-dependent protein kinase. *Eur J Pharmacol* 1993;61(suppl 1):272 (abstr.)

33. Kubo M, Nakaya Y, Matsuoka S, et al. Atrial natriuretic factor and isosorbide dinitrate modulate the gating of ATP-sensitive K^+ channels in cultured vascular smooth muscle cells. *Circ Res* 1994;74:471-476.

34. Escande D, Cavero I. K^+ channel openers and natural cardioprotection. *Trends Pharmacol. Sci* 1992;13:269-272.

35. Grover GJ. Protective effects of ATP sensitive potasium channel openers in models of myocardial ischaemia. *Cardiovasc Res* 1994;28:778-782.

36. Parratt JR, Kane KA. K_{ATP} channels in ischaemic preconditioning. *Cardiovasc Res* 1994;28: 783-787.

37. Liu Y, Sato T, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? *Circulation* 1998;97:2463-2469.

Figure Legends

Figure 1. The experimental protocol outlining the procedures performed in the two groups of anaesthetised dogs.

Figure 2. Hemodynamic effects [mean arterial blood pressure (MABP), heart rate (HR) and LV dP/dt_{max}] of ANP, given in a dose of 10 $\mu g\ kg^{-1}$ as intravenous bolus injection followed by infusion of 0.1 $\mu g\ kg^{-1}\ min^{-1}$. Data are presented were measured at baseline (open columns) and when maximum changes occurred during the infusion prior to coronary aretry occlusion (filled columns). Values are means ± s.e.m.; $^{*}P < 0.05$ cp pre-drug values.

Figure 3. Changes in diastolic coronary blood flow and resistance measured before (open columns) and 5 min after the onset of an infusion of ANP (filled columns). There is a transient increase in left circumflex diastolic coronary blood flow and a decrease in the coronary vascular resistance. Values are means ± s.e.m.; $^{*}P < 0.05$ cp to the values before giving ANP.

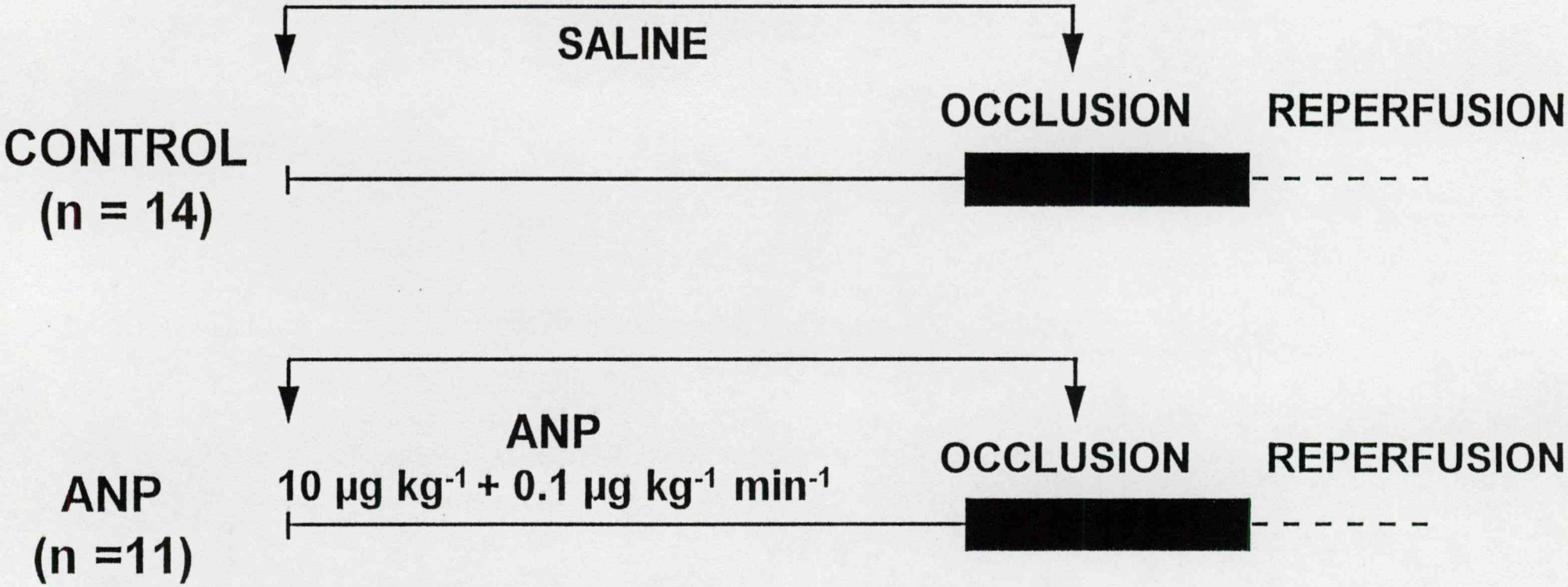
Figure 4. The distribution of ventricular arrhythmias in control dogs, and in dogs infused with ANP, during a 25 min coronary artery occlusion followed, at the end of this period, by reperfusion. ANP markedly reduced the severity of these arrhythmias and 7/11 dogs survived the combined ischaemia-reperfusion insult. Data are presented as ventricular premature beats (VPBs; light grey columns), ventricular tachycardia (VT; hatched columns) and ventricular fibrillation (VF; filled columns) during occlusion and reperfusion.

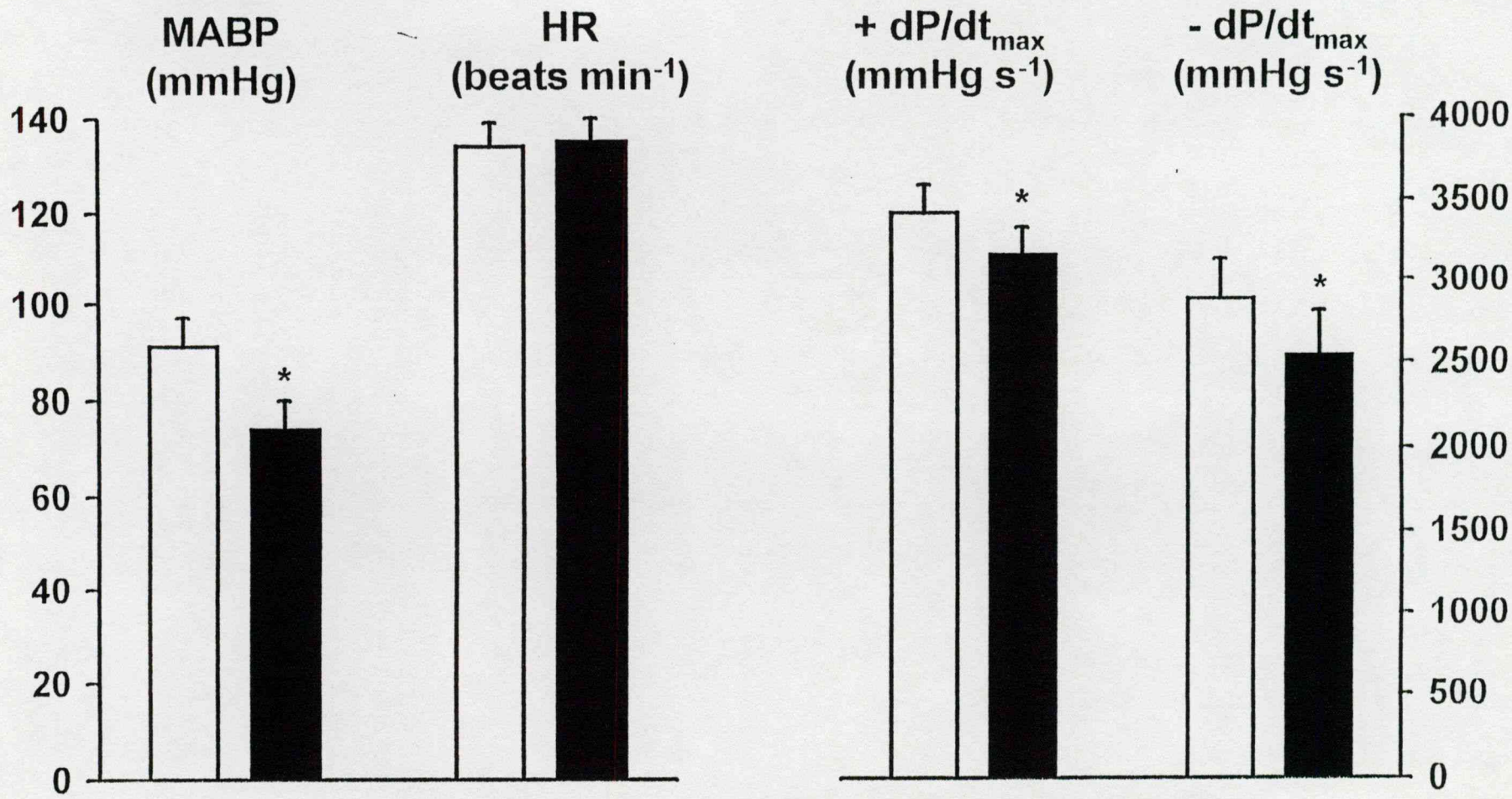
Figure 5. A summary of the effects of ANP (fille histograms), in comparison with control (saline infused) dogs (open histograms), on ventricular arrhythmias resulting from coronary artery occlusion and subsequent reperfusion. VPBs = ventricular premature beats; VT = ventricular tachycardia; VF = ventricular fibrillation (during occlusion and reperfusion) and survival. Values are means ± s.e.m. from 11 dogs; $^{*}P < 0.05$ cp to controls (n = 14).

Figure 6. Changes in epicardial ST-segment (mV) during a 25 min occlusion of the left anterior descending coronary artery in anaesthetised dogs given either saline (filled circles) or ANP (filled squares). The administration of ANP led to a reduction in the severity in this index of myocardial ischaemia. Values are means ± s.e.m.; $^{*}P < 0.05$ cp to controls.

Figure 7. Changes in the degree of inhomogeneity of electrical activation (ms) during a 25 min occlusion of the left anterior descending coronary artery in anaesthetised in control dogs (filled circles) and in dogs given ANP (filled squares). ANP significantly reduced this index of the severity of ischaemia. Values are means ± s.e.m.; $^{*}P < 0.05$ cp to controls.

EXPERIMENTAL PROTOCOL

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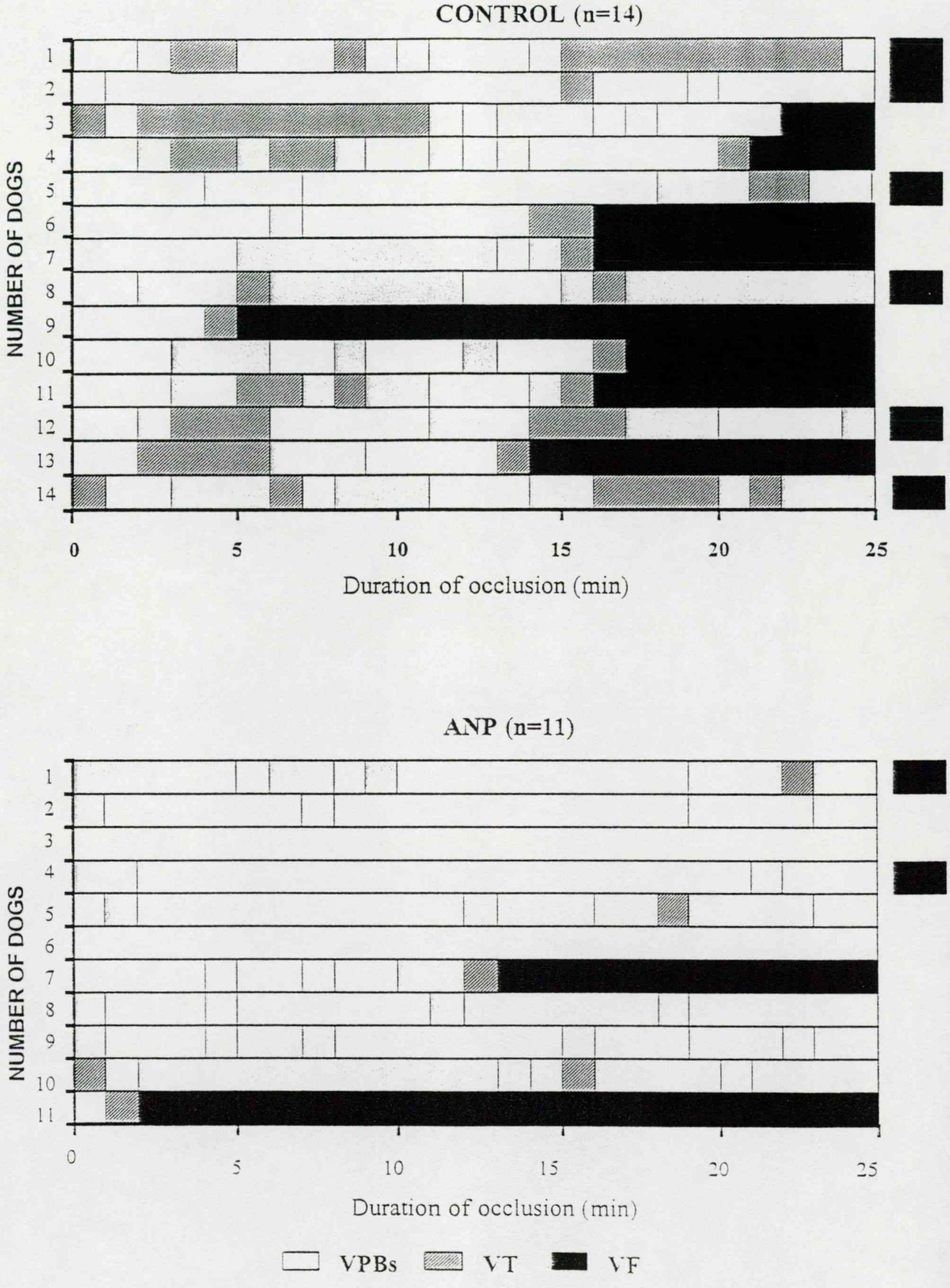
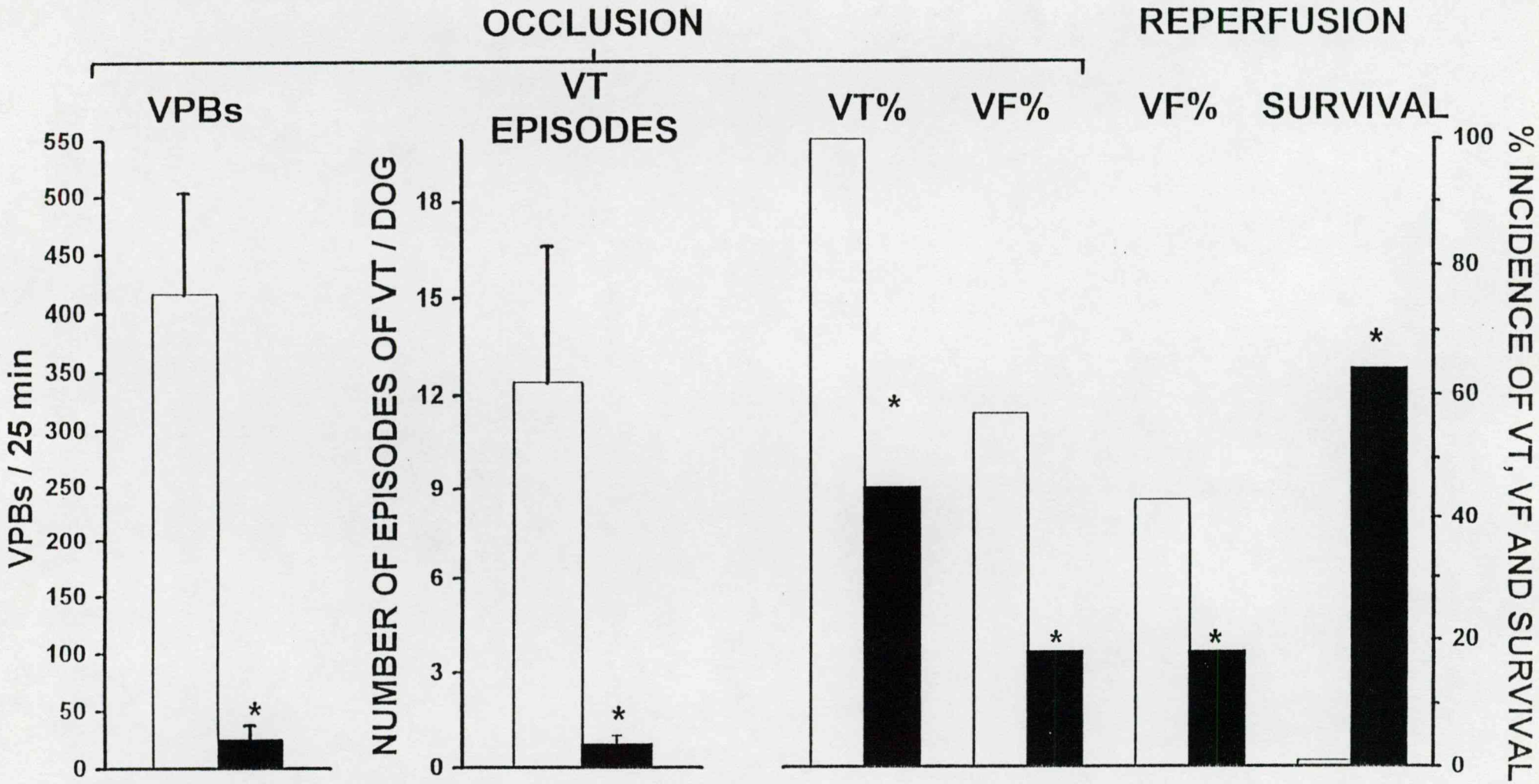
HR
(beats min-1)
+ dP/dtmax
(mmHg s-1)
- dP/dtmax
(mmHg s-1)
140
120
100
80
60
40
20
0
4000
3500
3000
2500
2000
1500
1000
500
0
*
*
*

DIASTOLIC CORONARY RESISTANCE
(mmHg ml-1 min-1)
1.00
0.80
0.60
0.40
0.20
0.00
*
*
160
140
120
100
80
60
40
20
0
(ml min-1)
DIASTOLIC CORONARY BLOOD FLOW


CONTROL (n=14)
NUMBER OF DOGS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0
5
10
15
20
25
Duration of occlusion (min)
ANP (n=11)
NUMBER OF DOGS
1
2
3
4
5
6
7
8
9
10
11
0
5
10
15
20
25
Duration of occlusion (min)
VPBs
VT
VF


OCCLUSION
REPERFUSION
VPBs
VT EPISODES
VT%
VF%
VF%
SURVIVAL
VPBs / 25 min
550
500
450
400
350
300
250
200
150
100
50
0
NUMBER OF EPISODES OF VT / DOG
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15
12
9
6
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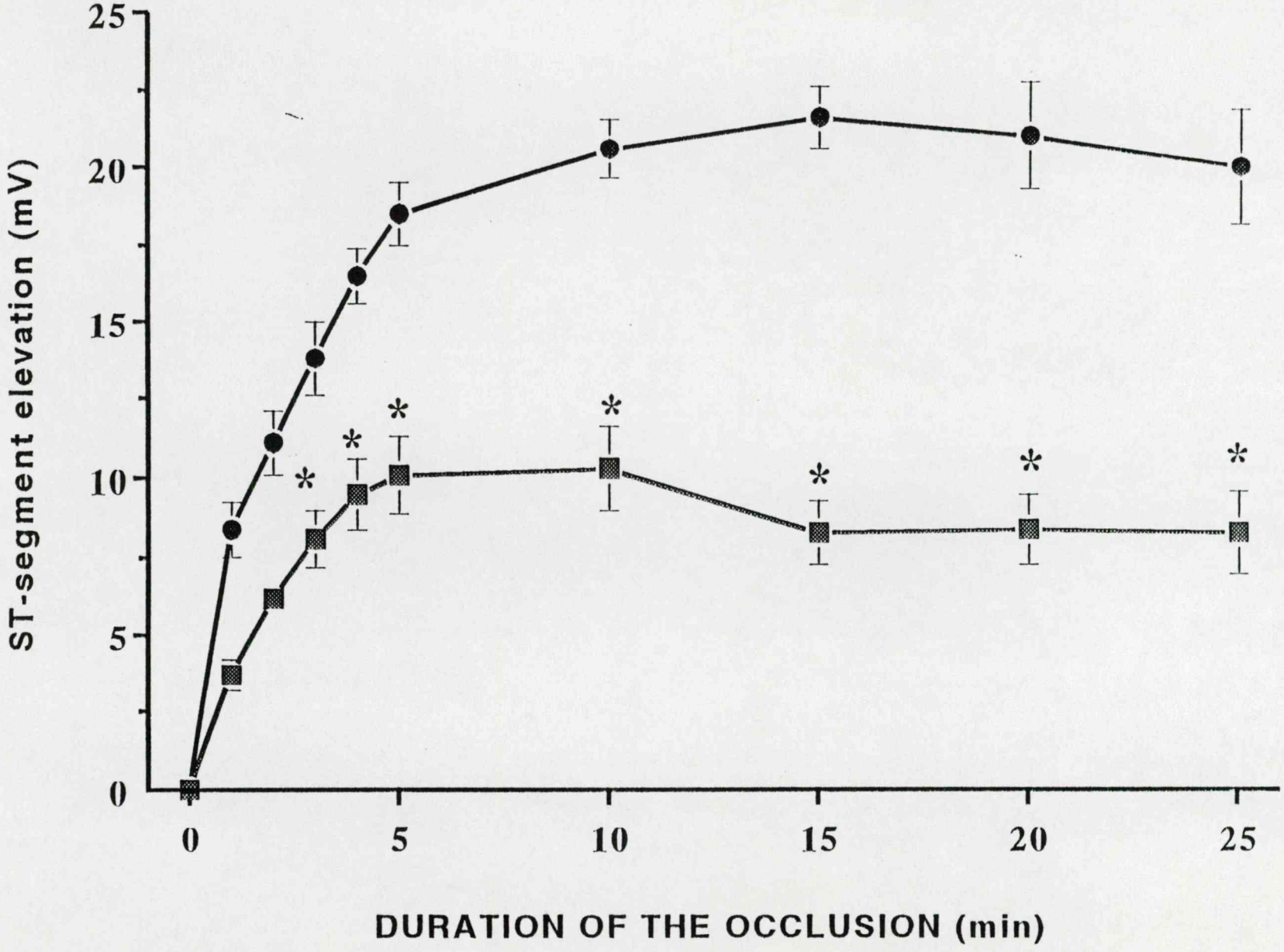
% INCIDENCE OF VT, VF AND SURVIVAL
100
80
60
40
20
0
*


25
20
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5
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ST-segment elevation (mV)
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5
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DURATION OF THE OCCLUSION (min)
*
*
*
*
*
*

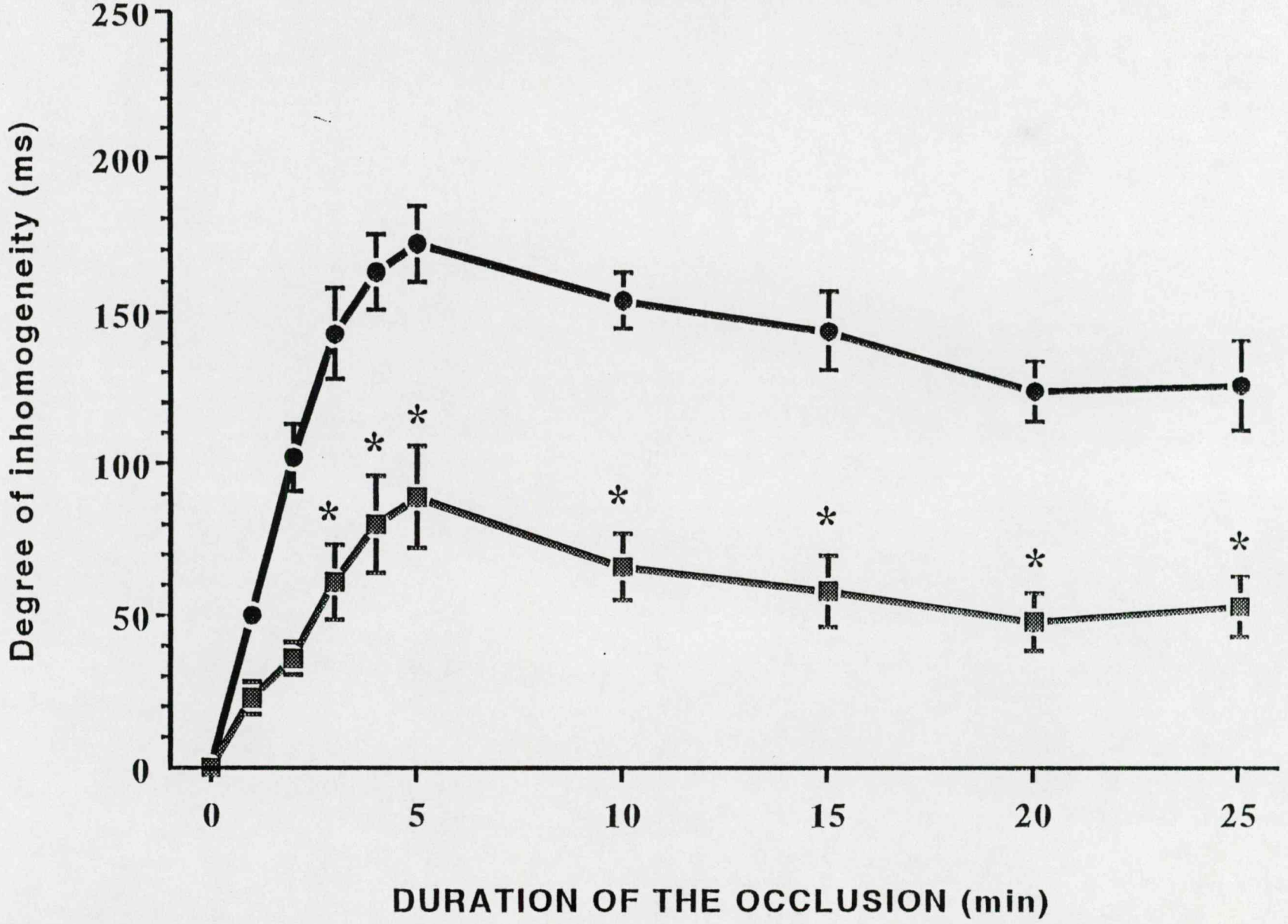
250
200
150
100
50
0
Degree of inhomogeneity (ms)
0
5
10
15
20
25
DURATION OF THE OCCLUSION (min)
*
*
*
*
*
*

Table 1.

Haemodynamic changes following coronary artery occlusion in dogs, pretreated with either saline or atrial natriuretic peptide (10 μg kg^{-1} + 0.1μg kg^{-1} min^{-1} i.v.)

	CONTROL (n=14)		ANP (n=11)	
	initial value	change	initial value	change
Arterial blood pressure				
systolic (mmHg)	118 ± 4.1	-14 ± 2.5*	104 ± 6	-9 ± 2*
diastolic (mmHg)	84 ± 4.1	-12 ± 3*	70 ± 5	-7 ± 2*
mean (mmHg)	96 ± 4	-12 ± 3*	81 ± 5	-8 ± 4*
LVSP (mmHg)	123 ± 8	-15 ± 4*	109 ± 4	-9 ± 2*
LVEDP (mmHg)	5.50 ± 0.43	12.00 ± 0.79*	5.00 ± 0.00	9.00 ± 0.9*#
$LVdP/dt_{max}$				
(+ve:mmHg s^{-1})	2359 ± 157	-589 ± 127*	3221 ± 174	-512 ± 95*
(-ve:mm Hg s^{-1})	2655 ± 231	-630 ± 135*	2592 ± 226	-278 ± 73*#
Heart rate (beats min^{-1})	154 ± 5	5 ± 1	137 ± 5	3 ± 1
LCX diastolic blood flow	82 ± 8	14 ± 3*	104 ± 8	33 ± 6*#
(ml min^{-1})				
LCX diastolic resistance	1.13 ± 0.12	-0.23 ± 0.12	0.72 ± 0.1	-0.23 ± 0.05*
(mmHg ml $^{-1}min^{-1}$)				

Values are means ± s.e.m. * $P < 0.05$ cp initial value; # $P < 0.05$ cp control group. For abbreviations see text.

CARDIOVASCULAR DRUGS AND THERAPY

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Delayed protection against ventricular arrhythmias by monophosphoryl lipid-A in a canine model of ischaemia and reperfusion

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Abstract

Bacterial endotoxin reduces the severity of ventricular arrhythmias which occur when a coronary artery is occluded several hours later. We have now examined in anaesthetised dogs the effects on ischaemia and reperfusion-induced arrhythmias, of a non-toxic derivative component of the endotoxin molecule of the lipid-A (monophosphoryl lipid-A). This was given intravenously, in doses of 10 and 100 $\mu g\ kg^{-1}$, 24 h prior to coronary artery occlusion. Arrhythmia severity was markedly reduced by monophosphoryl lipid-A. During ischaemia, ventricular premature beats were reduced from 315 ± 84 in the vehicle controls to 89 ± 60 (with the lower dose of monophosphoryl lipid-A) and 53 ± 23 ($P < 0.05$) with the higher dose. The incidence of ventricular tachycardia was reduced from 75% to 25% ($P < 0.05$) and 31% ($P < 0.05$), and the number of episodes of ventricular tachycardia from 13.4 ± 4.9 per dog to 1.1 ± 1.1 ($P < 0.05$) and 1.2 ± 0.9 ($P < 0.05$) after doses of 10 and 100 $\mu g\ kg^{-1}$, respectively. The incidence of ventricular fibrillation during occlusion and reperfusion in the control group was 96% (15/16), i.e., only 6% (1/16) dogs survived the combined ischaemia–reperfusion insult. Monophosphoryl lipid-A (100 $\mu g\ kg^{-1}$) significantly reduced the incidence of occlusion-induced ventricular fibrillation (from 50% to 7%; $P < 0.05$), and increased survival following reperfusion to 54% ($P < 0.05$). Monophosphoryl lipid-A also significantly reduced ischaemia severity as assessed from ST-segment elevation recorded from epicardial electrodes as well as the degree of inhomogeneity of electrical activation within the ischaemia area. There were no haemodynamic differences prior to coronary occlusion between vehicle controls and monophosphoryl lipid-A-treated dogs. These results demonstrate that monophosphoryl lipid-A reduces arrhythmia severity 24 h after administration. Although the precise mechanisms are still unclear, there is some evidence that nitric oxide and prostanoids (most likely prostacyclin) may be involved because the dual inhibition of nitric oxide synthase and cyclooxygenase enzymes by administration of aminoguanidine and meclofenamate abolished the marked antiarrhythmic protection resulted from monophosphoryl lipid-A treatment 24 h previously.

Keywords: Bacterial endotoxin; Monophosphoryl lipid-A; Ventricular arrhythmias; Ischaemia; Reperfusion; Nitric oxide (NO); Aminoguanidine; Meclofenamate

1. Introduction

The administration of lipopolysaccharide (endotoxin) derived from *Escherichia coli* results in a delayed protection of the heart against various consequences of acute myocardial ischaemia including necrotic cell death (Rowland et al., 1996; Wu et al., 1996), life-threatening ventricular arrhythmias (Wu et al., 1994, 1996) and the depressed recovery of contractile function which follows a prolonged period of ischaemia and reperfusion (Brown et al., 1989; McDonough and Causey, 1994). The most likely explanation for this paradoxical effect of endotoxin lies in its ability, cytokine mediated, to induce a nitric oxide synthase (iNOS) in a variety of cells including blood vessels (Julou-Schaeffer et al., 1990; Stoclet et al., 1993) and cardiac myocytes (Schulz et al., 1992, 1995). This induction (Radomski et al., 1990), as well as the cardioprotec-

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tive effects of endotoxin (Wu et al., 1994, 1996), are dexamethasone-sensitive. The result of this enzyme induction is a markedly increased generation of nitric oxide (NO) especially under conditions of ischaemia. There is evidence both for the release of NO during ischaemia (Depre and Hue, 1994; Node et al., 1995; Zweier et al., 1995) and for the ability of NO to reduce arrhythmia severity under conditions of ischaemia and reperfusion (recently reviewed by Parratt and Végh, 1996, 1997).

Much of the biological activity and toxicity of endotoxin resides in the lipid-A component of the molecule (Madonna et al., 1986) and there have been various successful attempts to detoxify it (e.g., Takayama et al., 1981). For example, monophosphoryl lipid-A (Qureshi et al., 1982; Takayama et al., 1984), which differs structurally from lipid-A by the absence of a phosphoester at the reducing end of the diglucosamine residue, is about a thousand times less toxic than the parent endotoxin molecule yet retains the tumour regression properties of bacterial lipopolysaccharide and the ability to induce tolerance to endotoxin itself (Astiz et al., 1995). It also reduces myocardial ischaemic damage in dogs (Yao et al., 1993a,b), rabbits (Baxter et al., 1996; Elliott et al., 1996) and rats (Wu et al., 1998) when administered 24 h prior to ischaemia and enhances the recovery of contractile function in rabbit hearts following a prolonged period of ischaemia and reperfusion (Zhao et al., 1996). Attention has been drawn to similarities between the delayed effects of endotoxin and monophosphoryl lipid-A and those of ischaemic preconditioning (Parratt and Szekeres, 1995; Przyklenk et al., 1996).

Up to the present, there have been no studies on the possible antiarrhythmic effect of monophosphoryl lipid-A in a large animal model of ischaemia–reperfusion. The purpose of the present study was to examine whether this compound, when given 24 h prior to an ischaemic episode, reduces the severity of those life-threatening ventricular arrhythmias that occur as a result of coronary artery occlusion and subsequent reperfusion. A preliminary account of these studies was given to the Bologna meeting of the International Society for Heart Research (Végh et al., 1996). We have also attempted to examine the possible mechanisms involved in the antiarrhythmic effect of monophosphoryl lipid-A by inhibiting the formation of NO (with aminoguanidine) and of cardioprotective prostanoids (with sodium meclofenamate) such as prostacyclin.

2. Material and methods

2.1. Animals and experimental design

These have been described in detail elsewhere (e.g., Végh et al., 1992) and will only be briefly described here. Mongrel dogs of both sexes, and with a mean body weight of 21.5 ± 0.9 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., 1992). The temperature was measured from the oesophagus and maintained by a heating pad between 36.8°C 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, respectively as previously described (Végh et al., 1992). The composite electrode 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 coronary artery occlusion, widening and fractionation of the summarised R-wave occurs, indicating that adjacent fibers are not simultaneously activated because of inhomogeneity of conduction. We expressed inhomogeneity of conduction as the greatest delay in activation (in ms) within the ischaemic area.

After a suitable stabilisation period (between 0.5 and 1 h), the anterior descending branch of the left coronary artery was occluded for a period of 25 min and after this time the ischaemic myocardium was rapidly reperfused. Dogs that were alive and predominantly in sinus rhythm 10 min later were designated as survivors from the combined ischaemia–reperfusion insult. In all dogs, Evans blue dye (or patent blue V dye) was infused into the occluded anterior descending branch to estimate the area at risk; this was expressed as a percentage of the left ventricular free wall together with the septum. Ventricular arrhythmias during ischaemia and reperfusion were analysed as previously described (Végh et al., 1992), following, in general, the recommendations laid down at the 'Lambeth Conventions' (Walker et al., 1988) except that no distinction was made between couplets and salvos, which were included as single ventricular ectopic (premature) beats, and that ventricular tachycardia was defined as a run of four or more ectopic beats at a rate faster than the resting sinus rate. In addition, we determined the number of episodes of ventricular tachycardia during coronary artery occlusion in each dog and the incidences of ventricular fibrillation both during occlusion and following reperfusion at the end of the occlusion period.

Systemic arterial blood pressure and systolic as well as end-diastolic left ventricular pressures were measured using suitably calibrated Statham P23XL transducers and recorded by a six channel haemodynamic system (System-6, Triton Technology, USA). They were recorded, together with left ventricular dP/dt and the output from the epi-

cardial and composite electrodes, on an eight channel Medicor R81 recorder. Heart rate was calculated from a limb lead electrocardiogram.

2.2. Experimental protocol

Monophosphoryl lipid-A was given in doses of either 100 μg kg^{-1} ($n = 13$) or 10 μg kg^{-1} ($n = 8$) by intravenous injection 24 h prior to anaesthesia and coronary artery occlusion. We used a total of 16 vehicle-treated control dogs suitably spaced among those given monophosphoryl lipid-A.

In order to examine the possible mechanisms of monophosphoryl lipid-A, two additional groups of dogs were included in this study. These dogs were given 100 μg kg^{-1} monophosphoryl lipid-A similar to that described above but, 24 h later, in five of these dogs, aminoguanidine, a relatively selective inhibitor of iNOS activity (e.g., Kis et al., 1999b) was administered in a dose of 50 mg kg^{-1} 30 min prior to the coronary artery occlusion. In six other dogs, in addition to aminoguanidine, meclofenamate, an inhibitor of cyclooxygenase (2 mg kg^{-1}, intravenously 20 min before the occlusion) was also given. The dose of aminoguanidine used completely abolished the protection against ventricular arrhythmias resulting from cardiac pacing, 24 h before an ischaemia–reperfusion insult (Kis et al., 1999b). Similarly, the dose of meclofenamate used markedly reduces the antiarrhythmic effect of ischaemic preconditioning, induced by brief coronary artery occlusions, in anaesthetised dogs (Végh et al., 1990). Although the experiments were carried out in Szeged, the protocol complies with UK Home Office Regulations (Project License No. 60%/00307).

2.3. Statistical analysis

The data were expressed as means (±S.E.M.) and differences between means were compared by analysis of variance (ANOVA for repeated measures) or the Student's *t*-test as appropriate. A One-way ANOVA was undertaken to determine whether or not there were significant haemodynamic differences between the groups. Ventricular premature beats were compared by using the Mann–Whitney Rank Sum test, and the incidences of ventricular tachycardia, ventricular fibrillation, and survival from the combined ischaemia–reperfusion insult, were compared using the Fisher Exact test. Differences between groups were considered significant when $P < 0.05$.

2.4. Drugs and materials

Monophosphoryl lipid-A was kindly provided in ampules by Drs. Gary Elliott and Patricia Weber of RIBI Immunochem Research, Hamilton, Montana. This was already dissolved in a mixture of 40% propyleneglycol, 10% ethanol and 50% water for injection. Aminoguanidine as the hemisulphate salt and meclofenamate as meclofenamic acid sodium salt were purchased from Sigma.

3. Results

3.1. Haemodynamic effects of monophosphoryl lipid-A and of coronary artery occlusion

The administration of monophosphoryl lipid-A had little effect on any haemodynamic parameter when measured, under anaesthesia, 24 h later (Table 1). Thus, there were no significant differences between monophosphoryl lipid-A-treated and control dogs with respect to arterial blood pressure, heart rate, left ventricular end-diastolic pressure or left ventricular dP/dt_{max} (positive or negative). There was also no significant difference between the groups in the haemodynamic effects of coronary artery occlusion, which were similar to those previously described in detail (Végh et al., 1992). For example, in the high dose monophosphoryl lipid-A group there were slight (less than 10 mm Hg) decreases in mean arterial pressure (e.g., 90 ± 5 to 86 ± 4 mm Hg; $P < 0.05$) and in left ventricular dP/dt_{max} (from 3135 ± 196 to 2923 ± 293 mm Hg s^{-1} (positive) and from 3042 ± 141 to 2792 ± 222 mm Hg s^{-1} (negative) and increases in heart rate (from 152 ± 8 to 164 ± 8 beats min^{-1}), and in left ventricular end-diastolic pressure (from 5.3 ± 1.1 to 18.8 ± 1.6 mm Hg; $P < 0.01$).

3.2. Haemodynamic changes following aminoguanidine, aminoguanidine together with meclofenamate administration and of coronary artery occlusion in monophosphoryl lipid-A-treated dogs

Aminoguanidine, given in a dose of 50 mg kg^{-1}, 30 min prior to coronary artery occlusion in five dogs treated with 100 μg kg^{-1} monophosphoryl lipid-A 24 h previously, significantly increased arterial blood pressure (from

Table 1
Haemodynamic parameters in anaesthetised dogs 24 h after the administration of monophosphoryl lipid-A (MLA: 10 or 100 μg kg^{-1} i.v.) or its vehicle control

	MLA		
	10 μg kg^{-1} ($n = 8$)	100 μg kg^{-1} ($n = 13$)	Vehicle ($n = 16$)
Arterial blood pressure (mm Hg)			
Systolic	129 ± 7	114 ± 7	121 ± 8
Diastolic	94 ± 6	78 ± 5	81 ± 7
Mean	106 ± 7	90 ± 5	94 ± 7
Heart rates (beats min^{-1})	154 ± 4	139 ± 2	163 ± 8
LVEDP (mm Hg)	5.8 ± 0.5	7.3 ± 0.9	5.0 ± 1.2
LV dP/dt_{max} (mm Hg s^{-1})	2182 ± 188	3135 ± 196	2753 ± 353

119 ± 11 to 135 ± 12 mm Hg systolic, from 86 ± 8 to 96 ± 9 mm Hg diastolic, and from 97 ± 9 to 109 ± 10 mm Hg mean; $P < 0.05$) and positive dP/dt_{max} (from 3467 ± 248 to 4055 ± 246 mm Hg s^{-1}; $P < 0.05$), without substantially influencing the other haemodynamic parameters. Similarly, in dogs treated with monophosphoryl lipid-A (100 μg kg^{-1}, 24 h before the occlusion) but given aminoguanidine together with meclofenamate prior to occlusion, the only significant changes were increases in arterial blood pressure (from 133 ± 6 to 156 ± 6 mm Hg systolic, from 82 ± 5 to 121 ± 7 mm Hg diastolic and from 107 ± 4 to 133 ± 7 mm Hg mean; $P < 0.05$) and in positive dP/dt_{max} (from 2673 ± 288 to 3305 ± 280 mm Hg s^{-1}; $P < 0.05$). These returned to initial values prior to the commencement of the coronary artery occlusion.

Occlusion of the left anterior descending coronary artery resulted in similar haemodynamic changes in all the groups. For example, in dogs treated with aminoguanidine, or with aminoguanidine, together with meclofenamate, there were decreases in mean arterial blood pressure of 14 ± 3 and 15 ± 3 mm Hg, respectively and in left ventricular dP/dt_{max} of 677 ± 143 and 590 ± 98 mm Hg s^{-1} (positive) and of 600 ± 143 and 495 ± 90 mm Hg s^{-1} (negative), respectively. There was also similar increases in heart rate (of 5 ± 3 and 10 ± 4 beats min^{-1}, respectively) and in left ventricular end-diastolic pressure (of 14 ± 1 and 15 ± 1 mm Hg, respectively).

3.3. Effects of monophosphoryl lipid-A on ventricular arrhythmias; comparison with controls

Coronary artery occlusion in the 16 vehicle control dogs led to pronounced ventricular ectopic activity commencing within 2–3 min of the onset of the occlusion and with a characteristic distribution of arrhythmias in two phases: phase 1a from 0–8 min and phase 1b from around 11–25 min. There was a somewhat quieter period in most of the dogs, between 7 and 11 min with rather few ventricular ectopic beats during this period. The distribution and time course of these arrhythmias in each of these dogs is shown in Fig. 1. In 8 of the 16 dogs fibrillated during the occlusion period, characteristically between 12 and 18 min, and nearly all the dogs had several episodes of ventricular tachycardia. Only 1 of the 16 dogs survived the ischaemia–reperfusion insult (i.e., 6%). The summarised effects of coronary artery occlusion on arrhythmia severity are shown in Fig. 2.

Prior treatment with monophosphoryl lipid-A, at both dose levels, reduced the severity of ventricular arrhythmias that occurred during ischaemia and reperfusion. There was

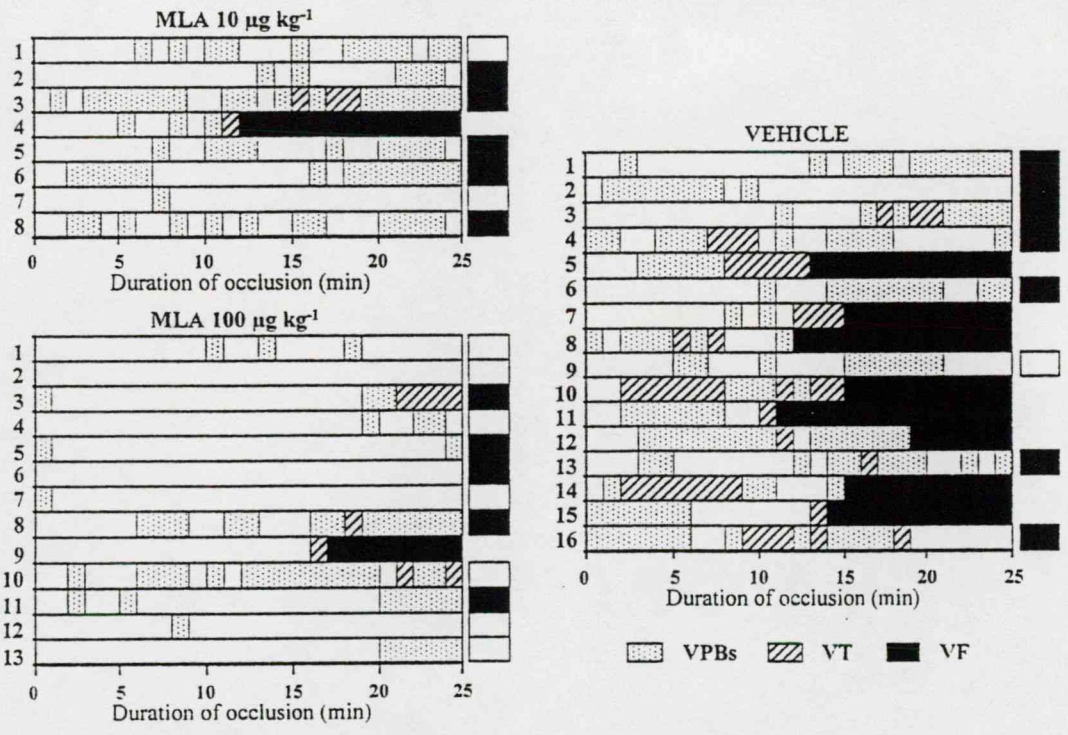

Fig. 1. The distribution of ventricular arrhythmias during a 25-min occlusion of the left anterior descending coronary artery (LAD) in anaesthetised dogs treated with monophosphoryl lipid-A (10 or 100 μg kg^{-1}, 24 h prior to occlusion) or the appropriate vehicle controls. The figures show the time course in each of the treated and vehicle control dogs of ventricular arrhythmias presenting as premature (ectopic) beats (VPBs; stippled bars), ventricular tachycardia (VT; cross-hatched bars) and ventricular fibrillation (VF; black bars). There are usually two distinct phases of arrhythmias in the controls (phase 1a from 3–10 or 11 min; phase 1b from 10 or 11–20 min) whereas phase 1a arrhythmias are not present in dogs treated with monophosphoryl lipid-A (100 μg kg^{-1}). To the right of each figure is shown the response to reperfusion: i.e., those dogs that fibrillated during reperfusion (black bars) and those that survived (open bars). Thus, survival from the combined occlusion–reperfusion was 1/16 in the controls but 7/13 in dogs given the higher dose of monophosphoryl lipid-A.

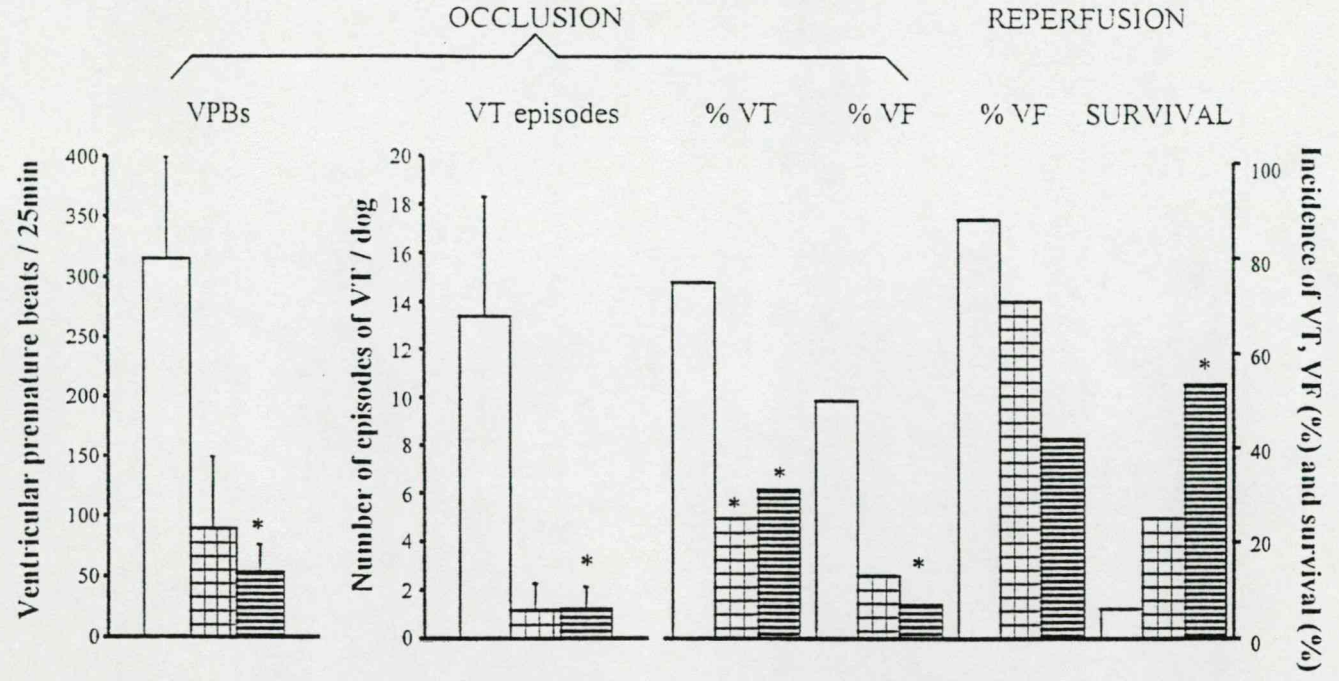

Fig. 2. Ventricular arrhythmias in anaesthetised dogs subjected to coronary artery occlusion after treatment with monophosphoryl lipid-A 10 μg kg^{-1} (cross-shaded histograms), 100 μg kg^{-1} (vertically shaded histograms) or the appropriate vehicle (open histograms). VPBs are the total number of ventricular premature beats during the occlusion period, VT = ventricular tachycardia, VF = ventricular fibrillation. Also shown is the survival from the combined ischaemia–reperfusion insult. * $P < 0.05$ compared to the vehicle controls.

a marked reduction in the number of ventricular premature beats which occurred during the occlusion period (from 315 ± 84 in controls to 89 ± 60; $P > 0.05$ and 53 ± 23; $P < 0.05$) in dogs treated with 10 and 100 μg kg^{-1} monophosphoryl lipid-A, respectively), a significant reduction in the occurrence of ventricular tachycardia (from

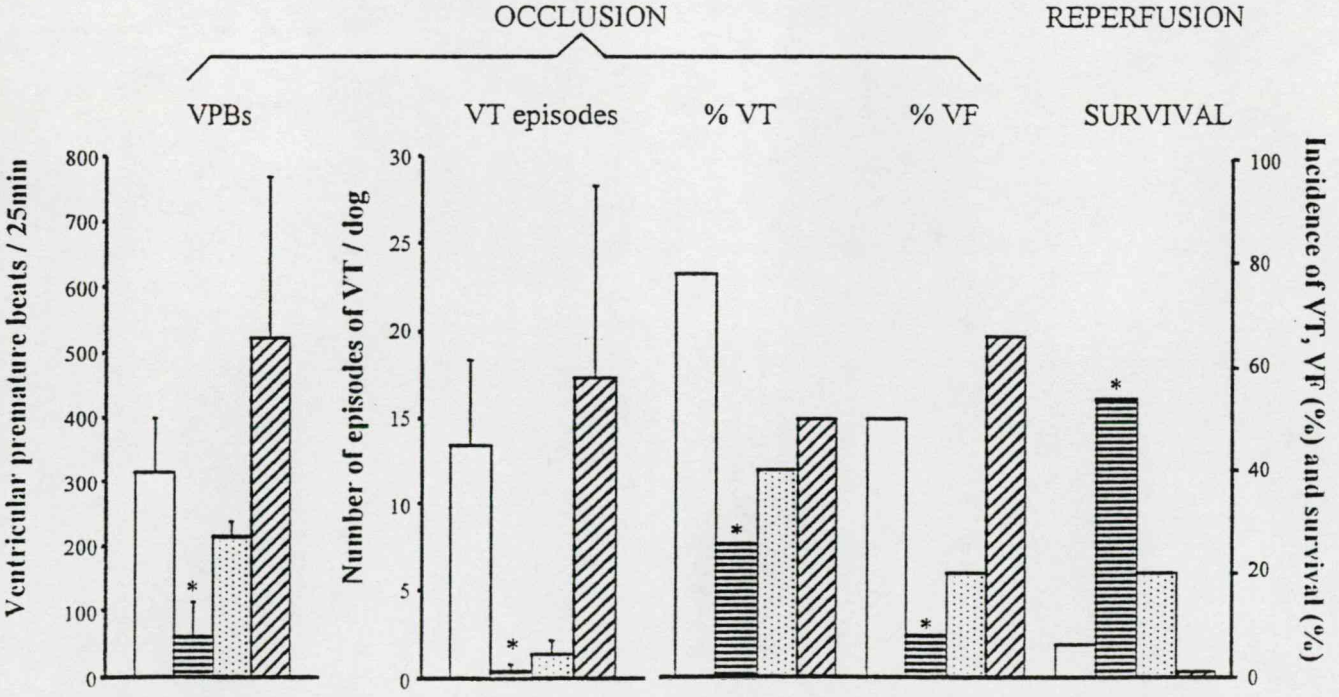

Fig. 3. Ventricular arrhythmias in anaesthetised dogs subjected to coronary artery occlusion after treatment with monophosphoryl lipid-A 100 μg kg^{-1} (vertically shaded histograms), and then given either aminoguanidine (stippled histograms) or aminoguanidine and meclofenamate (hatched histograms), as well as the appropriate vehicle (open histograms). VPBs are the total number of ventricular premature beats during the occlusion period, VT = ventricular tachycardia, VF = ventricular fibrillation. Also shown is survival from the combined ischaemia–reperfusion insult. * $P < 0.05$ compared to the vehicle controls.

75% to 25%; $P > 0.05$, and to 31%; $P < 0.05$) and in the number of the episodes of ventricular tachycardia (from 13.4 ± 4.9 episodes per dog to less than two episodes in dogs given monophosphoryl lipid-A; $P < 0.05$). There was also a significant ($P < 0.05$) reduction in the incidence of ventricular fibrillation during occlusion with the higher dose of monophosphoryl lipid-A (from 50% to 7%; Fig. 2). Of particular interest is the fact that of the 13 dogs given the higher dose of monophosphoryl lipid-A, six had less than six ectopic beats during the whole of the occlusion period and two of them had no ectopic beats whatsoever. Such a marked antiarrhythmic effect is rare even in dogs subjected to ischaemic preconditioning (Végh et al., 1992). The times during occlusion when these arrhythmias occurred in monophosphoryl lipid-A-treated dogs is compared to the vehicle controls in Fig. 1. Monophosphoryl lipid-A also markedly altered the distribution of ventricular premature beats during the occlusion period; there was almost no activity during phase 1a but a significant shift in the distribution of these arrhythmias to the later occlusion time (phase 1b; Fig. 1). Nevertheless, pronounced ventricular ectopic activity had largely disappeared before the end of the occlusion period. Ventricular fibrillation during reperfusion was also significantly reduced by the 100 μg kg^{-1} dose of monophosphoryl lipid-A (Fig. 2) and survival from the combined ischaemia–reperfusion insult was thus increased (54% vs. 6% in the controls; $P < 0.05$).

3.4. The severity of ventricular arrhythmias following inhibition of inducible nitric oxide synthase and cyclooxygenase enzymes in monophosphoryl lipid-A-treated dogs

In a further 11 dogs treated with the 100 μg kg^{-1} dose of monophosphoryl lipid-A, either aminoguanidine alone, or aminoguanidine together with meclofenamate, were administered prior to coronary artery occlusion. The results are summarised in Fig. 3. Whereas inhibition of the iNOS activity with aminoguanidine only attenuated the protective effect of monophosphoryl lipid-A, dual blockade of both the iNOS and cyclooxygenase enzymes completely abolished the protection against arrhythmias. Thus, compared to the monophosphoryl lipid-A alone, in the presence of aminoguanidine, there was only tendency for increases in the number of ventricular premature beats (from 89 ± 60 to 214 ± 60), in the number of episodes of ventricular tachycardia (from 0.8 ± 0.6 to 1.3 ± 0.8) and in the incidence of ventricular tachycardia (from 25% to 40%). Furthermore, the incidence of ventricular fibrillation (1/5) and survival (1/5) from the combined ischaemia–reperfusion insult were not significantly different in dogs treated with aminoguanidine from those not given aminoguanidine. However, when meclofenamate was given in addition to aminoguanidine, the number of ventricular premature beats and episodes of ventricular tachycardia was increased to 522 ± 246 and to 17.3 ± 10.9, respec-

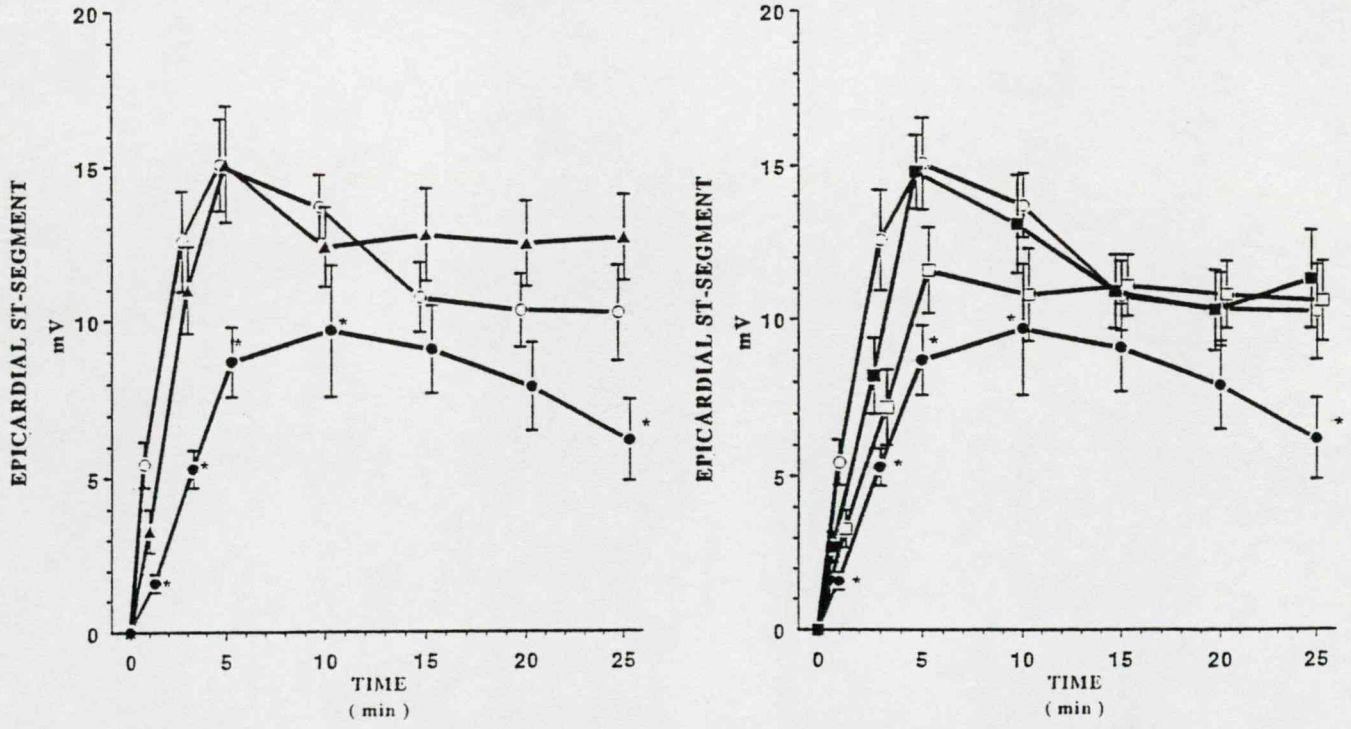

Fig. 4. Changes in the epicardial ST-segment (mV) during a 25-min coronary artery occlusion in dogs given 24 h previously, monophosphoryl lipid-A 100 μg kg^{-1} (filled circles), 10 μg kg^{-1} (filled triangles) or 100 μg kg^{-1} monophosphoryl lipid-A and then either aminoguanidine (open squares) or aminoguanidine and meclofenamate (filled squares), as well as the appropriate vehicle control (open circles). *$P < 0.05$ compared to the vehicle controls.

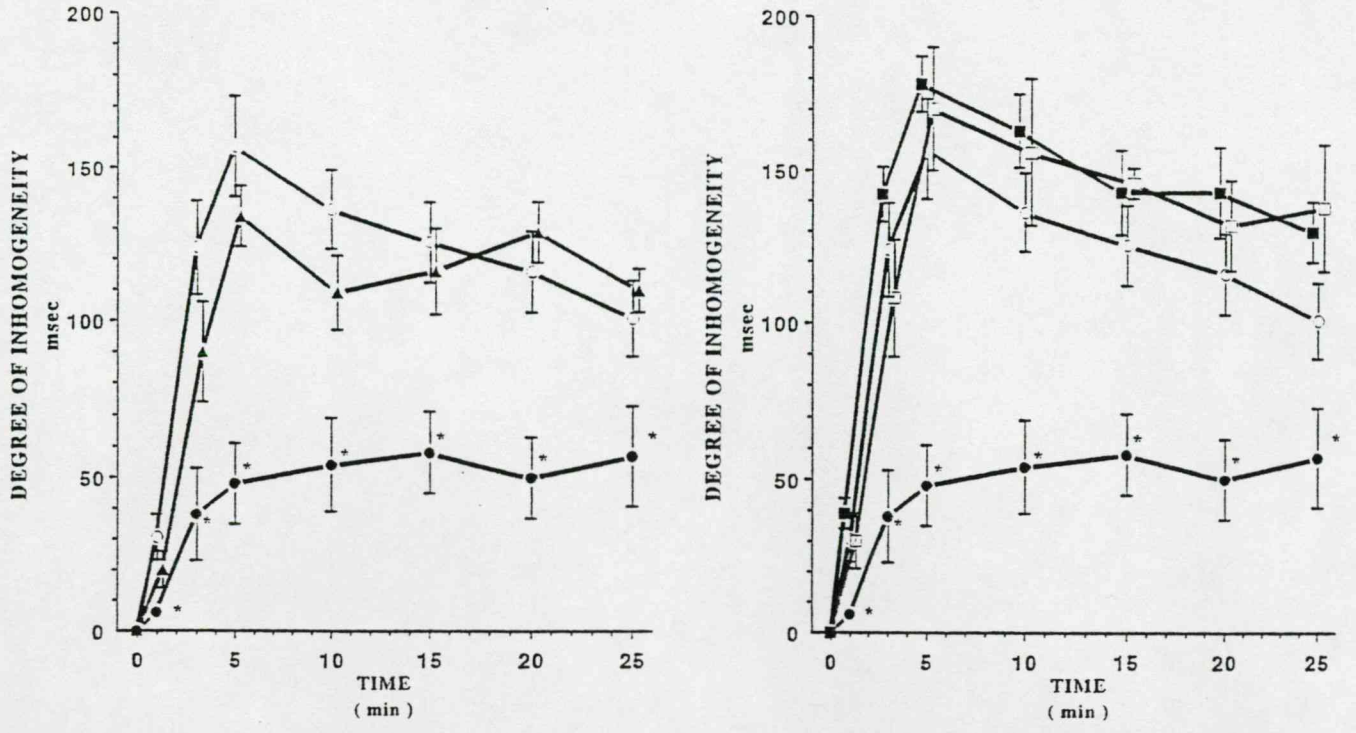

Fig. 5. Changes in the degree of inhomogeneity of electrical activation (ms) during a 25-min coronary artery occlusion in dogs given 24 h previously, monophosphoryl lipid-A 100 μg kg^{-1} (filled circles), 10 μg kg^{-1} (filled triangles) or 100 μg kg^{-1} monophosphoryl lipid-A and then either aminoguanidine (open squares) or aminoguanidine and meclofenamate (filled squares), as well as the appropriate vehicle control (open circles). $^{*}P < 0.05$ compared to the vehicle controls.

tively, values not significantly different from the controls (315 ± 84 and 11.3 ± 7.6, respectively). In the presence of aminoguanidine and meclofenamate, 66% of the dogs fibrillated during occlusion, and no dog survived following reperfusion (Fig. 3).

Neither aminoguanidine (Kis et al., 1999a,b) nor meclofenamate (Wainwright and Parratt, 1991) modify arrhythmia severity resulting from coronary artery occlusion.

3.5. Effects of monophosphoryl lipid-A on indices of ischaemia severity

In the controls, coronary artery occlusion led to marked epicardial ST-segment elevation (maximal at 10 min) and to increases in the degree of inhomogeneity of electrical activation within the ischaemic area (maximal at 5 min). These changes are illustrated in Figs. 4 and 5 respectively.

Treatment with monophosphoryl lipid-A markedly reduced these indices of ischaemia severity (Figs. 4 and 5). Aminoguanidine alone, or in combination with meclofenamate, attenuated or reversed these protective effects of monophosphoryl lipid-A.

There was no difference between the area at risk between any of the groups (40.1 ± 1.3% in the dogs given the higher dose of monophosphoryl lipid-A, 39.1 ± 1.7% in those given the lower dose of monophosphoryl lipid-A, 40.7 ± 0.7% in the vehicle controls, 41.2 ± 1.1% in dogs given 100 μg kg^{-1} monophosphoryl lipid-A and aminoguanidine, and 40.8 ± 2.6 in dogs given this higher dose of monophosphoryl lipid-A and then aminoguanidine and meclofenamate).

4. Discussion

4.1. Cardioprotection by monophosphoryl lipid-A

These results show that the prior administration of this non-toxic derivative of the lipid-A component of the endotoxin molecule markedly reduces the severity of ischaemia (and reperfusion)-induced ventricular arrhythmias in a canine model of ischaemia–reperfusion. The most likely explanation for this antiarrhythmic effect is that ischaemia severity, as demonstrated from recordings of the ST-segment of the epicardial electrocardiogram and from changes in the degree of inhomogeneity of electrical activation within the ischaemic area, is much less marked in the monophosphoryl lipid-A-treated dogs than it is in the vehicle controls. This, despite the fact that the area at risk from infarction is almost identical and that there is no evidence that monophosphoryl lipid-A increases coronary collateral blood flow (Mei et al., 1996). We did not investigate whether this very early reduction in ischaemia (and arrhythmia) severity would lead to a decrease in the area of the ischaemic zone that ultimately becomes necrotic.

Others have shown in doses somewhere between the two that we have used in the present study, that monophosphoryl lipid-A does reduce infarct size, when given several hours prior to coronary artery occlusion in dogs (Yao et al., 1993a,b; Przyklenk et al., 1996) and in rabbits (Baxter et al., 1996; Elliott et al.,1996). These effects on infarct size have been recently summarised and reviewed by Gross (1998). The delayed effects of monophosphoryl lipid-A are thus rather similar to those of endotoxin itself, which both reduces arrhythmia severity and myocardial ischaemic damage when given several hours prior to coronary artery occlusion in rats (Wu et al., 1996), although in doses considerably higher than those used in the canine and rabbit studies. This presumably reflects the differences in sensitivity to endotoxin, and thus to monophosphoryl lipid-A, between these species.

4.2. Relation of monophosphoryl lipid-A with other forms of delayed cardioprotection; possible role of NO

There are striking similarities between the effects of monophosphoryl lipid-A administration and the delayed cardioprotection associated with ischaemic preconditioning and with cardiac pacing (Parratt and Szekeres, 1995; Végh and Parratt, 1998). All these manifestations of ischaemic injury (arrhythmias, cellular damage, depressed recovery of contractile function) are beneficially modified both by monophosphoryl lipid-A and by ischaemic preconditioning perhaps suggesting that similar mechanisms are involved. Indeed, the reason why the cardioprotective effects of endotoxin were examined initially (Wu et al., 1994) was that one hypothesis for the antiarrhythmic effects of ischaemic preconditioning involves the formation of NO from endothelial cells, the generation of which is stimulated by bradykinin release (reviewed by Parratt and Végh, 1996, 1997). There is also recent evidence that the enhanced recovery of contractile function from a period of ischaemia that results from delayed preconditioning is also NO-mediated (Bolli et al., 1997, 1998). Because it is well-known that bacterial endotoxin induces NO synthase (see Section 1), and because both the antiarrhythmic effects of preconditioning (Végh et al., 1994) and of bacterial endotoxin (Wu et al., 1994, 1996) are attenuated by dexamethasone, it is possible that this delayed cardioprotection by monophosphoryl lipid-A is also NO-mediated. There is recent direct evidence for this. Zhao et al. (1997) have recently demonstrated in rabbits that the ability of monophosphoryl lipid-A to reduce infarct size is prevented by the prior administration of aminoguanidine, a reasonably selective inhibitor of the inducible isoform of NO synthase. However, unlike studies with endotoxin itself, increased iNOS activity was only demonstrated following ischaemia; levels were not increased in left ventricular samples taken prior to coronary artery occlusion. These changes in activity were also rather small and much less marked than the increases that occur in the heart and vessels following the administration of bacterial endotoxin. Similarly, Maulik et al. (1998) reported that in hearts, isolated from monophosphoryl lipid-A-treated rats, the recovery from ischaemia–reperfusion injury was markedly improved and this was due to an expression of the iNOS.

However, in the present experiments, we have found that aminoguanidine given to dogs treated with monophosphoryl lipid-A, in a dose that significantly attenuated the antiarrhythmic protection resulting from cardiac pacing (Kis et al., 1999b), only slightly modified the protective effect of monophosphoryl lipid-A (Fig. 3). Even a higher dose of aminoguanidine (100 mg kg^{-1}; data not shown) was unable to reverse the protection resulted from monophosphoryl lipid-A treatment. However, when in addition to aminoguanidine sodium meclofenamate, an inhibitor of the cyclooxygenase pathway, was given the antiarrhythmic protection resulting from monophosphoryl lipid-A was completely abolished. Neither aminoguanidine nor meclofenamate, when given to dogs not treated with monophosporyl lipid-A, modify the severity of ventricular arrhythmias resulting from coronary artery occlusion (Wainwright and Parratt, 1991; Kis et al., 1999b). These results indicate that endogenous substances other than NO such as prostanoids derived from cyclooxygenase activation are also involved in the antiarrythmic effect of monophosphoryl lipid-A. Further, it is likely that monophosphoryl lipid-A represents stronger stimulus for myocardial protection than does cardiac pacing, since the dose of aminoguanidine which almost completely abolished the protective effects of cardiac pacing against arrhythmias (Kis et al., 1999a) only slightly attenuated the antiarrhythmic effect of monophosphoryl lipid-A.

The time course for the protection afforded by monophosphoryl lipid-A has not been examined in the present study. If there are parallels with endotoxin itself, then one would expect that the time course of protection, against both arrhythmias and infarct size, would follow that for the induction of the enzyme. In the endotoxin studies referred above (Wu et al., 1994, 1996), the time course of cardioprotection by bacterial endotoxin was indeed examined; protection, at least against arrhythmias, was apparent 4 h after intraperitoneal injection, was maximal at 8 h, still present at 24 h but had disappeared 48 h after administration. We also do not know whether repeated administration of MLA would protect the myocardium for a longer period of time. In other studies of delayed cardioprotection against ventricular arrhythmias, for example, that induced by cardiac pacing, the time course of the protection is similar to that described for endotoxin but can be prolonged by repeating the preconditioning stimulus at a time when the protection afforded by the initial stimulus has faded (Kaszala et al., 1996; Kis et al., 1999a). It would be interesting to determine whether it is possible to maintain and prolong protection against life-threatening ventricular arrhythmias by repeating the monophosphoryl lipid-A stimulus.

There are other possible explanations for the beneficial effects of monophosphoryl lipid-A. In both dogs (Mei et al., 1996) and rabbits (Elliott et al., 1996), the protection induced by monophosphoryl lipid-A in reducing myocardial ischaemic damage is abolished by drugs (glibenclamide and 5-hydroxydecanoate) that block the ATP-sensitive potassium channels (reviewed by Gross, 1998). This again is similar to studies with ischaemic preconditioning (reviewed by Parratt and Kane, 1994 and by Grover, 1996) although there is uncertainty as to whether the antiarrhythmic effects of ischaemic preconditioning involve these channels (Végh et al., 1993).

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References

Astiz, M.E., Rackow, E.C., Still, G.J., Howell, S.T., Cato, A., Von Eschen, K.B., Ulrich, J.T., Rudbach, J.A., McMahon, G., Vargas, R., Stern, W., 1995. Pretreatment of normal humans with monophosphoryl lipid-A induces tolerance to endotoxin: a prospective, double blind, randomized, controlled trial. Crit. Care Med. 23, 9–17.

Baxter, G.F., Goodwin, R.W., Wright, M.J., Kerac, M., Heads, R.J., Yellon, D.M., 1996. Myocardial protection after monophosphoryl lipid-A: studies of delayed anti-ischaemic properties in rabbit heart. Br. J. Pharmacol. 117, 1685–1692.

Bolli, R., Bhatti, Z.A., Tang, X.-L., Zhang, Q., Guo, Y., Jadoon, A.K., 1997. Evidence that the late preconditioning against myocardial stunning in conscious rabbits is triggered by the generation of nitric oxide. Circ. Res. 81, 42–52.

Bolli, R., Dawn, B., Tang, X.-L., Qiu, Y., Ping, P., Zhang, J., Takano, H., 1998. Delayed preconditioning against myocardial stunning. In: Baxter, G.F., Yellon, D.M. (Eds.), Delayed Preconditioning and Adaptive Cardioprotection. Kluwer Academic Publishers, pp. 29–47.

Brown, J.M., Grosso, M.A., Terada, L.S., Whitman, G.J.R., Banerjee, A., White, C.W., Harken, A.H., Pepine, J.E., 1989. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischaemia–reperfusion injury of isolated rat heart. Proc. Natl. Acad. Sci. U.S.A. 86, 2526–2530.

Depre, C., Hue, L., 1994. Cyclic GMP in the perfused rat heart: effect of ischemia, anoxia and nitric oxide synthase inhibitor. FEBS Lett. 345, 241–245.

Elliott, G.T., Comerford, M.L., Smith, J.R., Zhao, L., 1996. Myocardial ischaemia/reperfusion protection using monophosphoryl lipid-A is abrogated by the ATP-sensitive potassium channel blocker, glibenclamide. Cardiovasc. Res. 32, 1071–1080.

Gross, G.J., 1998. Endotoxin, monophosphoryl lipid-A and delayed cardioprotection. In: Baxter, G.F., Yellon, D.M. (Eds.), Delayed Preconditioning and Adaptive Cardioprotection. Kluwer Academic Publishers, pp. 171–189.

Grover, G.J., 1996. Role of ATP-sensitive potassium channels in myocardial preconditioning. In: Wainwright, C.L., Parratt, J.R. (Eds.), Myocardial Preconditioning. Springer, Berlin, pp. 129–146.

Julou-Schaeffer, G., Gray, G.A., Fleming, I., Schott, C., Parratt, J.R., Stoclet, J.-C., 1990. Loss of vascular responsiveness induced by endotoxin involves the L-arginine pathway. Am. J. Physiol. 259, H1038–H1043.

Kaszala, K., Végh, Á., Papp, J.Gy., Parratt, J.R., 1996. Time-course of the protection against ischaemia and reperfusion-induced ventricular arrhythmias resulting from brief periods of cardiac pacing. J. Mol. Cell. Cardiol. 28, 2085–2095.

Kis, A., Végh, Á., Papp, J.Gy., Parratt, J.R., 1999a. Repeated pacing extends the time during which canine hearts are protected against ischaemia-induced arrhythmias: role of nitric oxide. J. Mol. Cell. Cardiol. 31, 1229–1243.

Kis, A., Végh, Á., Papp, J.Gy., Parratt, J.R., 1999b. Pacing-induced delayed protection against arrhythmias is attenuated by aminoguanidine, an inhibitor of nitric oxide synthase. Br. J. Pharmacol. 127, 1545–1550.

Madonna, G.S., Peterson, J.E., Ribi, E.E., Vogel, S.N., 1986. Early phase endotoxin tolerance induction by a detoxified lipid-A derivative, monophosphoryl lipid-A. Infect. Immun. 52, 6–11.

Maulik, N., Tosaki, Á., Elliott, G.T., Maulik, G., Das, D.K., 1998. Induction of iNOS gene expression by monophosphoryl lipid-A: a pharmacological approach for myocardial adaptation to ischemia. Drugs Exp. Clin. Res. 24, 117–124.

McDonough, K.H., Causey, K.M., 1994. Effects of sepsis on recovery of the heart from 50 min ischaemia. Shock 1, 432–437.

Mei, D.A., Elliott, G.T., Gross, G.J., 1996. K_{ATP} channels mediate late preconditioning against infarction produced by monophosphoryl lipid-A. Am. J. Physiol. 271, H2723–H2729.

Node, K., Kitakaze, M., Kosaka, H., Komamura, K., Minamino, T., Tada, M., Inoue, M., Hori, M., Kamada, T., 1995. Plasma nitric oxide end products are increased in the ischemic canine heart. Biochem. Biophys. Res. Commun. 211, 370–374.

Parratt, J.R., Kane, K.A., 1994. K_{ATP} channels in ischaemic preconditioning. Cardiovasc. Res. 28, 783–787.

Parratt, J.R., Szekeres, L., 1995. Delayed protection of the heart against ischaemia. Trends Pharmacol. Sci. 16, 352–355.

Parratt, J.R., Végh, Á., 1996. Endothelial cells, nitric oxide and ischaemic preconditioning. Basic Res. Cardiol. 91, 27–30.

Parratt, J.R., Végh, Á., 1997. Delayed protection against ventricular arrhythmias by cardiac pacing. Heart 78, 423–425.

Przyklenk, K., Zhao, L., Kloner, R.A., Elliott, G.T., 1996. Cardioprotection with ischaemic preconditioning and MLA: role of adenosine-regulating enzymes? Am. J. Physiol. 271, H1004–H1014.

Qureshi, N., Takayama, K., Ribi, E.E., 1982. Purification and structural determination of nontoxic lipid-A obtained from lipopolysaccharide of *Salmonella typhimurium*. J. Biol. Chem. 257, 11808–11815.

Radomski, M.W., Palmer, R.M.J., Moncada, S., 1990. Glucocorticoids inhibit the expression of an inducible, but not the constitutive nitric oxide synthase in vascular endothelial cells. Proc. Natl. Acad. Sci. U.S.A. 87, 10043–10047.

Rowland, R.T., Cleveland, J.C., Meng, C., Ao, L., Harken, A.H., Brown, J.M., 1996. A single endotoxin challenge induces delayed myocardial protection against infarction. J. Surg. Res. 63, 193–198.

Schulz, R., Nava, E., Moncada, S., 1992. Induction and potential biological relevance of Ca^{2+}-independent nitric oxide synthase in the myocardium. Br. J. Pharmacol. 105, 575–580.

Schulz, R., Panas, D.L., Catena, R., Moncada, S., Olley, P.M., Lopaschuk, G.D., 1995. The role of nitric oxide in cardiac depression induced by interleukin-1β and tumor necrosis factor-α. Br. J. Pharmacol. 114, 27–34.

Stoclet, J.-C., Fleming, I., Gray, G.A., Julou-Schaeffer, G., Schneider, F., Schott, C., Parratt, J.R., 1993. Nitric oxide and endotoxaemia. Circulation 87, V77–V80. (suppl. V).

Takayama, K., Ribi, E.E., Cantrell, J.L., 1981. Isolation of a nontoxic

lipid-A fraction containing tumour regression activity. Cancer Res. 41, 2654–2657.

Takayama, K., Qureshi, N., Ribi, E.E., Cantrell, J.L., 1984. Separation and characterization of toxin and nontoxic forms of lipid-A. Rev. Infect. Dis. 6, 439–443.

Végh, Á., Parratt, J.R., 1998. Delayed preconditioning against ventricular arrhythmias. In: Baxter, G.F., Yellon, D.M. (Eds.), Delayed Preconditioning and Adaptive Cardioprotection. Kluwer Academic Publishers, pp. 63–91.

Végh, Á., Szekeres, L., Parratt, J.R., 1990. Protective effects of preconditioning of the ischaemic myocardium involve cyclo-oxygenase products. Cardiovasc. Res. 24, 1020–1023.

Végh, Á., Komori, S., Szekeres, L., Parratt, J.R., 1992. Antiarrhythmic effects of preconditioning in anaesthetised dogs and rats. Cardiovasc. Res. 26, 487–495.

Végh, Á., Papp, J.Gy., Szekeres, L., Parratt, J.R., 1993. Are ATP sensitive potassium channels involved in the pronounced antiarrhythmic effects of preconditioning? Cardiovasc. Res. 27, 638–643.

Végh, Á., Papp, J.Gy., Parratt, J.R., 1994. Prevention by dexamethasone of the marked antiarrhythmic effects of preconditioning induced 20 h after rapid cardiac pacing. Br. J. Pharmacol. 113, 1081–1082.

Végh, Á., Papp, J.Gy., Elliott, G.T., Parratt, J.R., 1996. Pretreatment with monophosphoryl lipid-A (MPL-C) reduces ischaemia reperfusion-induced arrhythmias in dogs. J. Mol. Cell. Cardiol. 28, A56.

Wainwright, C.L., Parratt, J.R., 1991. Failure of cyclo-oxygenase inhibition to protect against arrhythmias induced by ischaemia and reperfusion: implications for the role of prostaglandins as endogenous myocardial protective substances. Cardiovasc. Res. 25, 93–100.

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.G., Lab, M.J., Manning, A.S., Northover, B.J., Parratt, J.R., Riemersma, R.A., Riva, E., Russel, 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.

Wu, S., Furman, B.L., Parratt, J.R., 1994. Attenuation by dexamethasone of endotoxin protection against ischaemia-induced ventricular arrhythmias. Br. J. Pharmacol. 113, 1083–1084.

Wu, S., Furman, B.L., Parratt, J.R., 1996. Delayed protection against ischaemia-induced ventricular arrhythmias and infarct size limitation by the prior administration of *Escherichia coli* endotoxin. Br. J. Pharmacol. 118, 2157–2163.

Wu, S., Furman, B.L., Parratt, J.R., 1998. Monophosphoryl lipid-A reduces both arrhythmia severity and infarct size in a rat model of ischaemia. Eur. J. Pharmacol. 345, 285–287.

Yao, Z., Auchampach, J.A., Pieper, G.M., Gross, G., 1993a. Cardioprotective effects of monophosphoryl lipid-A, a novel endotoxin analogue, in the dog. Cardiovasc. Res. 27, 832–838.

Yao, Z., Rasmussen, J.L., Hirt, J.L., Mei, D.A., Pieper, G.M., Gross, G.J., 1993b. Effects of monophosphoryl lipid-A on myocardial ischaemia/reperfusion injury in dogs. J. Cardiovasc. Pharmacol. 22, 653–663.

Zhao, L., Kirsch, C.C., Hagen, S.R., Elliott, G.T., 1996. Preservation of global cardiac function in the rabbit following protracted ischaemia/reperfusion using monophosphoryl lipid-A (MLA). J. Mol. Cell. Cardiol. 28, 197–208.

Zhao, L., Weber, P.A., Smith, J.R., Comerford, M.L., Elliott, G.T., 1997. Role of inducible nitric oxide synthase in pharmacological "preconditioning" with monophosphoryl lipid-A. J. Mol. Cell. Cardiol. 29, 1567–1576.

Zweier, J.L., Wang, P., Kuppusamy, P., 1995. Direct measurement of nitric oxide generation in the ischemic heart using electron paramagnetic resonance spectroscopy. J. Biol. Chem. 270, 304–307.

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Isosorbide-2-mononitrate reduces the consequences of myocardial ischaemia, including arrhythmia severity: implications for preconditioning

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Short running title: isosorbide-2-mononitrate and myocardial ischaemia

Abstract

The effects of the intracoronary administration of isosorbide-2-mononitrate (ISMN; 3 $\mu g\ kg^{-1}\ min^{-1}$), a major metabolite of isosorbide dinitrate, were examined in chloralose-urethane anaesthetized dogs before and during a 25 min, acute occlusion of the left anterior descending coronary artery. The only significant haemodynamic effects of ISMN administration were a slight (-11 ± 2 mmHg) decrease in arterial blood pressure and a decrease (< 12 %) in diastolic coronary vascular resistance. Coronary occlusion in the presence of ISMN led to a markedly reduced incidence and severity of ventricular arrhythmias compared to those in control, saline-infused dogs. There were fewer ectopic beats (62 ± 35 v 202 ± 72; $P< 0.05$), a lower incidence (25 % v 75 %; $P< 0.05$) and number of episodes (0.7 ± 0.4 v 4.3 ± 2.1; $P< 0.05$) of ventricular tachycardia and fewer dogs fibrillated during the ischaemic period (17 % v 82 %; $P< 0.05$). More dogs given ISMN survived the combined ischaemia-reperfusion insult (50 % v 0 %; $P< 0.05$). Changes in ST-segment elevation (recorded by epicardial electrodes) and in the degree of inhomogeneity of electrical activation within the ischaemic area were much less pronounced throughout the occlusion period in dogs given ISMN. These results add weight to the hypothesis that the previously reported antiarrhythmic effects of ischaemic preconditioning, and of the intracoronary administration of nicorandil, involve nitric oxide.

Key Words: isosorbide-2-mononitrate, myocardial ischaemia, ventricular arrhythmias, ischaemic preconditioning, nitric oxide, reperfusion

1. Introduction

In two recent studies [1, 2] designed to analyse the effect of opening K_{ATP} channels (with cromakalim and nicorandil given by local coronary infusion) on the consequences of coronary artery occlusion, and especially arrhythmia severity, the conclusion was reached that opening these channels prior to ischaemia does not greatly influence the ischaemic process in anaesthetised dogs. The fact that the administration of nicorandil, but not cromakalim, actually benefited the ischaemic myocardium was attributed to its ability to donate nitric oxide (NO). If this conclusion is valid then other drugs with a similar ability to donate NO should also reduce the consequences of coronary artery occlusion in the same canine ischaemia-reperfusion model. Further, such studies would bear on the hypothesis that NO is involved in the protection against ischaemia-induced arrhythmias afforded by ischaemic preconditioning [3, 4, 5, 6] an hypothesis the depends on the generation of NO by coronary vascular endothelial cells as a result of early, ischaemia-induced bradykinin release [7, 8]. This hypothesis has yet to be directly tested, at least in the experimental model in which it was initally proposed, by infusing an NO-donor locally into the blood supply to the potentially ischaemic myocardium. We have tested these possibilities by examining the effects on the ischaemic canine myocardium of isosorbide-2-mononitrate which, in isolated bovine coronary arteries acts, like NO itself, by elevating cyclic GMP through the cysteine-dependent stimulation of soluble guanylate cyclase [9].

The 5-and 2-mononitrates of isosorbide, which are the main metabolites of isosorbide dinitrate [10] were originally studied as alternatives to conventional antianginal drugs, the potential clinical advantage being that they are just as effective as the parent compound [11] yet have longer elimination half-lives [12]. More recently a combination of oral isosorbide mononitrate

and captopril has been shown to reduce the incidence of ventricular arrhythmias in the early phase of acute clinical myocardial infarction [13]. The vascular effects of isosorbide-2-mononitrate are basically similar to those of the parent compound [14, 15], but the drug is particularly interesting in that it has been shown to be more active than the parent isosorbide dinitrate, or the 5-mononitrate metabolite, in inhibiting platelet aggregation and thromboxane generation [16] partially through its ability to synergize with prostacyclin [17].

There have been two previous canine studies examining the effects of isosorbide-2-mononitrate, both of which used a conscious dog model [18, 19]. These showed, as expected, that the compound reduced arterial blood pressure, and the electrocardiographic (ST-segment) consequences of intermittent and brief coronary artery occlusions [18], and increased heart rate and plasma renin activity [19]. There have been no studies designated to determine the effects of this compound on coronary vascular resistance, or on the severity of ventricular arrhythmias during periods of coronary artery occlusion and reperfusion, although there is one study [20] which showed that isosorbide-2-mononitrate reduced mortality in rats after an acute myocardial infarction, an effect that might have been the result of a reduction in the incidence of ventricular fibrillation.

2. Methods

2.1. Animals and experimental design

We used mongrel dogs, of either sex, and with a body weight excess of 17 kg (mean 26.7 ± 5.8 kg), anaesthetised with a mixture of chloralose and urethane (60 and 200 mg kg^{-1} i.v., respectively). The anaesthesia was maintained by the administration of an additional slow injection of the same anaesthetics (dose volume of 0.5 ml kg^{-1}) when it was necessary. The dogs were ventilated with room air using a Harvard Respirator at a rate and volume sufficient to maintain arterial blood gases and pH within normal limits [21]. A thoracotomy was performed at the fifth intercostal space and the anterior descending branch of the left coronary artery (LAD) prepared for occlusion just proximal to the first diagonal branch and a silk thread was placed around it. Myocardial ischaemia was produced by tightening the thread around the coronary artery by means of a plastic tube pushed gently to the myocardial surface. The occlusion was kept for 25 min. After this time the coronary artery was rapidly reperfused by pulling the tube from the myocardial surface and loosening the thread around the coronary artery.

In twelve dogs a side branch of this artery, proximal to the occlusion, was catheterised, using a thin polyethylene catheter (internal diameter 2 mm), for the intracoronary administration of isosorbide-2-mononitrate, which was given in a dose of 3 μg kg^{-1} min^{-1} by means of slow infusion pump (rate 0.5 ml min^{-1}), commencing 20 min before the occlusion and maintained throughout the entire occlusion period. This dose was calculated from that used in a previous study [15]. We have also considered our previous studies with nicorandil and cromakalim and

tried to select a dose of isosorbide-2-mononitrate which produced a similar increase in coronary blood flow. The coronary artery was then occluded for 25 min, followed by rapid reperfusion. A further twelve dogs which were given saline for the same period and rate, and subjected to coronary artery occlusion and reperfusion served as controls.

Polyethylene catheters (internal diameter 5 mm) were inserted into the right femoral artery for monitoring arterial blood pressure, into the left ventricle (for the measurement of left ventricular systolic and diastolic pressures and left ventricular dP/dt) and the right femoral vein (for anaesthetic administration). Blood flow was measured with an electromagnetic flow probe coupled to a Statham SP 2202 flowmeter. 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 [21]. This electrode 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 large single spike. However, following occlusion, widenning and fractionation of the summarized R-waves occurs, indicating that adjacent fibres are not activated simultaneously because of inhomogeneity of conduction. We expressed this degree of inhomogeneity as the delay in activation (ms) within the ischaemic area at different times during the occlusion. All parameters, together with a limb lead electrocardiogram, were recorded on an eight channel Medicor R81 recorder. Ventricular arrhythmias were assessed as described previously [21, 1]. In brief, the total number of ventricular premature beats, the incidence and number of episodes of ventricular tachycardia (defined as a run of four or more ventricular premature beats at a rate faster than the resting heart rate), and the incidence of ventricular fibrillation were assessed. Because there is some evidence that the area at risk can modify the severity of ventricular arrhythmias following coronary artery occlusion, this was measured at the end of each experiment by injecting patent blue V dye into the re-occluded coronary artery; it was expressed

as a percentage of the left ventricular wall together with the septum. There were no significant differences between the two groups in respect to body weights (controls, 26 ± 3 kg and isosorbide-2-mononitrate treated dogs 25 ± 3 kg) and in the area at risk (controls, 36.7 ± 2.8 % and isosorbide-2-mononitrate treated dogs 35.4 ± 2.1%).

2.2. Ethics

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

2.3. Statistics

All data are expressed as means ± s.e.m. and the differences between means were compared by Student's *t* test. Ventricular premature beats were compared using the Mann-Whitney U test and were presented as mean ± s.e.m. for sake of simplicity. For comparison of incidences of arrhythmias (ventricular tachycardia, ventricular fibrillation and survival from the combined occlusion-reperfusion insult) the Fisher exact probability test was used. Differences between groups were considered significant when P was < 0.05.

3. Results

3.1. The haemodynamic effects of intracoronary isosorbide-2-mononitrate

The intracoronary infusion of isosorbide-2-mononitrate resulted in a slight reduction in mean arterial blood pressure (96 ± 2 mmHg prior to infusion and 86 ± 3 mmHg; $P < 0.05$ immediately before coronary artery occlusion, i.e. after 30 min of infusion), in left ventricular systolic pressure (from 114 ± 5 mmHg to 103 ± 5 mmHg; $P < 0.05$). There were no significant changes in positive left ventricular dP/dt_{max} (2625 ± 229 to 2530 ± 240 mmHg s^{-1}), end-diastolic pressure (5.2 ± 0.4 to 5.6 ± 0.3 mmHg) or heart rate (130 ± 5 to 132 ± 6 beats min^{-1}). There was also no effect on the epicardial ST-segment or on the degree of inhomogeneity of electrical activation (69 ± 5 ms to 72 ± 6 ms). Diastolic coronary vascular resistance decreased slightly but significantly from 5 min after the onset of infusion (from 0.64 ± 0.06 to 0.60 ± 0.05 mmHg ml^{-1} min at this time; $P < 0.05$), and especially just prior to coronary artery occlusion (e.g. 0.57 ± 0.04 mmHg ml^{-1} min, $P < 0.05$ at 20 min). This decrease in resistance was due both to an increase in coronary blood flow (of 12 % from the control flow (diastolic) of 139 ± 11 ml min^{-1}) and to a reduction in coronary perfusion pressure. These changes were quantitatively similar to those observed in the previous study with nicorandil [1].

3.2. Haemodynamic changes after coronary artery occlusion

In both saline control and isosorbide-2-mononitrate-treated groups the left anterior descending coronary artery was occluded for a period of 25 min; after this time the myocardium was rapidly reperfused. In control dogs coronary artery occlusion resulted in a slight reduction in arterial

blood pressure (from 108 ± 5 to 102 ± 7 mmHg; $P < 0.05$), in left ventricular dP/dt_{max} (positive 2806 ± 361 to 2534 ± 358 mmHg s^{-1}; $P < 0.05$ and negative 3885 ± 417 to 3389 ± 393 mm Hg s^{-1}; $P < 0.05$) and a marked increase in left ventricular end-diastolic pressure (from 5 ± 1 to 20 ± 1 mmHg; $P < 0.05$). Heart rate was unchanged. Similar changes were observed following coronary artery occlusion in those dogs in which isosorbide-2-mononitrate was given prior to and during the occlusion, except that the increase in left ventricular end-diastolic pressure was significantly less pronounced (5 ± 1 to 13 ± 2 mmHg; $P < 0.05$) than in the control group.

3.3. Severity of myocardial ischaemia following coronary artery occlusion; the effect of isosorbide-2-mononitrate

We used two indices to assess the severity of myocardial ischaemia; ST-segment elevation electrocardiograms recorded from the epicardial surface, and the degree of inhomogeneity of electrical activation, both measured within the ischaemic area. The changes observed are shown in Figures 1 and 2. In control dogs coronary artery occlusion resulted in a significant ST-segment elevation recorded from the epicardial electrodes and a marked increase in the degree of inhomogeneity of electrical activation within the area supplied by the occluded vessel. Administration of isosorbide-2-mononitrate markedly reduced both the elevation in ST-segment (Figure 1) and in the degree of inhomogeneity of electrical activation within the ischaemic area (Figure 2).

3.4. Effects of isosorbide-2-mononitrate on the incidence and severity of ischaemia and reperfusion-induced ventricular arrhythmias

In control dogs, occlusion of the left anterior descending coronary artery for 25 min resulted in severe ventricular arrhythmias; the distribution of these arrhythmias in each dog is shown in Figure 3, and the results are summarised in Figure 4. There were 202 ± 72 VPBs over the 25 min occlusion period (Figure 4). The incidence of ventricular fibrillation was 82 % (most of these dogs fibrillated between 15 and 20 min of the ischaemia; Figure 3), and 75 % of the animals exhibited ventricular tachycardia with a mean of 4.3 ± 2.1 episodes per dog (Figure 4). The two dogs that survived the occlusion period fibrillated within 1-2 min of reperfusion; thus in the control group there were no survivors from the combined ischaemia-reperfusion insult.

In contrast, the severity of ventricular arrhythmias was markedly reduced in those dogs which received intracoronarily isosorbide-2-mononitrate (Figures 3 and 4). Compared to the controls, the number of ventricular premature beats was markedly suppressed (62 ± 35, $P < 0.05$), there were fewer episodes of ventricular tachycardia (0.7 ± 0.4; $P < 0.05$), and lower incidences of ventricular tachycardia (25 %; $P < 0.05$) during coronary artery occlusion. Furthermore, in dogs treated with isosorbide-2-mononitrate only 2 dogs out of 12 (17%; $P < 0.05$) fibrillated during occlusion (Figures 3 and 4). When the ischaemic myocardium was rapidly reperfused another 4 dogs fibrillated (40 %; $P < 0.05$), thus the survival from the combined ischaemia-reperfusion insult was 50 % in the isosorbide-2-mononitrate treated dogs (compared to 0 % in the controls; $P < 0.05$).

4. Discussion

These studies demonstrate that the local intracoronary administration of the NO donor isosorbide-2-mononitrate reduces the severity of the ischaemic changes which result from coronary artery occlusion. It is this reduction in the degree of ischaemia that is probably responsible for the marked suppression of arrhythmias that result, in this model, from both coronary artery occlusion and reperfusion, rather than a more direct antiarrhythmic effect of the drug. Although there are no published studies with isosorbide-2-mononitrate, those with intravenous nitroglycerin in closed-chest, chloralose anaesthetised dogs, revealed no direct effect on ventricular fibrillation threshold in the normal myocardium [22, 23], although it did partially inhibit the decrease in ventricular fibrillation threshold that results from coronary artery occlusion [24], especially if the hypotensive effect is prevented by phenylephrine [23]. This particular combination also markedly reduced the incidence of spontaneous ventricular fibrillation during occlusion of the left anterior descending coronary artery in conscious dogs; in this study the effects of nitroglycerin alone were not examined [25]. Nitroglycerin also reduced the incidence of complex ventricular arrhythmias which occurred in patients with ischaemic heart disease during treadmill exercise [26] and following acute myocardial infarction [27]. In contrast, it seems to be ineffective in reducing arrhythmia severity during occlusion and reperfusion in anaesthetised rats [28, 29]. More recently, however, Bilinska and colleagues [30] have found that both nitroglycerin and the NO donor SIN-1 mimic the protective effect of ischaemic preconditioning on reperfusion arrhythmias in rat isolated hearts. Because of the pronounced effects of isosorbide-2-mononitrate on the ischaemic myocardium demonstrated in the present study, and the additional beneficial effects of this drug outlined in the Introduction (eg. pronounced inhibition of platelet aggregation), it would be worthwile exploring the effects

of this particular NO donor under those clinical conditions of ischaemia that might result in life-threatening ventricular arrhythmias.

The mechanism of these beneficial effects of NO donors has been ascribed, albeit without supportive experimental evidence, to coronary vasodilatation [23]. In the present study the increase in blood flow following local isosorbide-2-mononitrate administration was significant but quite small (< 20 %) and this degree of coronary vasodilatation seems unlikely to explain the very marked arrhythmia suppression. As one might expect, the elevated left ventricular filling pressure that results from ischaemia was less than in control dogs also subjected to coronary artery occlusion. This would presumably result in an increase in subendocardial driving pressure under these conditions [31], despite the slight reduction in coronary artery pressure. However, our previous studies [1, 2] with levcromakalim and nicorandil would argue against this mild degree of coronary vasodilatation being a major factor responsible for the reduction in arrhythmia severity; locally administered levcromakalim increased coronary blood flow to a similar extent to that in the present studies with isosorbide-2-mononitrate yet failed to influence either the severity of the ischaemic changes following coronary artery occlusion or ischaemia-induced ventricular arrhythmias.

There are two repercussions of the present results. First, it supports the argument [1] that the suppression of ventricular arrhythmias by nicorandil is due to its ability to donate nitric oxide to the ischaemic myocardium rather than to any effect on K_{ATP} channels. Second, the results bear on the hypothesis that the pronounced antiarrhythmic effects of ischaemic preconditioning in this species, induced either by brief periods of coronary artery occlusion or by cardiac pacing, are due to bradykinin-mediated nitric oxide release, probably from coronary vascular endothelial

cells [4, 8]. Up to the present evidence for this hypothesis [8] is primarily pharmacological. The protection is prevented (i) by drugs that block bradykinin B2 receptors, stimulation of which leads to nitric oxide release [7], (ii) by inhibitors of the L-arginine-nitric oxide pathway [3] and (iii) by the local intracoronary administration of methylene blue, an inhibitor of soluble guanylyl cyclase [32]. There is also evidence which comes from studies in which the release of these mediators and the resultant increase in myocardial cGMP have been measured [7]. The present studies, no provide direct evidence that, in the same canine model as that used for our previous preconditioning studies, the local intracoronary administration of a NO donor leads to the same characteristics of myocardial protection as that of preconditioning itself; that is, a reduction in epicardial ST-segment elevation, a decrease in the inhomogeneity of electrical activation within the ischaemic area and a markedly reduced severity of ventricular arrhythmias. Whether this protection is due to an increase in myocardial cGMP, and a resultant decrease in calcium entry, or to some ability of NO donors to protect endothelial cells against the consequences of ischaemia [33], and thus conserve their ability to produce endogenous protective mediators, warrants further investigation.

Acknowledgements

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References

1. Végh Á, György K, Papp JGy, Parratt JR. Nicorandil suppressed ventricular arrhythmias in a canine model of myocardial ischaemia. *Eur J Pharmacol* 1996;305:163-168.
2. Végh Á, Papp JGy, György K, Kaszala K, Parratt JR. Does the opening of ATP-sensitive K^+ channels modify ischaemia-induced ventricular arrhythmias in anaesthetised dogs? *Eur J Pharmacol* 1997;333:33-38.
3. Végh Á, Szekeres L, Parratt JR. Preconditioning of the ischaemic myocardium; involvement of the L-arginine nitric oxide pathway. *Br J Pharmacol* 1992;107:648-652.
4. Parratt JR., Végh Á. Endothelial cells, nitric oxide and ischaemic preconditioning. *Basic Res Cardiol* 1996;91:27-30.
5. Kis A, Végh A, Papp JGy, Parratt JR. Pacing-induced delayed protection against arrhythmias is attenuated by aminoguanidine, an inhibitor of nitric oxide synthase. *Br J Pharmacol*, 1999;127:1545-1550.
6. Kis A, Végh A, Papp JGy, Parratt JR Repeated cardiac pacing extends the time during which canine hearts are protected against ischaemia-induced arrhythmias: Role of nitric oxide. *J Mol Cell Cardiol*, 1999;31:1229-1241.
7. Parratt JR, Végh Á, Zeitlin IJ, et al. Bradykinin and endothelial-cardiac myocyte interactions in ischaemic preconditioning. *Am J Cardiol* 1997;80(3A):124A-131A.
8. Parratt JR, Végh Á. Coronary vascular endothelium, preconditioning and arrhythmogenesis. In: M.J. Lewis, A.M. Shah (Eds.) *Endothelial Modulation of Cardiac Function*, Harwood Academic Publishers, 1997:237-255.
9. Kukovetz WR, Holzmann S, Romarin C. Mechanism of vasodilation by nitrates: Role of cyclic GMP. *Cardiology*, 1987;74:Suppl.1, 12-19.

10. Sisenwine SF, Ruelius HW. Plasma concentration and urinary excretion of isosorbide dinitrate and its metabolites in the dog. *J Pharmacol Exp Ther* 1971;176:296-301

11. Stauch M, Grewe N. Die Wirkung von Isosorbiddinitrat, Isosorbid-2- und 5-Mononitrat auf das Belastungs-EKG und auf die Hämodinamik während Vorhofsstimulation bei Patienten mit Angina Pectoris. *Z Kardiol* 1979;68:687-693.

12. Tauchert M, Jansen W. Development of mononitrates. *Cardiology*, 1987; 74: Suppl. 1, 3-5.

13. Pipilis A, Flather M, Collins R, et al. Effects on ventricular arrhythmias of oral captopril and of oral mononitrate started early in acute myocardial infarction: results of randomized placebo contolled trial. *Br Heart J* 1993; 69:161-165.

14. Bogaert MG, Rosseel MT. Vascular effects of the dinitrate and mononitrate esters of isosorbide, isomannide and isoiodide. *Arch Pharmacol* 1972;275:339-344.

15. Wendt RL. Systemic and coronary vascular effects of the 2-and the 5-mononitrate esters of isosorbide. *J Pharmacol Exp Ther*, 1972;180:732-742.

16. DeCaterina R, Lombardi M, Bernini W, et al. Inhibition of platelet function during in vivo infusion of isosorbide mononitrates: relationship between plasma drug concentration and hemodynamic effects. *Am Heart J* 1990;119:855-862.

17. DeCaterina R, Giannessi D, Mazzone A, Bernini W. Mechanism for the in vivo antiplatelet effects of isosorbide dinitrate.*Eur Heart J* 1988;9:Suppl.A,45-49.

18. Strein K, Sponer G, Bartsch W, Müller-Beckman B, Dietmann K. Electrocardiographic analysis of the effects of isosorbide-5-mononitrate on regional myocardial ischemia in conscious dogs. *J Pharmacol Exp Ther* 1984;229:787-792.

19. Bacher S, Kraupp O, Beck A, Skoda H, Raberger G. Characterisation of vasodilators by comparison of their effects on blood pressure, counterregulation and myocardial oxygen demand in conscious normotensive dogs. *Arzneim-Forch/Drug Res*, 1985;35:288-291.

20. Leitold Von M, Laufer H. Vergleichende antianginöse, hamodynamische und pharmakokinetische Wirkung von Isosorbid-2-mononitrat und Isosorbiddinitrat an der Ratte. *Arzneim-Forsch/Drug Res* 1983;33:1117-1121.

21. Végh Á, Komori S, Szekeres L, Parratt JR. Antiarrhythmic effects of preconditioning in anaesthetised dogs and rats. *Cardiovasc Res* 1992;26:487-495.

22. Kent KM, Smith ER, Redwood DR, Epstein SE. Beneficial electrophysiologic effects of nitroglycerin during acute myocardial infarction. *Am J Cardiol,* 1974;33:513-516.

23. Stockmann MB, Verrier RL, Lown B. Effect of nitroglycerin on vulnerability to ventricular fibrillation during myocardial ischaemia and reperfusion. *Am J Cardiol* 1979; 43:233-238.

24. Levites R, Boderheimer MM, Helfant RH. Electrophysiologic effects of NTG during experimental coronary occlusion. *Circulation,* 1975;52:1050-1055.

25. Borer JS, Kent KM, Goldstein RE, Epstein SE. Nitroglycerin-induced reduction in the incidence of spontaneus ventricular fibrillation during coronary artery occlusion in dogs. *Am J Cardiol* 1974;33:517-520.

26. Margonato A, Bonetti F, Mailhac A, Vicedomini G, Cianflone C, Chierchia SL. Intravenous nitroglycerin suppresses exercise-induced arrhythmias in patients with ischaemic heart disease: implications for long-term treatment. *Eur Heart J* 1991;12:1278-1282.

27. Mihalick MJ, Rasmusson S. Knoebel SB. The effect of nitroglycerin on premature ventricular complexes in acute myocardial infarction. *Am J Cardiol* 1974;33:157 (abstr).

28. Kane KA, Parratt JR, Williams FM. An investigation into the characteristics of reperusion-induced arrhythmias in the anaesthetised rat and their susceptibility to antiarrhythmic agents. *Br J Pharmacol* 1984;82:349-357.

29. Barnes CS, Coker SJ. Failure of nitric oxide donors to alter arrhythmias induced by acute myocardial ischaemia or reperfusion in anaesthetized rats. *Br J Pharmacol* 1995;114:349-356.

30. Bilinska M, Maczewski M, Beresewicz A. Donors of nitric oxide mimic effects of ischaemic preconditioning on reperfusion-induced arrhythmias in isolated rat heart. *Mol Cell Biochem* 1996;160-161:265-271.

31. Marshall RJ, Parratt JR. Drug-induced changes in blood flow in the acutely ischaemic canine myocardium: relationship to subendocardial driving pressure. *Clin Exp Pharmacol Physiol* 1974;1:99-112.

32. Végh Á, Papp JGy, Szekeres L, Parratt JR. The local administration of methylene blue prevents the pronounced antiarrhythmic effect of ischaemic preconditioning. *Br J Pharmacol* 1992;107:910-911.

33. Siegfried MR, Erhardt J, Rider T, Ma X-L, Lefer A. Cardioprotection and attenuation of endothelial dysfunction by organic nitric oxide donors in myocardial ischemia-reperfusion. *J Pharmacol Exper Ther* 1992;260:668-675.

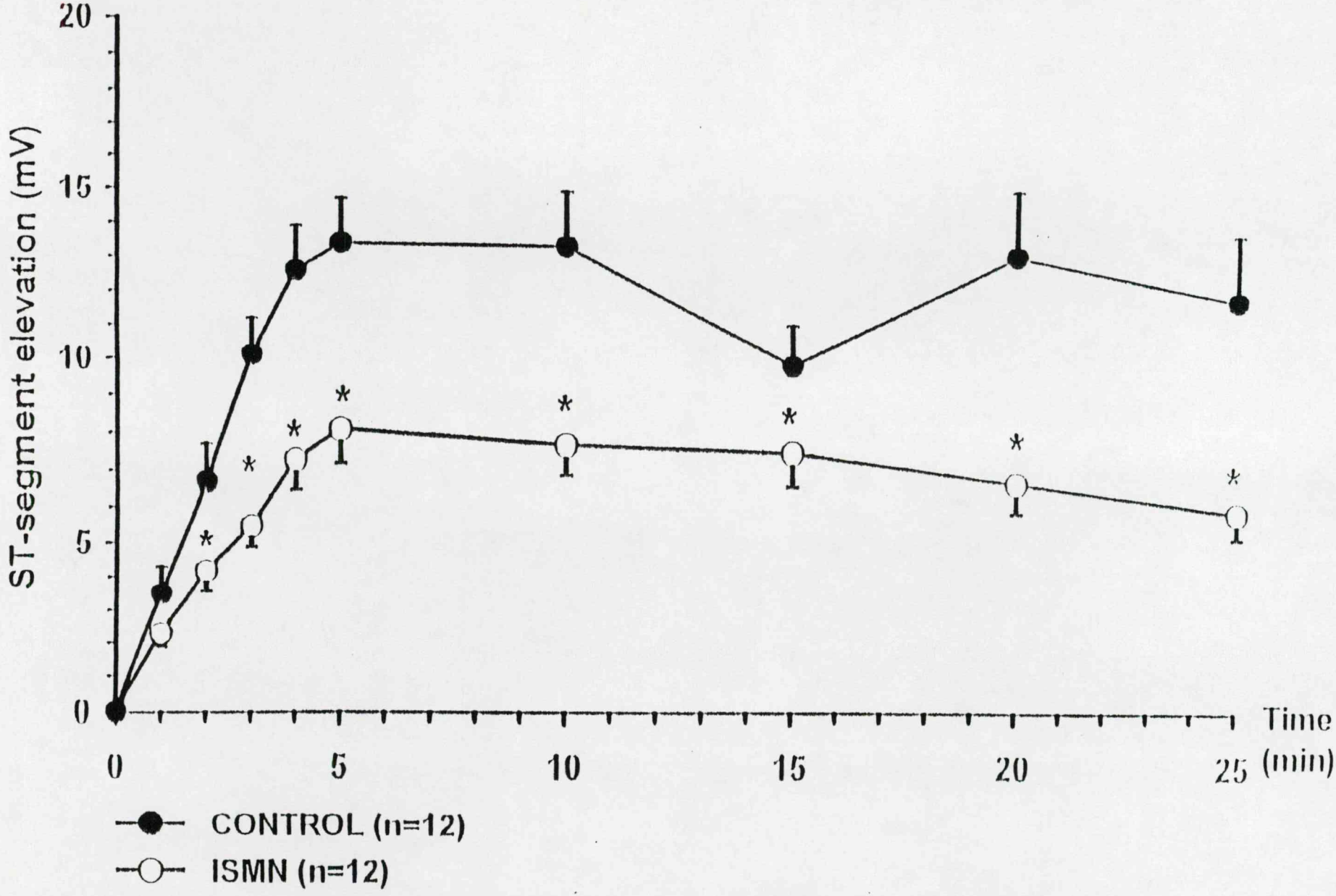
Figure legends

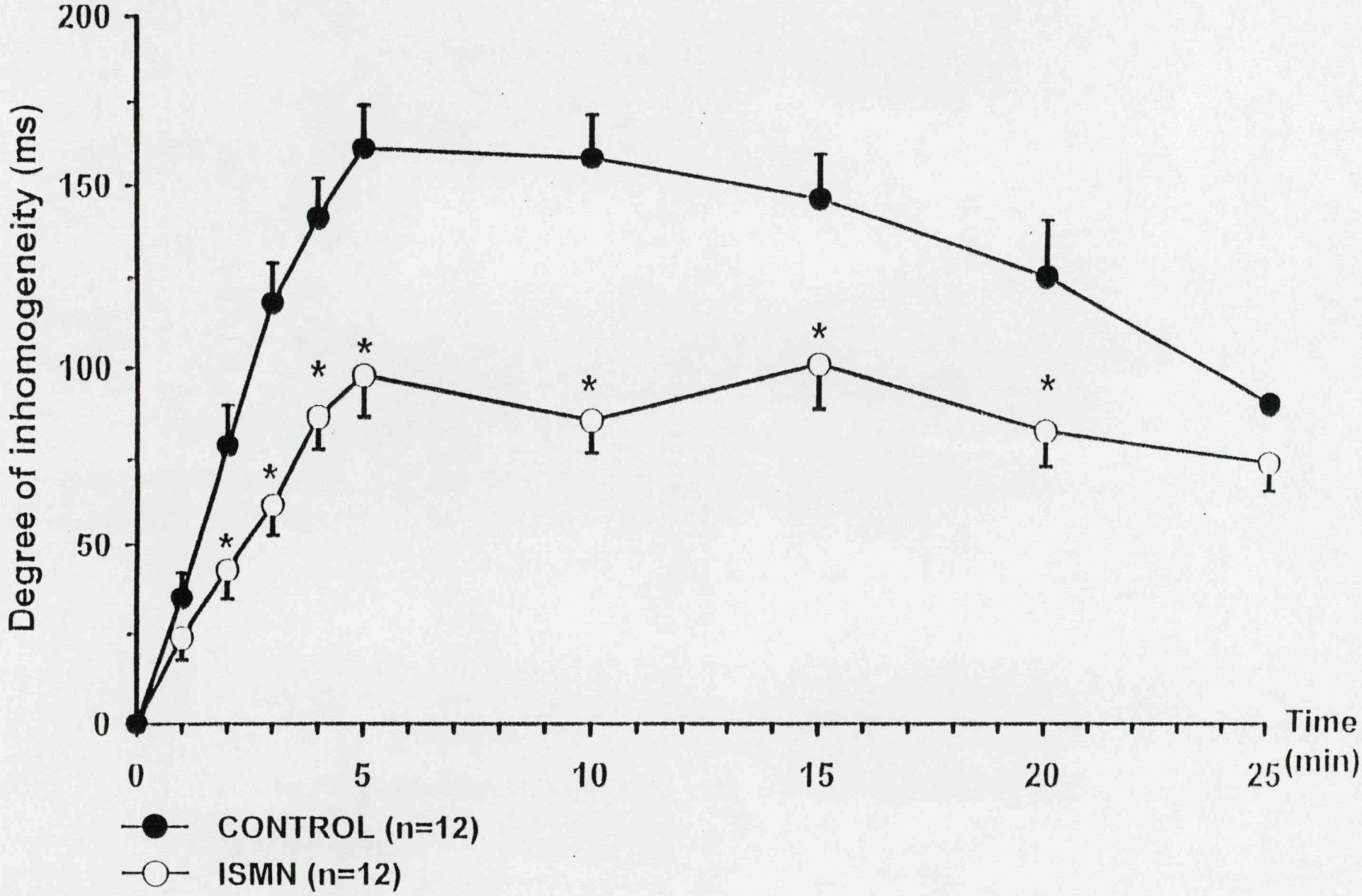
Figure 1. Changes in ST-segment elevation (mV), recorded from epicardial electrodes, after coronary artery occlusion in control dogs and in dogs given isosorbide-2- mononitrate 3 $\mu g\ kg^{-1}\ min^{-1}$ by intracoronary infusion. Isosorbide-2-mononitrate markedly reduced the extent of ST-segment elevation following coronary artery occlusion. $^{*}P < 0.05$ in comparison with value in untreated control dogs. Values are means ± s.e.m.

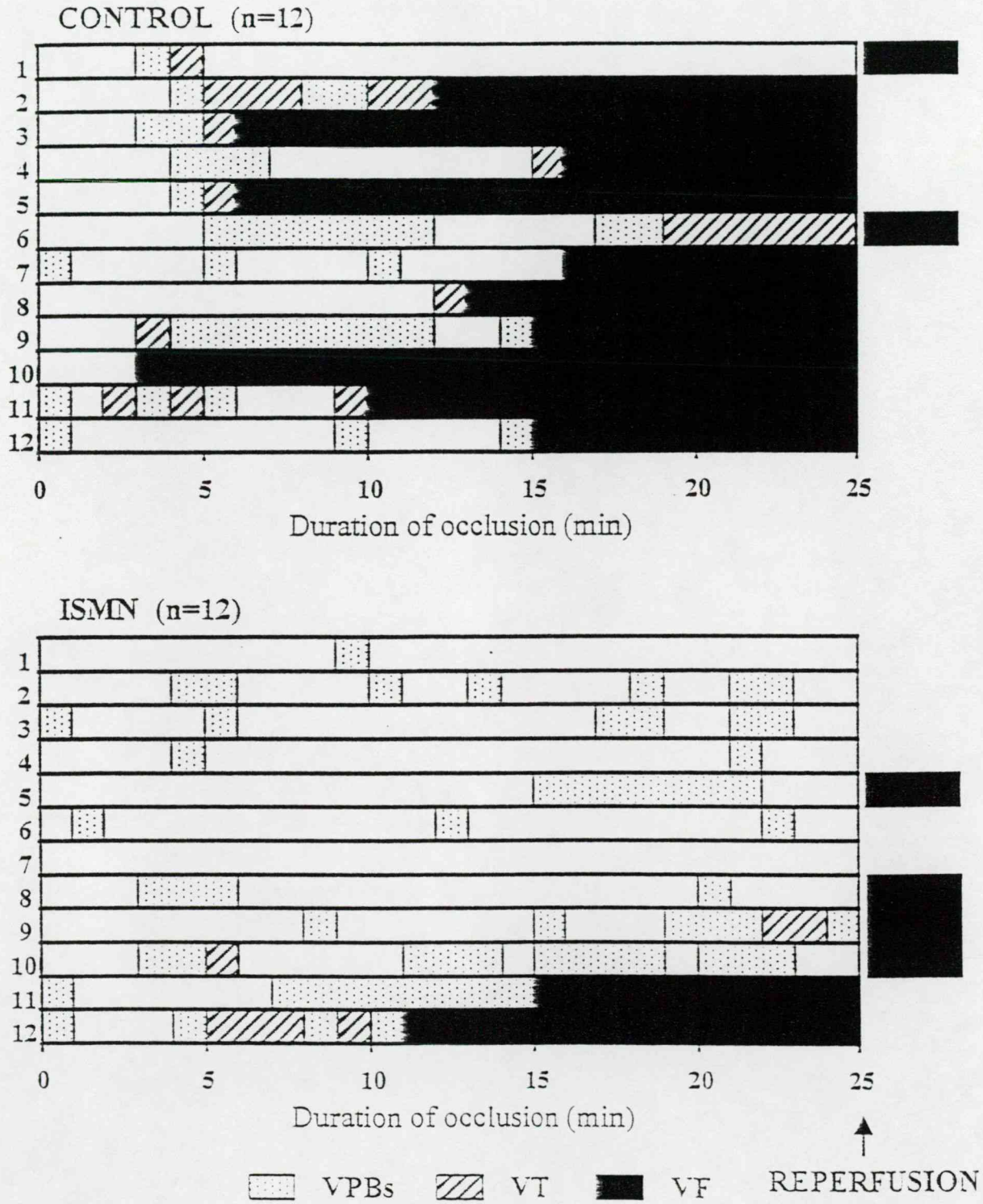
Figure 2. Changes in the inhomogeneity of electrical activation (in ms) within the area supplied by the left anterior descending coronary artery when this artery is occluded (at time zero). The marked increase in inhomogeneity that occurs in control dogs was reduced by isosorbide-2-mononitrate 3 $\mu g\ kg^{-1}\ min^{-1}$ given by intracoronary infusion. $^{*}P < 0.05$ in comparison with value in untreated control dogs. Values are means ± s.e.m.

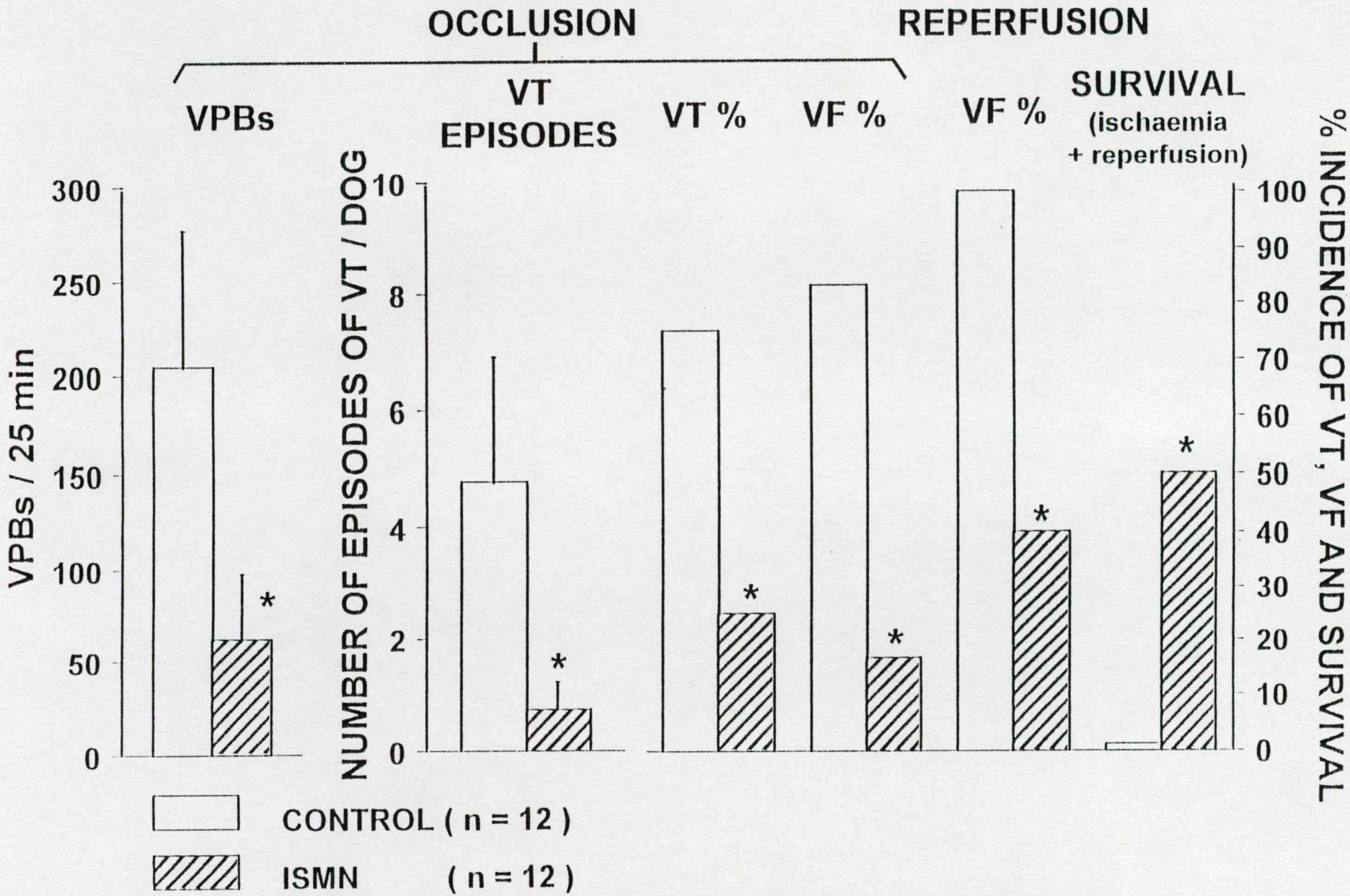
Figure 3. The distribution of ventricular arrhythmias; ventricular premature beats (VPBs), ventricular tachycardia (VT) and ventricular fibrillation (VF) during a 25 min occlusion of the left anterior descending coronary artery in anaesthetised dogs; this was followed by reperfusion. Ten of the 12 controls fibrillated during the occlusion, and all but three had a period of ventricular tachycardia. None of these controls survived the combined ischaemia-reperfusion insult. These arrhythmias were markedly suppressed by ISMN (isosorbide-2-mononitrate). Thus, only two dogs fibrillated during the occlusion period, three exhibited a brief period of ventricular tachycardia and there was a 50 % survival from the ischaemia-reperfusion insult.

Figure 4. 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 incidence of ventricular tachycardia (VT), the incidence of ventricular fibrillation (VF) and survival from the combined ischaemia-reperfusion insult in control dogs and in dogs given isosorbide-2-mononitrate 3 $\mu g\ kg^{-1}\ min^{-1}$ by intracoronary infusion. Isosorbide-2-mononitrate markedly reduced arrhythmia severity and increased survival from the ischaemia-reperfusion insult.


ST-segment elevation (mV)
20
15
10
5
0
0
5
10
15
20
25
Time
(min)
CONTROL (n=12)
ISMN (n=12)


Degree of inhomogeneity (ms)
200
150
100
50
0
0
5
10
15
20
25
Time
(min)
CONTROL (n=12)
ISMN (n=12)


CONTROL (n=12)
1
2
3
4
5
6
7
8
9
10
11
12
0
5
10
15
20
25
Duration of occlusion (min)
ISMN (n=12)
1
2
3
4
5
6
7
8
9
10
11
12
0
5
10
15
20
25
Duration of occlusion (min)
REPERFUSION
VPBs
VT
VF


OCCLUSION
REPERFUSION
VPBs
VT EPISODES
VT %
VF %
VF %
SURVIVAL (ischaemia + reperfusion)
VPBs / 25 min
NUMBER OF EPISODES OF VT / DOG
% INCIDENCE OF VT, VF AND SURVIVAL
300
250
200
150
100
50
0
10
8
6
4
2
0
100
90
80
70
60
50
40
30
20
10
0
*
CONTROL (n = 12)
ISMN (n = 12)

CARDIOVASCULAR DRUGS AND THERAPY

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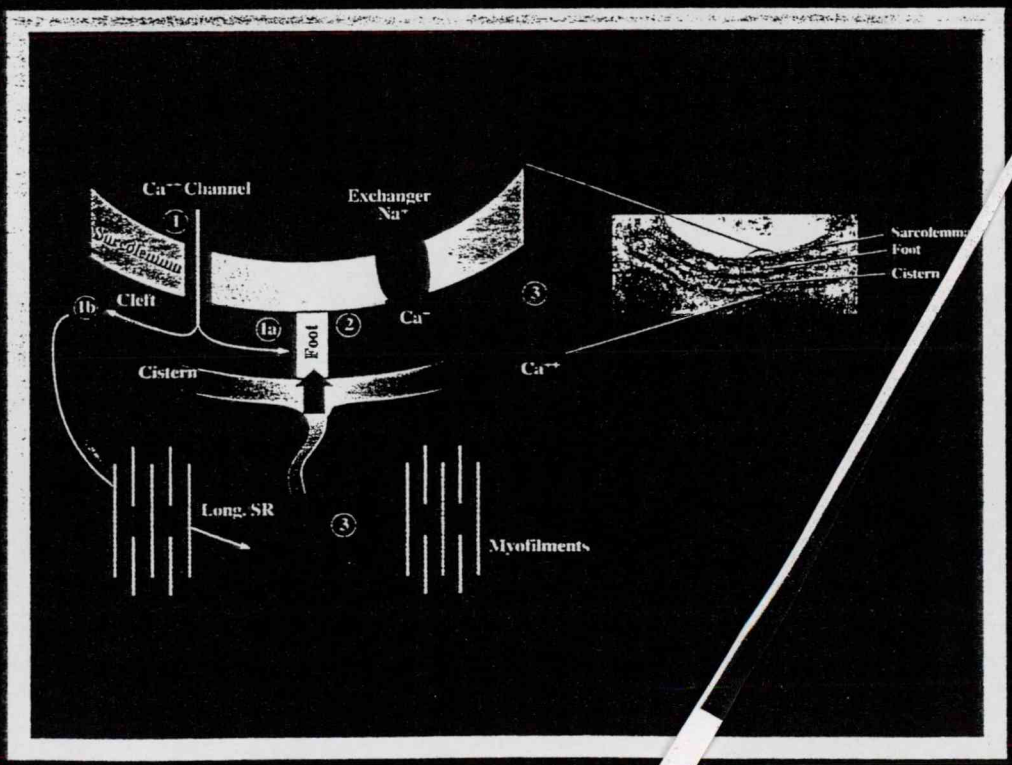

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ANTIBODIES AGAINST MYOSIN IN SERA OF PATIENTS WITH IDIOPATHIC PAROXYSMAL ATRIAL FIBRILLATION Sa56

Jean-Michel Maixent, Franck Paganelli, Jorge Scaglione, Samuel Lévy. Dept of cardiology, Faculty of Medicine, Marseille France

This study investigated the presence of antibodies in sera of patients with idiopathic paroxysmal atrial fibrillation. Circulating autoantibodies against myosin have been detected in patients with idiopathic dilated cardiomyopathy, myocarditis and other cardiac diseases. An SDS-PAGE procedure, followed by Western blotting with homogenates and membrane fractions of human left ventricular and atrial specimen as antigens, was used to analyze sera of 7 patients with idiopathic paroxysmal atrial fibrillation and 5 age-matched control subjects. Cardiac immunoglobulin G reactivity against myosin heavy chain were detected in 5 of 7 patients (71 %) as compared to only one of the 5 (20 %) control subjects ($p<0.05$). All 5 patients who showed reactivity against myosin heavy chain had also in their sera cardiac specific reactivity which exhibited both reactivities to ventricular and atrial cardiac myosin heavy chain isoforms.This study demonstrated the presence of circulating immunoreactivity against myosin in patients with idiopathic paroxysmal atrial fibrillation. A cardiac specificity of this immune reaction could be specified.

NA,K-ATPASE AND MITOCHONDRIAL ATPASE ACTIVITIES DURING PACING-INDUCED ATRIAL FIBRILLATION Sa57

Odile Barbey, Karine Robert, Alain Gerbi, Philippe Ricard, Samuel Lévy, Jean-Michel Maixent. Dept of cardiology, Faculty of Medicine, Marseille France

The purpose of this study was to examine the effects of atrial fibrillation on ATPase activities in a sheep model of sustained atrial fibrillation induced by temporary pacing at a rate of 600 beats/min through the high right atrium. Nine adult sheep were assigned to the control group (n=3) or to the atrial fibrillation group (n=6). Anterior right atrium and apex of left ventricle specimen were removed for membrane isolation and measurements of enzymatic activity and expression of catalytic α-subunit of the Na,K-ATPase by immunodetection. All sheep developed multiple episodes of sustained and reproducible atrial fibrillation defined as lasting ≥ 5 min with a mean total duration of 110 min over a 2 hour period. The atrial and ventricular F_o-F_1-ATPase activity became both activated ($p<0.05$) without significant modification of Na,K-ATPase activity or expression of its catalytic subunit. These results may provide a biochemical basis of atrial fibrillation-induced cardiac cellular alterations

GENETIC AND ALLELIC HETEROGENEITY IN FRENCH FAMILIES WITH LONG QT SYNDROME Sa58

Pascale Guicheney[1], Claire Donger[1], Myriam Berthet[1], Isabelle Denjoy[2], Pascale Richard[3], Bernard Hainque[3], Ketty Schwartz[1], Philippe Coumel[2]. [1] INSERM U153, Hôpital Pitié-Salpêtrière, [2] Service de Cardiologie, Hôpital Lariboisière, [3]Service de Biochimie, Hôpital Pitié-Salpêtrière, France.

Four disease loci have been reported for congenital Long QT syndrome (LQTS) on chromosomes 11p15.5 (LQT1), 7q35-36 (LQT2), 3p21-24 (LQT3), and 4q25-27 (LQT4). Three morbid genes have been identified : two potassium channels, KVLQT1 and HERG for LQT1 and LQT2 respectively, and a sodium channel SCN5A for LQT3. By linkage analysis and mutation detection, we determined that LQTS was due to missense mutations in KVLQT1 or HERG in 20 and 5 LQTS families, respectively. The morphological aspect of the QRS complexes was evaluated from the 24 hour electrocardiographic recordings for all the propositus. The T-wave patterns clearly differed between the two groups. KVLQT1 mutations were associated with wide-based monophasic T-waves while HERG mutations were associated with abnormal biphasic T-wave patterns. Phenotype-genotype analyses showed a great phenotypic variability (symptoms or not, number of syncopes, sudden death) among the patients even with the same mutation suggesting that other factors than the mutation itself influence the risk of sudden death.

CAN THE ALREADY PRECONDITIONED MYOCARDIUM BE ADDITIONALLY PROTECTED BY PRECONDITIONING 'AT A DISTANCE'? Sa59

Ali M. Rastigar, Végh Ágnes, Julius Gy. Papp, James Parratt. Dept of Pharmacology, A. Szent-Györgyi Med. Univ. Szeged, Hungary.

The myocardium can be preconditioned by partial or complete occlusion of an adjacent coronary artery (*Przyklenk et al., 1993*). We have investigated in pentobarbitone anaesthetised dogs, whether the myocardium, already preconditioned by brief LAD coronary artery occlusions, can be further protected if the LCX artery is also partially constricted. Preconditioning was induced by two 5 min occlusions of the LAD, with a 30 min interval between followed 50 min later by a third 5 min occlusion. In some dogs, between the second and third occlusions, the LCX was partially constricted for 20 min. In both groups the first occlusion of the LAD resulted in a more marked elevation in epicardial ST-segment (16.6 ± 0.6 and 17.9 ± 1.5 mV) and increase in the degree of inhomogeneity (190 ± 13 and 199 ± 10 msec) than the second occlusion (ST-segment: 10.6 ± 1.3 and 12.8 ± 1.2 mV, inhomogeneity: 115 ± 9 and 140 ± 16 msec, respectively, $P<0.05$). In the presence of LCX constriction, the third occlusion further increased both parameters (20.1 ± 1.5 mV, 209 ± 16 msec) cf 10.3 ± 1.2 mV and 115 ± 8 msec ($P<0.05$) in the absence of LCX constriction. We conclude that constricting the LCX does not yield additional protection to the already preconditioned area; rather the ischaemic changes are worsened.

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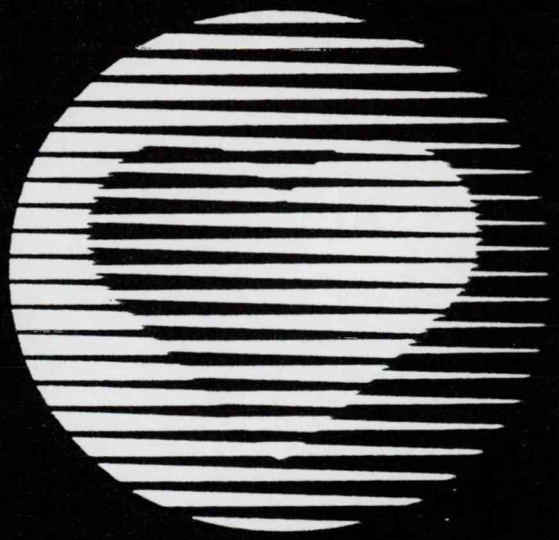

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EFFECT OF A CALCIUM-SENSITIZING AGENT, LEVOSIMENDAN, ON THE POST-CARDIOPLEGIC INOTROPIC RESPONSE OF THE ISOLATED GUINEA PIG HEART 317

A. Loenner, S. Genade, F. Colesky. Dept. Medical Physiology, Univ. of Stellenbosch Faculty of Medicine; MRC Exp. Biol. Programme, Tygerberg, Rep. of South Africa.

Myocardial contractility during reperfusion after coronary artery bypass graft operations is often depressed, particularly in patients treated with Ca^{2+}- and β-adrenergic blockers and inotropic support may be required for resumption of function. In this study the effects of levosimendan, a Ca^{2+} sensitizing agent, on the inotropic response of hearts after normothermic cardioplegic arrest was evaluated. Methods: Isolated perfused working guinea pig hearts were subjected to normothermic (45 min, 37°C) cardioplegic arrest using St Thomas Hospital solution no. 2. Dose-response curves with levosimendan (10^{-9}-10^{-7} M) were conducted during reperfusion in the presence and absence of nifedipine (10^{-6} M) and propranolol (10^{-7} M). Results: Normothermic cardioplegic arrest for 45 min caused a 67% reduction in cardiac output during reperfusion. This was prevented completely by prior simultaneous administration of the blockers. Levosimendan (10^{-9}-10^{-7} M) caused a dose-dependent improvement in functional performance in the absence of the blockers, reaching a maximum at 10^{-7} M (133% increase in cardiac output). In the presence of the blockers a 100 fold higher dosage of levosimendan was required to initiate stimulation of contractility. Conclusions: Although levosimendan was able to significantly stimulate contractility after normothermic cardioplegic arrest, the dosage has to be increased in the presence of β- and Ca^{2+}-blockers.

NITROGLYCERINE (NTG)-DERIVED NO OPENS K_{ATP} CHANNELS VIA A cGMP-INDEPENDENT MECHANISM IN THE RAT HEART. 318

Tamas Csont, Zoltán Szilvássy*, & Peter Ferdinandy. Depts Biochem., *Medicine I, Albert Szent-Gyorgyi Med. Univ., Szeged, Hungary

We have recently demonstrated a direct myocardial anti-ischemic action of NTG (BJP 15:1129-1131, 1995). Now, we examined if this effect is mediated by NTG-derived NO, involves cardiac cGMP system and activation of K_{ATP}-channels. Isolated working hearts of male Wistar rats were subjected to 10-min coronary occlusion in the presence of solvent, 10^{-7} M NTG, 10^{-4} M methylene blue, 10^{-5} M glibenclamide, the combination of NTG + methylene blue, and NTG + glibenclamide (n=7 in each group). Neither compound affected preischemic myocardial function and coronary flow. During coronary occlusion, NTG improved ischemic aortic flow from its control value of 20.4±1.2 to 33.5±1.5 mL/min ($p<0.05$) and decreased lactate dehydrogenase release from 212±54 to 32±18 mU/min/g. Cardiac NO content assessed by electron spin resonance analysis of N-methyl-glucoseamine-dithiocarbamate-Fe^{2}-NO complex was markedly increased after NTG administration, however, NTG failed to change significantly cardiac cGMP concentration (control: 0.042±0.006, NTG: 0.038±0.008 pmol/mg) measured by radioimmunoassay. In the presence of the NO scavenger methylene blue, the anti-ischemic effect of NTG was inhibited. Similarly to methylene blue, the K_{ATP}-blocker glibenclamide abolished the anti-ischemic effect of NTG. It is concluded, that NTG derived NO opens K_{ATP}-channels via a cGMP independent mechanism, thereby leading to an anti-ischemic effect in the rat heart. (Supported by grants from OTKA F19946, D23736; OMFB 05201950616; FKFP 1284/1997, and the Hungarian Space Res. Office)

2-ISOSORBIDE MONONITRATE REDUCES THE CONSEQUENCES OF ISCHAEMIA IN ANAESTHETISED DOGS 319

Katalin György, Ágnes Végh, Mohamed Ali Rastigar, Julius Gy. Papp, James R. Parratt. Depts. of Pharmacology, A. Szent-Györgyi Med. Univ. Szeged, Hungary, Univ. Strathclyde, Glasgow, UK.

We have shown previously, that nicorandil, a nitric oxide (NO) donor and K_{ATP} opener, but not chromakalim, a pure K_{ATP} activator, markedly reduced the severity of ventricular arrhythmias in a canine model of ischaemia-reperfusion. The present experiments were designed to examine, whether isosorbide 2-mononitrate (2-ISMN), a nitric oxide donor, modifies the severe consequences of myocardial ischaemia, resulting from a 25 min occlusion and reperfusion of the left anterior descending coronary artery (LAD). In chloralose-urethane anaesthetised mongrel dogs, 2-ISMN (3 μg kg^{-1} min^{-1}, n = 12) or saline (control group, n = 12) was infused intracoronarily, 20 min prior to and then throughout the entire 25 min occlusion period. 2-ISMN resulted in a slight reduction in arterial blood pressure (-11 ± 2 mmHg, $P < 0.05$), without modifying heart rate, coronary blood flow or coronary resistance. Compared to the controls, occlusion of the LAD in the presence of 2-ISMN resulted in a reduced number of VPBs (62 ± 35 vs 202 ± 72, $P < 0.05$) and episodes of VT (0.7 ± 0.5 v. 4.3 ± 2.1, $P < 0.05$), and lower incidences of VT (25 % vs 58 %, $P < 0.05$) and VF (17 % vs 92 %, $P < 0.05$). Survival from the combined ischaemia-reperfusion insult was also increased (50 % vs 0 %, $P < 0.05$). In dogs given 2-ISMN, the epicardial ST-segment elevation and degree of electrical inhomogeneity were less marked than in the controls. We conclude from these results that as with nicorandil, the cardioprotective effects are mainly due to the ability of 2-ISMN to donate NO to the ischaemic myocardium.

Supported by the Hungarian Scientific Research Foundation (OTKA), the British Council and the Hungarian Cultural and Education Ministry (No 14 and FKFP 1290/1997).

EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITION OF DIFFERENT TIME COURSE ON INFARCT SIZE IN RAT HEART 320

Hitoshi Matsuoka, Kaoru Shimada, Hitoshi Hasegawa, Hiroyuki Tsuya, Fukiko Kinno, Ken Terata, Mamoru Miura, Second Dept of Intern Med, Akita University, Akita, Japan

Nitric oxide(NO) has been demonstrated both protective and deleterious effects during acute phase of reperfusion (Rep) following myocardial ischemia. However, roles of NO in subacute phase of Rep remains unknown. To investigate roles of NO during acute and subacute phase of Rep, 30 male 12 week Sprague-Dawley rats were divied into 3 groups(Grs). The left coronary artery was occluded for 30 min followed by 24 hr of Rep. In Gr N, 50 mg/hr of N-nitro-L-arginine methylester (L-NAME) was administered intravenously beginning 15 min before coronary occlusion until 15 min after Rep. In Gr NP, L-NAME was administered intravenously (50 mg/hr) from 30 min until 90 min after Rep, thereafter (30 mg/kg/day) intraperitoneally until 24 hours after Rep. In Gr C, rats were served as control. Blood pressure and heart rate were measured. At 24 hr of Rep rats were sacrificed and the heart was excised. Histological infarct size (IS) was determined. Inducible NO synthase (iNOS) was examined histochemically. There were no differences in rate pressure products during ischemia and Rep among these groups. Significant IS reduction was obtained in Gr N (14.5±1.9%)($p<0.05$, but IS was similar in Gr N (40.0±4.9%) compared with Gr C (47.5±6.0%). Immunohistochemical staining for iNOS revealed positive reactivity in the damaged myocytes in each Gr. These results suggest that NO produced in acute phase of Rep are more deleterious than NO produced in subacute phase of Rep in relation to Rep injury in rat heart.

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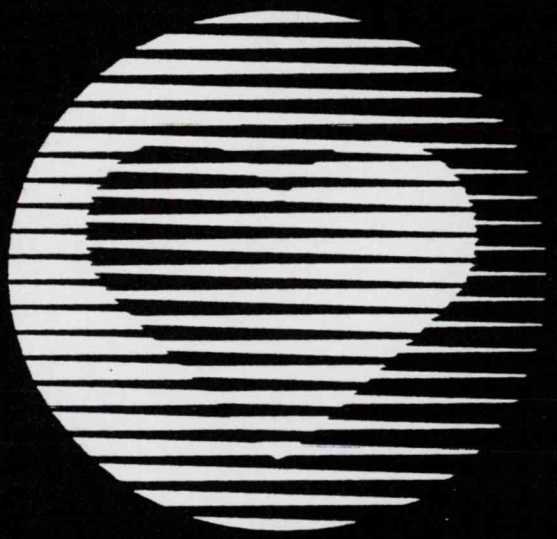

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ISCHEMIA MODULATES DISCONTINUOUS ACTION POTENTIAL CONDUCTION 13

Ronald W. Joyner[1], Ronald Wilders[1,2], E. Etienne Verheijck[1,2], Rajiv Kumar[1], David Goiod[1], Antoni van Ginneken[2], and Habo Jongsma[2]. [1]The Children's Heart Center, Emory University, Atlanta, GA, USA. [2]Dept. of Med. Physiology and Sports Med., Utrecht University, and [3]Academic Medical Center, University of Amsterdam, Dept. of Physiology, The Netherlands.

Background: Ischemia may occur in tissue with prior injury and spatially inhomogeneous membrane properties and cell-cell coupling. Changes in action potential (AP) conduction with ischemia, which can be associated with release of catecholamines, may be particularly important in tissue with discontinuous conduction. **Methods and Results:** We electrically coupled isolated guinea pig ventricular myocytes to a mathematical model of a ventricular myocyte to assess alterations in required coupling conductance (G_c) for AP conduction when the real cell was exposed to hypoxia, acidosis, and elevated $[K^+]_o$ to simulate ischemia. The "ischemic" solution increased critical G_c from 6.2±0.1 to 7.4±0.2 nS (n =11, $p<0.05$) and decreased maximum conduction delay from 31±1 to 23±1 ms (n =11, $p<0.05$). Ischemic solution plus 1 μM noradrenaline decreased critical G_c from 5.9±0.2 to 5.0±0.1 nS (n =8, $p<0.05$) and increased maximum conduction delay from 31±2 to 54±4 ms (n =8, $p<0.05$). **Conclusions:** Release of catecholamines with ischemia, in a setting of partially uncoupled cells, may play a major role in producing long conduction delays which may allow reentrant pathways.

ACTION POTENTIAL LENGTHENING OPPOSES REPERFUSION ARRHYTHMIAS 14

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Action potential lengthening delays I_{Na} recovery from inactivation and may indirectly alleviate Na loading during ischemia and reperfusion. We investigated the effects of blocking I_{K1} with Ba^{2+} (100 μM) on the electrical response to regional ischemia and reperfusion in ten spontaneously beating rat hearts. Transmembrane potentials and the ECG were recorded. In the controls, the resting potential (RP) depolarized by 13 ± 0.7 mV during ischemia. Reperfusion induced ventricular tachyarrhythmias within 1 min in 90% of hearts. Ba^{2+} lengthened the action potential duration (APD_{90}) from 78.5 ± 3 to 144.8 ± 24 ms and enhanced ischemic depolarization (23.8 ± 1.8 mV). The action potential upstrokes were notched. The combined duration of tachycardia and fibrillation episodes on reperfusion decreased from 319 ± 47 s (over 10 min of observation) to 49 ± 15 s. All the hearts recovered sinus rhythm. Our data show that modulation of I_{Na} by changes in APD may significantly modify arrhythmogenesis on reperfusion.
Supported by CONICET and CIUNC.

DOES INHIBITION OF BRADYKININ CATABOLISM MODIFY THE SEVERITY OF ARRHYTHMIAS IN MYOCARDIAL ISCHAEMIA 15

Mohamed Ali Rastigar, Ágnes Végh, Julius Gy. Papp, [*]James R. Parratt, [**]Claudio Semeraro, [**]Francesco Marchini. Depts. of Pharmacology, A. Szent-Györgyi Med. Univ. Szeged, Hungary, [*]Univ. Strathclyde, Glasgow, UK and [**]Zambon, Milan, Italy.

It is well documented that bradykinin (BK) is involved in the cardioprotective effect of ischaemic preconditioning. It is also established that angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP) are the two main enzymes involved in BK degradation. The present study aimed to examine whether Z13752A, a dual ACE/NEP inhibitor modifies the severity of ventricular arrhythmias resulting from a 25 min occlusion and reperfusion of the left anterior descending coronary artery (LAD) in anaesthetised dogs. Z13752A was infused (128 $\mu gkg^{-1}min^{-1}$ iv., n=12) 60 min prior to a 25 min occlusion of the LAD. Z13752A resulted in a fall in arterial blood pressure (-11±3 mmHg, $P<0.05$) and a reduction in the resistance of the LAD and left circumflex (LCX) coronary arteries (by 12 % and 16 %, respectively; $P<0.05$). Compared to the controls (n=20), occlusion of the LAD following Z13752A administration, resulted in a reduced number of VPBs (91±41 vs 390±74, $P<0.05$) and VT episodes (0.8±0.3 v. 9.0±1.9, $P<0.05$) and lower incidences of VT (42% vs 100%, $P<0.05$) and VF (25% vs 40%, $P<0.05$). Survival from the combined ischaemia-reperfusion insult was also increased (50% vs 0%, $P<0.05$). The occlusion-induced epicardial ST-segment elevation and the degree of inhomogeneity, were also less marked in Z13752A treated dogs. These results indicate, that dual blockade of ACE and NEP provides marked protection against the severe consequences of myocardial ischaemia, perhaps by elevating BK levels and/or prolonging the protective effect of BK.
Supported by the Hungarian Scientific Research Foundation (OTKA), the British Council and the Hungarian Culture and Education Ministry (No 14 and FKFP 1290/1997).

HEART FAILURE ARRHYTHMIA MECHANISMS 16

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Despite frequent arrhythmia and sudden death, attempts to study arrhythmia mechanisms in HF patients have been unsuccessful due to the inability to induce arrhythmia using electrical stimulation. We found that the rapidly paced dog heart model of HF, is prone to arrhythmia, apparently not due to reentry or automaticity, suggesting that triggered activity induced afterdepolarization may play a role. We determined the susceptibility of the HF dog to triggered activity elicited by the administration of cesium chloride (CsCl). HF was produced by pacing the hearts at 240 beats/min for 3-4 weeks. At restudy, the minimal dose of CsCl which produced ventricular tachycardia was significantly lower in the HF than the control dogs (1.02±0.02 vs 1.21±0.07 mMol/kg, $p<0.05$). Epicardial mapping during CsCl-induced ventricular tachycardia showed activation patterns consistent with multifocal origin. In *in vitro* microelectrode studies, within 30 minutes, CsCl superfusion (5 mMol/L) produced triggered activity in 7 of 8 Purkinje fibres from 4 HF dogs and ventricular myocytes from 2 HF dogs. In contrast, CsCl induced triggered activity in only 1 of 8 Purkinje fibers from the hearts of 4 control dogs even with continuous superfusion for up to 60 minutes ($p<0.01$). A slowed pacing stimulus increased the number of early afterdepolarizations. These results demonstrate that the pacing-induced HF dog has an increased sensitivity to VT due to triggered activity induced by CsCl. Furthermore, this suggests that triggered activity may play an important role in arrhythmogenesis of heart failure.
Supported by the Heart and Stroke Foundation of Ontario

SZENT-GYÖRGYI ALBERT ORVOSTUDOMÁNYI EGYETEM

SZOTE IV. Ph.D Előadói Napok

PROGRAMFÜZET ÉS ELŐADÁSKIVONATOK

Szeged

1998. június 4-5.

Mohamed Ali Rastigar, Ágnes Végh, Julius Gy. Papp,*James R. Parratt
Department of Pharmacology, Albert Szent-Gyorgyi Medical University Szeged, and
**University of Strathclyde, Glasgow, UK.*

THE ANTIARRHYTMIC EFFECTS OF ANP IN A CANINE MODEL OF ISCHAEMIC-REPERFUSION

The effects of atrial natriuretic peptide (ANP) was examined in chloralose-urethane anaesthetised mongrel dogs. After thoracotomy, human synthetic ANP was infused intravenously ($10\mu g\ kg^{-1} + 0.1\mu g\ kg^{-1}\ min^{-1}$, n=12), 30 min before and 10 min after the 25 min occlusion of the left anterior descending coronary artery (LAD). Control dogs (n=20) were subjected to simply a 25 min occlusion of the LAD, followed by reperfusion. ANP infusion resulted in a fall in mean arterial blood pressure (-17 ±2 mmHg, $P<0.05$) and a moderate reduction in diastolic coronary resistance (-0.11 ±0.06 mmHg ml^{-1} min, ns). During ischaemia, the increase in LVEDP was less marked in the ANP treated than in the control dogs (9.0 ± 0.9 vs 12.7 ± 0.6 mmHg, $P<0.05$). Compared to the controls, in the presence of ANP , there was a reduced number of VPBs (129 ± 99 vs 390 ± 74, $P<0.05$) and episodes of VT (4.3 ± 3.5 vs. 9.0 ± 1.9, $P<0.05$) , a lower incidences of VT (50% vs 100%, $P< 0.05$) and VF (17% vs 40%, $P<0.05$) during occlusion. Survival from the combined ischaemia-reperfusion insult was also increased (58% vs 0%, $p<0.05$). The severity of myocardial ischaemia, assessed from changes in epicardial ST-segment and degree of inhomogeneity, were significantly less marked in dogs given ANP. We conclude, that ANP may protect the myocardium against the severe consequences of myocardial ischaemia, resulting from coronary artery occlusion and reperfusion in canine.

Témavezető : Dr. Végh Ágnes

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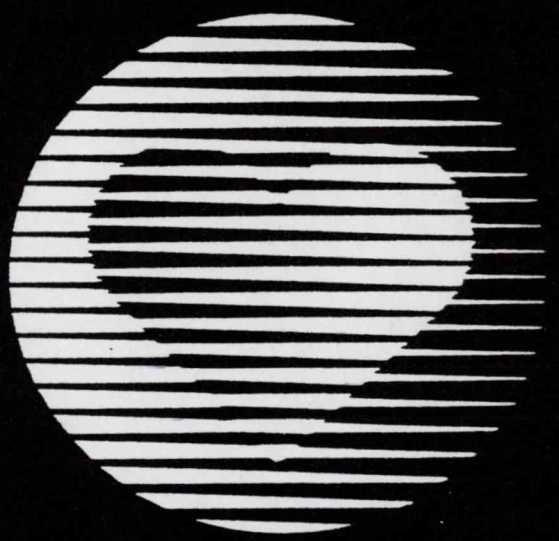

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17 Na^+/K^+ ATPase α ISOFORMS AND ARRHYTHMIAS IN THE EARLY STAGES OF GUINEA PIG HEART FAILURE.

Pascal Trouve. François Carré. Christophe Leclercq. Damien Bonnet. Thierry Dakhli and Daniele Charlemagne. INSERM U127. Hopital Lariboisière. Paris. France.

We studied the expression of Na^+/K^+ ATPase α isoforms and arrhythmias in the early stages of guinea pig (GP) heart failure. Left ventricular hypertrophy was induced by abdominal aortic stenosis (AS) for 6, 12 and 20 months. Anatomical and haemodynamic data indicated the development of heart failure after 20 months of stenosis (sham-operated vs. AS group): LV/Bwt (1.6±0.2 vs. 2.5±0.6mg/g, $p<0.01$), RV/Bwt (0.4±0.06 vs. 0.5±0.1mg/g, $p<0.05$), mean arterial pressure (51±16 vs. 72±16mmHg, $p<0.05$), LVEDP (13±4 vs. 20±7mm Hg, $p<0.05$). Holter monitoring showed a high frequency of atrio-ventricular block and occurrence of supraventricular and ventricular arrhythmias in hypertrophied hearts. mRNA levels of α2 and α3 Na^+/K^+ ATPase isoforms were increased after 6, 12 and 20 months of AS (+80%, +320%, +194%, $p<0.05$ and +60%, +287%, +119%, respectively, both $p<0.05$). Western blot analysis demonstrated α1 and α2 isoforms in GP heart for the first time and an increased level of α2 protein at 12 and 20 months of stenosis (+40% and +220% respectivly, $p<0.05$). In conclusion, we have demonstrated upregulation of α2 and α3 Na^+/K^+ ATPase isoforms in the early stages of GP heart failure associated with an increased incidence of arrhythmias. These two events should be correlated.

18 SPATIAL DISORGANISATION OF THE CARDIAC INTERCELLULAR JUNCTIONS IN DILATED CARDIOMYOPATHY.

Sawa Kostin. Stefan Hein. Jutta Schaper.
Max-Planck-Institute. Bad Nauheim. Germany

The purpose of this study was to investigate the distribution pattern of *fascia adherens* (FA), desmosomes (D) and gap junctions (GJ) in patients with dilated cardiomyopathy. Left ventricular samples from six explanted hearts obtained at transplantation were immunolabelled for proteins forming FA (N-cadherin), D (desmoplakin) or GJ (connexin 43) and examined by confocal laser microscopy. Altered patterns of cardiac intercellular junction distribution were observed in areas featuring myofibre disarray and at the myocardial interface with areas of replacement fibrosis. In the latter areas, connexin 43 GJ, as well as immunolabelled D, were arranged in longitudinally orientated arrays along the lateral sarcolemma, forming aberrant side to side connections. This abnormality extended in parallel with increasing myocardial scarring. Severe junctional abnormalities were detected in zones of myofibre disarray, including random distribution of GJ, side to side GJ and D, intersecting megadisk structures containing FA, D and GJ. Myocardial areas free from structural damage have a normal pattern of the distribution of immunolabelled FA, D and GJ.

Conclusion. The disorganisation of the intercellular junctions associated with myocardial scarring and myofibre disarray may play an important role in the development of a ventricular arrhythmogenic substrate in patients with dilated cardiomyopathy.

19 THE ROLE OF MITOCHONDRIAL GENES IN VENTRICULAR FIBRILLATION

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Ischemia/reperfusion stimulates expression of several early response genes and stress protein genes like proto-oncogenes and heat-shock proteins. Sequencing of 28 confirmed positive clones showed that mitochondrial-encoded genes constituted a major part of upregulated genes in isolated rat hearts (n=6 in each group) subjected to 30 min global ischemia followed by 2 hours of reperfusion. The expression (upregulation) of ATP synthase subunit 6 (ATPase 6) and cytochrome oxidase subunit III (C III), using Northern blot, has been detected after 30 min of ischemia followed by reperfusion in nonfibrillated hearts. These genes were downregulated in fibrillated myocardium. Thus, upon reperfusion, the relative band intensity of ATPase 6 and C III were increased from corresponding nonischemic control values of $6x10^{-4}$±$0.5x10^{-4}$ and $2.9x10^{-4}$±$0.3x10^{-4}$ pixel units to $7.3x10^{-4}$±$0.4x10^{-4}$ (NS) and $5.1x10^{-4}$±$0.4x10^{-4}$ ($p<0.05$) pixel units, respectively, in nonfibrillated myocardium. In fibrillated myocardium, the downregulation of these genes were detected. Thus, in fibrillated myocardium, the band intensity of ATPase 6 and C III was reduced from their nonischemic control values of $6x10^{-4}$±$0.5x10^{-4}$ and $2.9x10^{-4}$±$0.3x10^{-4}$ pixel units to $0.6x10^{-4}$±$0.2x10^{-4}$ and $1.2x10^{-4}$±$0.3x10^{-4}$ pixel units, respectively. These results have been confirmed using polymerase chain reaction (PCR) technique. The expression of these mitochondrial genes was the same in both nonfibrillated and fibrillated diabetic hearts. Our results show that the aforementioned mitochondrial genes can play an crucial role in the regulation of ventricular fibrillation in the ischemic/reperfused myocardium. (Supported by grants from OTKA F19946; OMFB 05201950515; FKFP 1254/1997 and Volkswagen Stiftung I/71-193)

20 THE ANTIARRHYTHMIC EFFECTS OF ANP IN A CANINE MODEL OF ISCHAEMIA-REPERFUSION

Agnes Végh, Mohamed Ali Rastigar, Julius Gy. Papp, James K. Parratt. Depts. of Pharmacology, A. Szent-Györgyi Med. Univ. Szeged, Hungary and Univ. Strathclyde, Glasgow, UK.

The effects of atrial natriuretic peptide (ANP) were examined in chloralose-urethane anaesthetised mongrel dogs. After thoracotomy, human synthetic ANP was infused intravenously ($10\ \mu g\ kg^{-1} - 0.1\ \mu g\ kg^{-1}\ min^{-1}$; n = 12), 30 min before and for 10 min after the 25 min occlusion of the left anterior descending coronary artery (LAD). Control dogs (n = 20) were simply subjected to a 25 min occlusion of the LAD, followed by reperfusion. ANP infusion resulted in a fall in mean arterial blood pressure (- 17 ± 2 mmHg, $P < 0.05$) and a slight reduction in diastolic coronary resistance (- 0.11 ± 0.06 mmHg $ml^{-1}\ min^{-1}$, ns). During ischaemia, the increase in LVEDP was less marked in the ANP treated dogs than in the controls (9.0 ± 0.9 vs 12.7 ± 0.6 mmHg, $P < 0.05$). Compared to the controls, ANP reduced the number of VPBs (129 ± 99 vs 390 ± 74, $P < 0.05$) and episodes of VT (4.3 ± 3.5 v. 9.0 ± 1.9, $P < 0.05$), and there were lower incidences of VT (50 % vs 100 %, $P < 0.05$) and VF (17 % vs 40 %, $P < 0.05$) during occlusion. Survival from the combined ischaemia-reperfusion insult was also increased (58 % vs 0 %, $P < 0.05$). The severity of myocardial ischaemia, assessed from changes in epicardial ST-segment and degree of inhomogeneity, were significantly less marked in dogs given ANP. We conclude that ANP may protect the myocardium against the severe consequences of myocardial ischaemia, resulting from coronary artery occlusion and reperfusion.
Supported by the Hungarian Scientific Research Foundation (OTKA), the British Council and the Hungarian Cultural and Education Ministry (No 14 and FKFP 1290/1997).

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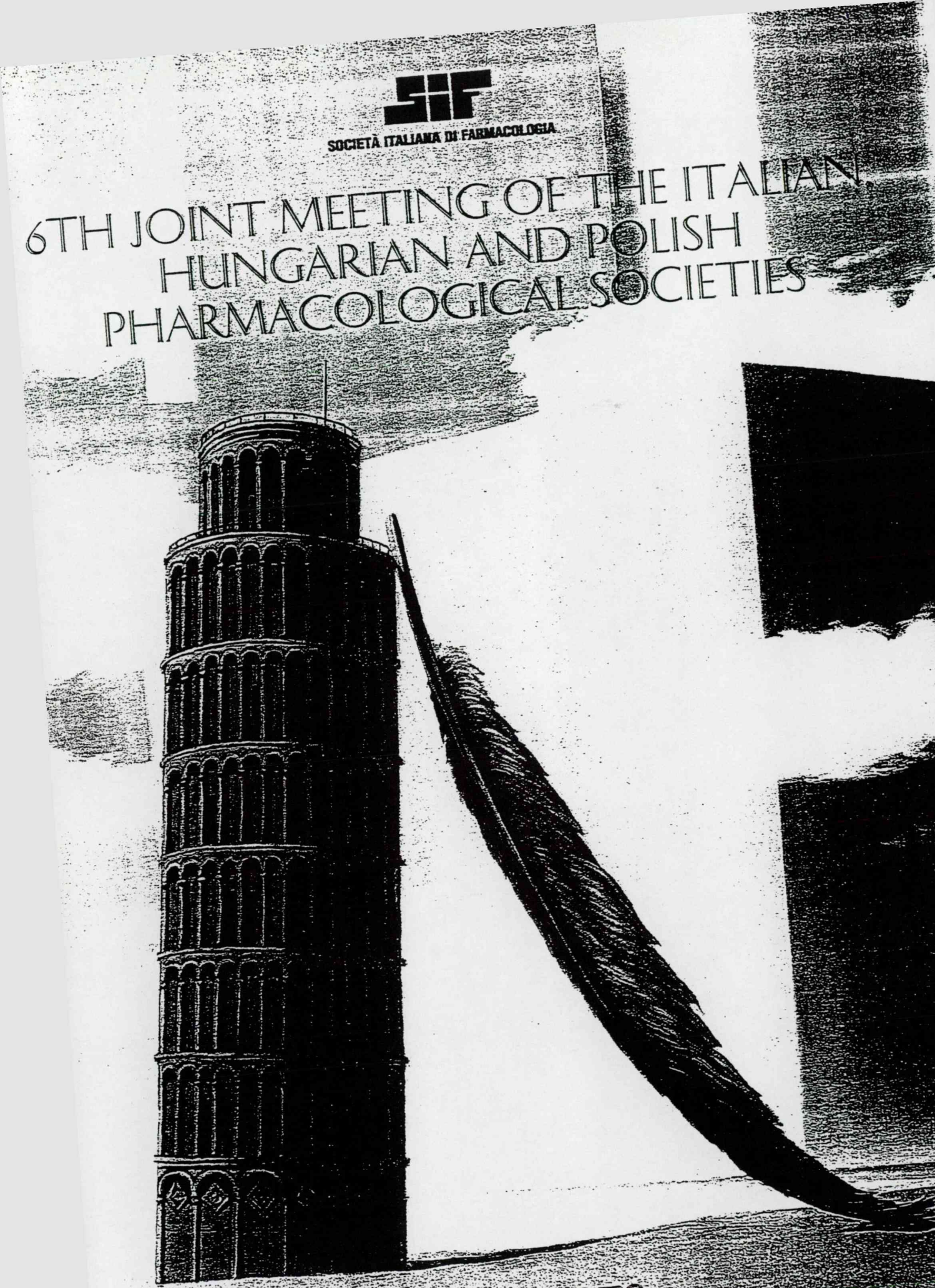
6TH JOINT MEETING OF THE ITALIAN, HUNGARIAN AND POLISH PHARMACOLOGICAL SOCIETIES



ABSTRACTS

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P88

PRECONDITIONING WITH SEROTONIN PROTECTS AGAINST ISCHEMIA-REPERFUSION INJURY IN ISOLATED RAT HEARTS

Mergveri K, Kovács A, Tihanyi K, Markó B, Egyed A, Szénási G
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The substantial serotonin (5-HT) content in the heart may be involved in the pathogenesis of cardiac ischemic injury. In fact, pretreatment with 5-HT prolonged time to contracture (TTC) during global ischemia that was completely abolished by the 5-HT_{2A} antagonists ketanserine and cinanserine in isolated rat hearts. Moreover, the application of the above 5-HT_{2A} antagonists led to a greater functional recovery from global ischemia during reperfusion, and systemic 5-HT depletion was also cardioprotective. Our hypothesis was that preconditioning (PC) with 5-HT also contributes to better recovery of contractile function during reperfusion in 5-HT depleted hearts. Therefore, we tested if PC with 5-HT improves functional recovery in rat hearts, and PC with ischemia (I) and phenylephrine (PHE) was also performed for comparison. Isolated rat hearts were subjected to three cycles of 5-min global ischemia or vehicle/drug infusion, separated by two cycles of 5-min reperfusion (REP) and a single 10-min REP before a 25-min prolonged ischemia. The hearts were then reperfused for 30 min and left ventricular developed pressure (LVDP) and end-diastolic pressure (EDP) were measured at the end of REP. Results are shown in the Table. (* = $p < 0.05$ vs. vehicle 0.2 μM ascorbic acid).

	Vehicle	IPC	5-HT			PHE	
			1 μM	0.1 μM	10 nM	1 μM	0.1 μM
TTC	8.4±1.3	4.6±0.3*	7.4±1.3	6.4±0.5	7.3±0.7	11±2	7.4±0.5
LVDP	49±4	83±4*	57±7	83±2*	66±4*	82±4*	66±7*
EDP	53±4	37±5*	48±3	36±3*	43±7	36±5*	45±6

Compared to the vehicle 5-HT at 0.1 μM exerted the same protective effect at the end of the REP as did IPC or PHE at 1 μM. The dose-response curve for 5-HT was bell shaped suggesting that the proischemic effect of 5-HT at high concentration may have compensated for PC. In conclusion, 5-HT induces PC at low concentration in isolated rat hearts. Since protein kinase C (PKC) plays a central role in PC, we suppose that 5-HT PC activated phospholipase C-inositol phosphate-diacylglycerol-PKC cascade at myocardial 5-HT_{2A} receptors.

P89

METABOLIC PROTECTION BY ADENOSINE OF THE HYPOXIC AND REPERFUSED MYOCARDIUM

Szabó JZs, Cseppento Á, Ujfalusi A, Szentmiklósi AJ
Debreceni Orvostudományi Egyetem Gyógyszertani Intézete, Debrecen, Magyarország

The influence exerted on the hypoxic and reperfused myocardium by adenosine was investigated in electrically driven left atrial myocardium and in isovolumically perfused Langendorff heart preparations of guinea pigs. The aim of our work was to study the effect of an in vivo methylxanthine treatment on the hypoxia-tolerance of the myocardium in vitro.

Methods: mechanical activity of left atrial myocardium was measured in guinea pigs treated i.p. once daily for 10 days with solvent or aminophylline (APH, 25 mg/kg ip). In Langendorff preparations, adenosine (15μM) or/and 8-phenyl-theophylline (8-PT) was present in the nutrient solution during the full experimental period. The experiments were terminated by freeze-clamping the ventricles used for biochemical determinations. The prevention of the hypoxia-induced changes in parameters measured was regarded as criterion of cardioprotection.

Results: in left atrial myocardium obtained from APH-treated guinea pigs, no change in the hypoxia-induced decrease of mechanical activity and the rate of recovery under reoxygenation could be detected. In Langendoff heart preparations adenosine significantly increased myocardial ATP, phosphocreatine and glycogen, moderated lactate production and effectively prevented the hypoxia-induced changes of these parameters. In vitro, 8-PT blocked all of the salutary effect of endogenous and exogenous adenosine.

Conclusion: in addition to the known favourable effect of adenosine on the coronary perfusion, a receptor-mediated metabolic protection may also contribute to the increase of the hypoxia-tolerance of the myocardium. The methylxanthines as potentially risk factors in the development of ischemic heart diseases, - on the basis of these experiments - cannot be justified.

Supported by grants from OTKA (T 016 848) and ETT (307/96)

P90

INHIBITION OF BRADYKININ CATABOLISM REDUCES THE SEVERITY OF VENTRICULAR ARRHYTHMIAS RESULTING FROM ISCHAEMIA-REPERFUSION IN ANAESTHETISED DOGS

Rastigar MA, Végh A, Papp JGy, *Parratt JR, °Semeraro C, °Marchini F
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It is well documented that bradykinin (BK) is involved in the cardioprotective effect of ischaemic preconditioning. It is also established that angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP) are the two main enzymes involved in BK degradation. The present study aimed to examine whether Z13752A, a dual ACE/NEP inhibitor modifies the severity of ventricular arrhythmias resulting from a 25 min occlusion and then reperfusion of the left anterior descending coronary artery (LAD) in chloralose-urethane anaesthetised mongrel dogs. Z13752A was infused (128 μg kg^{-1} min^{-1} iv., n = 12) 60 min prior to occlusion of the LAD. Then the infusion was stopped and dogs were subjected to a 25 min occlusion and then reperfusion of the LAD. Z13725A resulted in a fall in mean arterial blood pressure (-11 ± 3 mmHg, $P < 0.05$) and a reduction in the resistance of the LAD and left circumflex (LCX) coronary arteries (by 12 % and 16 %, respectively; $P < 0.05$). Compared to the controls (n = 20), occlusion of the LAD following Z13725A administration, resulted in a reduced number of VPBs (91 ± 41 vs 390 ± 74, $P < 0.05$) and VT episodes (0.8 ± 0.3 v. 9.0 ± 1.9, $P < 0.05$) and lower incidences of VT (42 % vs 100 %, $P < 0.05$) and VF (25 % vs 40 %, $P < 0.05$). Survival from the combined ischaemia-reperfusion insult was also increased (50 % vs 0 %, $P < 0.05$). The occlusion-induced epicardial ST-segment elevation and the degree of electrical inhomogeneity were also significantly less marked in Z13752A treated dogs than in the controls. These results indicate that dual blockade of ACE and NEP provides marked protection against the severe consequences of myocardial ischaemia, perhaps by elevating BK levels and/or prolonging the protective effect of BK.

Supported by the Hungarian Scientific Research Foundation (OTKA), the British Council and the Hungarian Ministry of Culture and Education (No 14 and FKFP 1290/1997).

P91

ATRIAL NATRIURETIC PEPTIDE INFUSION REDUCES THE SEVERITY OF VENTRICULAR ARRHYTHMIAS IN A CANINE MODEL OF ISCHAEMIA-REPERFUSION

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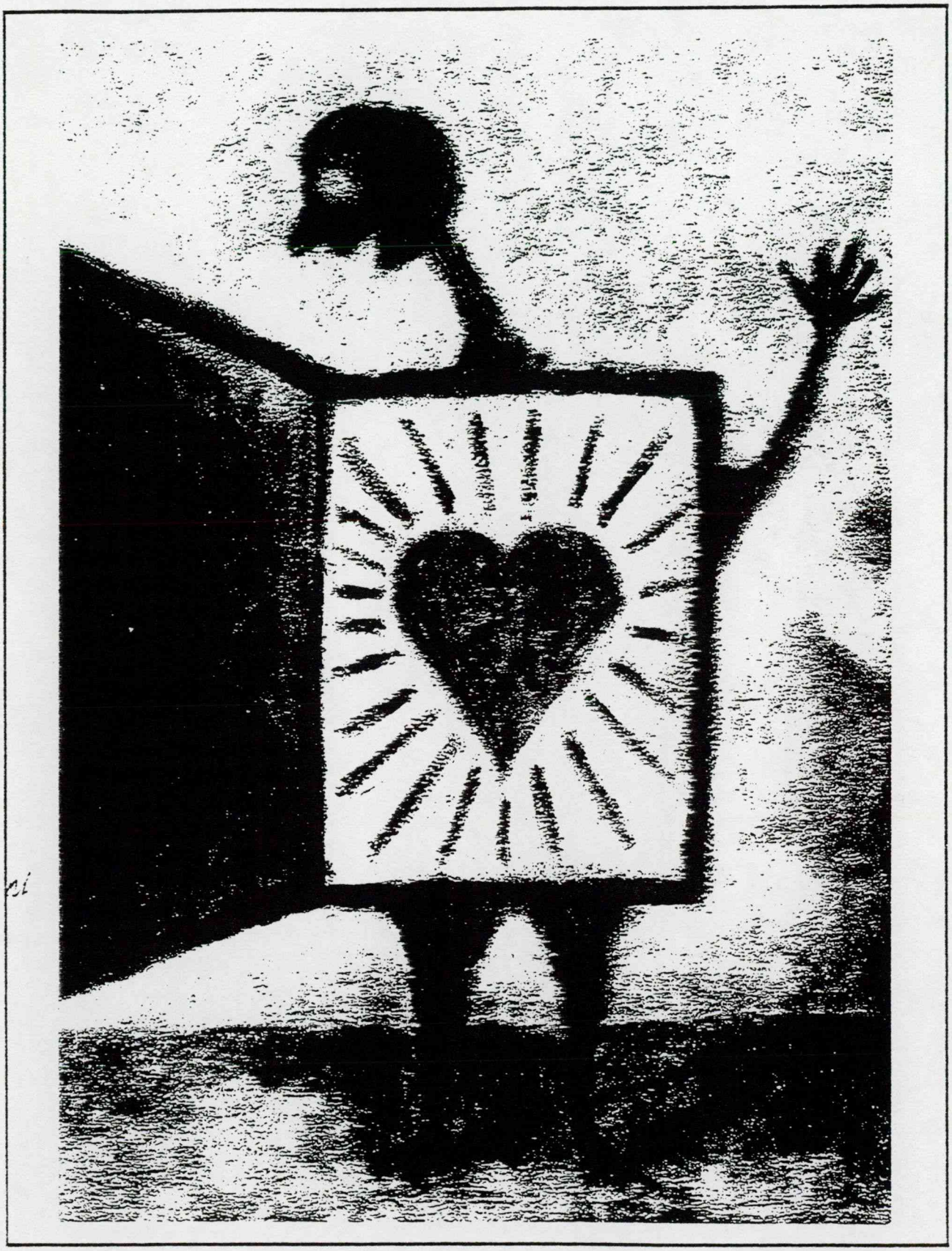
The effects of atrial natriuretic peptide (ANP) on the severity of ventricular arrhythmias resulting from coronary artery occlusion and reperfusion were examined in chloralose-urethane anaesthetised mongrel dogs. After thoracotomy, human synthetic ANP was infused intravenously (10 μg kg^{-1} + 0.1 μg kg^{-1} min^{-1}; n = 12) 30 min before and for 10 min after the 25 min occlusion of the left anterior descending coronary artery (LAD). Control dogs (n = 20) were simply subjected to saline infusion and then occlusion of the LAD, followed by reperfusion. ANP infusion resulted in a fall in mean arterial blood pressure (-17 ± 2 mmHg, $P < 0.05$) and a slight reduction in diastolic coronary resistance (-0.11 ± 0.06 mmHg ml^{-1} min^{-1}, ns). During ischaemia, the increase in LVEDP was less marked in the ANP treated dogs than in the controls (9.0 ± 0.9 cp 12.7 ± 0.6 mmHg, $P < 0.05$). Compared to the controls, ANP reduced the number of VPBs (129 ± 99 cp 390 ± 74, $P < 0.05$) and episodes of VT (4.3 ± 3.5 cp 9.0 ± 1.9, $P < 0.05$), and there were lower incidences of VT (50 % cp 100 %, $P < 0.05$) and VF (17 % cp 40 %, $P < 0.05$) during occlusion. Survival from the combined ischaemia-reperfusion insult was also increased (58 % cp 0 %, $P < 0.05$). The severity of myocardial ischaemia, assessed from changes in epicardial ST-segment and degree of inhomogeneity, was significantly less marked in dogs given ANP. We conclude that ANP may protect the myocardium against the severe consequences of myocardial ischaemia, resulting from coronary artery occlusion and reperfusion.

Supported by the Hungarian Scientific Research Foundation (OTKA), the British Council and the Hungarian Ministry of Culture and Education (No 14 and FKFP 1290/1997)

British Society for Cardiovascular Research



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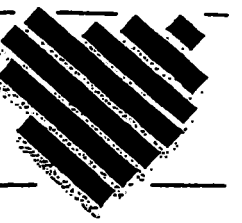

Myocardial Responses to Sub-lethal Ischaemia: Stunning, Hibernation and Preconditioning

Organiser: Dr G F Baxter

Assistant: Ms A Burdus

The Hatter Institute for Cardiovascular Studies
Centre for Cardiology
University College London Hospitals and Medical School
London WC1E 6DB

P4.

Does ACE or NEP inhibition provide additional protection to ischaemic preconditioning?

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The aim of the present study was two fold: first to analyse the time-course of the antiarrhythmic protection induced by a single brief (5min) preconditioning (PC) ischaemic episode, and second whether this protection when it has already faded can be enhanced or prolonged by increasing bradykinin levels either with the ACE inhibitor enalaprilate or with the dual ACE/NEP inhibitor Z13752A. Nine groups of chloralose-urethane anaesthetised dogs were used. Control dogs (n=16) were infused for 60 min and then subjected to a 25 min occlusion of the left anterior descending coronary artery (LAD), followed by reperfusion. In the time course study, dogs were preconditioned by a single 5 min occlusion of LAD and then at various times afterwards, (5 min, n=9; 15 min, n=8; 30 min, n=8; 60 min, n=12, respectively) these dogs were subjected to prolonged ischaemia. In two further groups either Z13752A, (128 µg kg-1 min-1, n=9) or enalaprilate (5 µg kg-1 min-1, n=10) was infused over 60 min, prior to occlusion of the LAD. Preconditioned dogs with a 60 min reperfusion were also given Z13752A (n=12), and enalaprilate (n=14). Compared to the controls, ventricular premature beats (VPBs: 353 ± 79 v. 83 ± 37, 72 ± 27, 32 ± 9), ventricular tachycardia episodes (VT: 10.7 ± 3.3 v.1.6 ± 0.7, 0.25 ± 0.16, 0.38 ± 0.18); the incidences of both VT (100% v.62%, 25%, 37%) and ventricular fibrilation (VF: 44% v 0% in all groups) were markedly reduced, and survivals (0% v. 63%, 38%, 38%) were increased when the reperfusion intervals between the PC occlusion and prolonged occlusion were 5, 10 and 30 min, respectively. However, the protection was abolished when the time interval between PC and the prolonged ischaemia was increased to 60 min, (VPBs: 273 ± 87, VT: 8 ± 4, VT: 58%, VF: 17%, survival: 17%). Z13752A and enalaprilate given alone, before 25 min occlusion of the LAD significantly reduced the severity of ventricular arrhythmias (VPBs: 91 ± 40, 103 ± 42, VT: 0.22 ± 0.15, 1.8 ± 1.2, VT: 22%, 44%, VF: 0%, 30%, survival: 67%, 50%, respectively). Howevér, when these drugs were infused in PC dogs with 60 min reperfusion, Z13752A provided no further protection, (VPBs: 56 ± 24, VT: 0.33 ± 0.18: VT: 25%, VF: 8.3%, survival: 33%, P> 0.05), whereas, enalaprilate attenuated rather than prolonged the protective effect of PC, (VPBs: 252 ± 73, VT: 2.5 ± 1.2, VT: 54%, VF: 38%, survival: 0%). We concluded from these results that niether enalaprilat nor the dual ACE/NEP inhibitor Z13752A, was able to provide additional protection to ischaemic Preconditioning.

SZENT-GYÖRGYI ALBERT ORVOSTUDOMÁNYI EGYETEM

SZOTE V. Ph.D Előadói Napok

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INVOLVMENT OF BRADYKININ IN THE ANTIARRHYTHMIC EFFECT OF Z-13752A, A NOVEL NEUTRAL ENDOPEPTIDASE INHIBITOR

We have shown previously that Z13752A, dual ACE-NEP inhibitor markedly reduced the severity of ventricular arrhythmias resulting from a 25 min occlusion and reperfusion of the left anterior descending coronary artery in anaesthetised dogs.Now we hypothesized that inhibition of NEP/ACE by Z13752A a novel NEP/ACE inhibitor would afford cardioprotection to ischemic myocardium and may modify ischaemia-induced ventricular arrhythmias and these effects are mediated by kinins.

Four groups of chloralose-urethane anaesthetised mongrel dogs were used, group 1: Twenty animals were given saline, group 2: Ten animals 0.3 $mg.kg^{-1}$ i.v bolus of icatibant (Hoe-140) kinin-receptor antagonist, 10 min before prolonge occlusion, group 3: Nine dogs were given 128 $\mu g.kg^{-1}.min^{-1}$ Z13752A in an i.v infusion, over a 60 min period, immediatly prior to a 25 min occlusion of the left antirior decending branch of coronary artery (LAD). group. To test whether the effect of Z13752A could be suppressed by blocking kinins, icatibant was administered to twelve dogs, befor LAD occlusion.

To see 128 $\mu g.kg^{-1}.min^{-1}$ i.v bolus of Z13752A can inhibite both NEP and ACE, we studied effect of Z13752A on blood-pressure responce to intravenous Angiotensin I and angiotensin II by comparing to enalaprilate (ACEi).

Z13752A significantly reduced number of VPBs (90.8 ± 40.5) and episodes of VT (0.22 ± 0.15) and lower incidence of VT (22%) and VF (0%), compared to the controls (VPBs:390 ± 74, $P<0.05$; episodes of VT:9 ± 1.9, $P<0.05$; incidence of VT:100% , $P<0.05$ and VF 40%), survival from the combined ischaemia-reperfusion insult was also increased significantly following Z13752A (68% vs 0% , $P<0.05$) treatment and it was also significan reduction in ST-segment elevation and degree of electrical inhomogenity during 25 min occlusion in Z13752A group.

If we give kinin receptor antagonist icatibant, before occlusion, result is not significant different by comparing to control. Cardioprotective effect of Z13752A are blocked by icatibant.

Thus NEP participates in catabolism of kinins in the heart: inhibition of NEP may increase cardiac kinins, which are responsible for the cardioprotective effect of Z13752A.

Supervisor: Ágnes Végh, PhD

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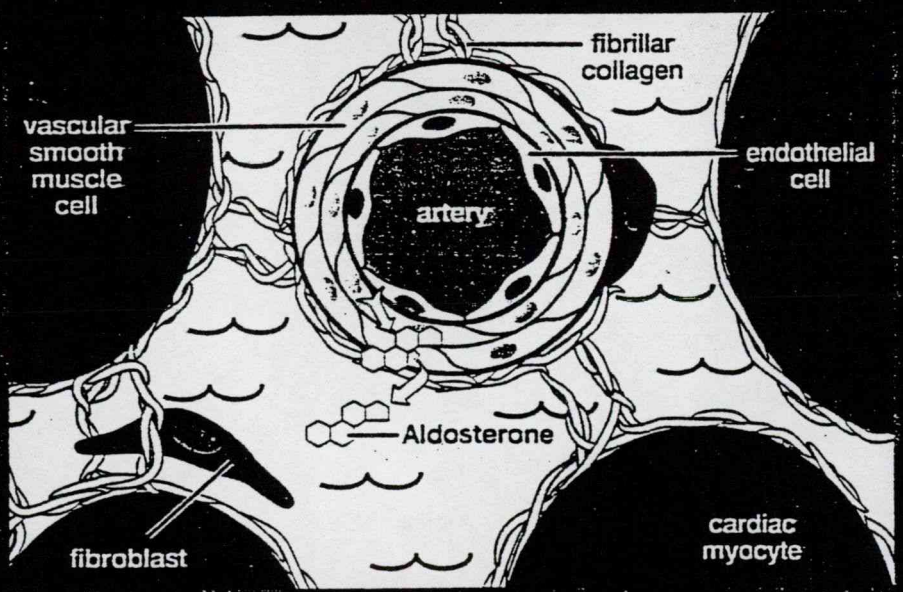

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THE ROLE OF BRADYKININ IN THE ANTIARRHYTHMIC EFFECT OF THE NEUTRAL ENDOPEPTIDASE INHIBITOR, Z-13752A

Th69

Mohamed Ali Rastigar, Agnes Végh, Julius Gy. Papp, [+]Francesco Marchini, [+]Lorenzo Pradella, James R. Parratt. Depts. of Pharmacology and Pharmacotherapy, A. Szent-Györgyi Med. Univ. Szeged, Hungary [+]Univ. of Strathclyde, Glasgow, UK. and [+]Zambon, Milan, Italy

We have shown previously that Z13752A, a dual ACE/NEP inhibitor markedly reduced the severity of ventricular arrhythmias resulting from a 25 min occlusion and reperfusion of the left anterior descending coronary artery (LAD) in anaesthetised dogs. Now we have examined whether bradykinin plays a role in this protective effect of Z13752A. For this purpose, in chloralose-urethane anaesthetised dogs, icatibant (0.3 $mgkg^{-1}$, iv.) was given 10 min before occlusion of the LAD, either in the absence (n=10) or in the presence of Z13752A (128 $\mu gkg^{-1}min^{-1}$ iv. infusion, over a 60 min period prior to the occlusion, n=12). Control (n=20) and Z13752A treated dogs (n = 9) were given either saline or Z13752A infusions (as above), and simply subjected to a 25 min occlusion-reperfusion insult. Compared to the controls, Z13752A reduced the number of VPBs (91±41 vs. 390±74, $P<0.05$) and episodes of VT (0.2±0.2 vs. 9.1±1.9, $P<0.05$) and there were lower incidences of VT (22% vs. 100%, $P<0.05$) and VF (0% vs. 40%, $P<0.05$) during occlusion. Survival from the combined ischaemia-reperfusion insult was increased (68% vs. 0%, $P<0.05$) following Z13752A administration. Although icatibant itself did not influence the severity of ventricular arrhythmias, it markedly attenuated the antiarrhythmic effect of Z13752A. Thus, the number of VPBs (632±300) and episodes of VT (22.5±13.8), the incidences of VT (100%) and VF (58%) during occlusion were significantly ($P<0.05$) higher than in the Z13752A treated dogs and these were similar to that in the controls. In the presence of icatibant no dog treated with Z13752A survived the combined occlusion-reperfusion insult. We concluded from these results that the marked antiarrhythmic effect of Z13752A is mediated through bradykinin.

Supported by the Hungarian Scientific Research Foundation (OTKA), the British Council and the Hungarian Ministry of Culture and Education.

ABOLISHING THE ANTIARRHYTHMIC EFFECT OF ISCHAEMIC PRECONDITIONING BY CAPTOPRIL IN RATS

Th70

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The aim of the present experiments was to investigate whether reflex activation of the renin-angiotensin system during preconditioning contributes to the protection against ischaemia-reperfusion induced arrhythmias. In anaesthetized, artificially ventilated rats the left main coronary artery was ligated for 6 min, followed by reperfusion. A single preconditioning ischaemia, i.e. occluding the coronary artery for 2 min, 10 min before the test ischaemia, markedly decreased the incidence of ventricular fibrillation (VF; 33% vs. 88% in control ischaemia-reperfusion) and ventricular tachycardia (VT; 48% vs. 94%), and increased the survival rate (SR; 86% vs. 29%). Captopril pretreatment (10 mg/kg orally, 1 h prior to test ischaemia) alone did not influence significantly the occurrence of VF, VT and SR (81%, 86% and 19%, respectively). However, captopril pretreatment significantly abolished the protection by preconditioning, increased the incidence of VF and VT (56% and 75%, respectively) and decreased SR (50%). These results suggest that production of angiotensin II due to the reflex activation of the renin-angiotensin system during ischaemic preconditioning may contribute to the development of the protection against ischaemia-reperfusion induced arrhythmias in the rat.

This work was supported by OTKA (T 22300) and ETT (06-127) grants.

INVESTIGATION OF THE ANTI- AND PROARRHYTHMIC EFFECT OF LINOLEIC ACID RICH DIET IN RABBITS

Th71

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New-Zealand white rabbits were fed control pellet or a diet supplemented with 10% sunflower seed oil (SSO, containing 58% linoleic acid) for one month. Myocardial ischaemia was produced by ligating the first branch of the left circumflex coronary artery for 10 min, followed by 10 min of reperfusion. The SSO diet significantly increased the number of animals developing no arrhythmias during the 10 min myocardial ischaemia (53% vs 17% in control animals, $P<0.05$). Also, SSO diet significantly decreased the incidence of ventricular fibrillation and increased survival (7% vs. 73% and 100% vs. 36% in control animals, respectively, $P<0.05$) as well as the number of animals without any arrhythmia during reperfusion (60% vs. 0% in the control group, $P<0.05$). In another set of experiments animals were administered cumulative doses of D-sotalol (3, 10 and 30 mg/kg, i.v.) during α_1-adrenergic receptor stimulation with phenylephrine (15 µg/kg/min). Torsade de pointes developed in 60% of control animals vs. 9% in animals fed SSO diet ($P<0.05$). Our results suggest that SSO diet may not only protect against arrhythmias during myocardial ischaemia but can also decrease the proarrhythmic potency of class III antiarrhythmic drugs.

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NO-DEFFICIENT HEART IS PRONE TO LOW K^+-INDUCED VENTRICULAR FIBRILLATION

Th72

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Long lasting NO-defficiency is accompanied by structural remodeling and myocardial fibrosis of the rat heart, which might deteriorate intercellular communication at the gap junctions and facilitate arrhythmias. We investigated vulnerability of L-NAME treated (40mg/kg daily in drinking water for 4-5weeks) rat hearts to low K^+-induced ventricular fibrillation (VF). The hearts were perfused in Langendorff mode at 37°C and 65mmHg with oxygenated Krebs-Henseleit (5.4mM K^+) solution followed by K^+ defficient (0.8mM K^+) one. Atrial and ventricular ECG and incidence of VF were continuously monitored. K^+ defficient perfusion induced sustained VF in 83% of L-NAME treated rats in comparing to 33% of nontreated hearts. VF preceeded hypokalemia-related subcellular injury of the cardiomyocytes, enzyme histochemical (SDH, PhPh, 5-NC) and gap junction connexin43 alterations, which were heterogenously distributed within myocardium and more pronounced in NO-defficient hearts.Results indicate that long lasting inhibition of NO production renders the heart more vulnerable to low K^+-induced malignant ventricular arrhythmias.

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PM113

INVOLVEMENT OF BRADYKININ IN THE ANTIARRHYTHMIC EFFECT OF Z-13752A, A NOVEL NEUTRAL ENDOPEPTIDASE INHIBITOR

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We have shown previously that Z13752A, a dual ACE/NEP inhibitor markedly reduced the severity of ventricular arrhythmias resulting from a 25 min occlusion and reperfusion of the left anterior descending coronary artery (LAD) in anaesthetised dogs. Now we have examined whether bradykinin plays a role in this protective effect of Z13752A. For this purpose, in dogs anaesthetised with chloralose and urethane, icatibant, a bradykinin B2 receptor antagonist, was given intravenously in a dose of 0.3 mg. kg^{-1}, 10 min before the occlusion of the LAD, both in the absence (n=10) and in the presence of Z13752A (128 $\mu g\ kg^{-1}\ min^{-1}$ iv. infusion, over a 60 min period just prior to the occlusion, n=12). Control (n=20) and Z13752A treated dogs (n=9) were given either saline or Z13752A infusions (as above), and simply subjected to a 25 min occlusion-reperfusion insult. Compared to the controls, Z13752A markedly reduced the number of the ventricular premature beats (VPBs; 91±41 vs. 390±74, P<0.05) and episodes of ventricular tachycardia (VT; 0.2±0.2 vs. 9.1±1.9, P<0.05) and there were lower incidences of VT (22% vs. 100%, P<0.05) and ventricular fibrillation (VF; 0% vs. 40%, P<0.05) during occlusion. Survival from the combined ischaemia-reperfusion insult was increased (68% vs. 0%, P<0.05) following Z13752A administration. Although icatibant itself did not influence the severity of occlusion/reperfusion-induced ventricular arrhythmias (VPBs: 524±166, VT episodes: 25.0±10.1, VT%: 73 %, VF%: 45 %, survival: 9 %), it markedly attenuated the antiarrhythmic effect of Z13752A. Thus, the number of VPBs (632±300) and episodes of VT (22.5±13.8), the incidences of VT (100%) and VF (58%) during occlusion were significantly (P<0.05) higher than in the Z13752A treated dogs and these were similar to that in the controls. In the presence of icatibant no dog treated with Z13752A survived the combined occlusion-reperfusion insult. We concluded from these results that the marked antiarrhythmic effect of Z13752A is mediated through bradykinin.

Supported by the Hungarian Scientific Research Foundation (OTKA), the British Council and the Hungarian Ministry of Culture and Education.

PM114

ANTIARRHYTHMIC EFFICACY OF ANALOGUES OF LIDOCAINE, PROCAINAMIDE AND PROPRANOLOL CONTAINING THE QUATERNARY NITROGEN AND FRAGMENTS OF K_{ATP} CHANNEL BLOCKERS.

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It was estimated, that the quaternization of aliphatic amino function of lidocaine (I), procainamide (II), propranolol (III), quinidine (IV) increased the acute toxicity and antiarrhythmic effect in comparison with their tertiary analogues. We synthesized the structural analogues of I-IV and bretylium, containing fragments of K_{ATP} channel blockers. The analogues of I, containing 4-methyl, alkoxy, alkoxycarbonyl, acetylamino, p-toluenesulfonamido and methanesulfonamido substituents in the anilide fragment were constructed. On the calcium chloride-induced arrhythmia model in rats the quaternary ammonium compounds showed the greater antiarrhythmic efficacy in comparison with tertiary analogues, I-IV, bretylium and in most cases prolonged the survival rate of animals by 60-90 percent against 10 percent in control. The ammmonium derivatives of I, containing 4-methyl- , p-toluenesulfonamido- or 4-methanesulfonamidoanilide fragment and allyl, benzyl, butyl or heptyl radicals in ammonium function, demonstrate the most efficacy on the strophantin model in guinea-pigs. They fully protected from ventricular tachycardia and ventricular fibrillation and also increased arrhythmogenic treshold for strophantin. It was established, that quaternary nitrogen atom is associated with greater antiarrhythmic efficacy which depends upon the structure of the cation function and on its compatibility with other pharmacophores as well. In conclusion, the presence of fragments of K_{ATP} channel blockers remarcably increased the antiarhythmic potency and decreased the acute toxicity of compounds. Derivatives of I, containing the 4-methanesulfonamido group in anilide fragment, exhibit the greater antiarrhythmic effect and significantly lesser acute toxicity in comparison with 4-methylanilide analogues.

PM115

GENE EXPRESSION RELATED TO FIBRILLATION IN ISCHEMIC/REPERFUSED ISOLATED RAT HEARTS

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The up- and down-regulation of mitochondrial genes related to ventricular fibrillation (VF) in ischemic/reperfused nondiabetic and diabetic myocardium were studied. We have done subtractive screening, Northern blotting, and reverse transcription polymerase chain reaction (RT-PCR) of mitochondrial genes expressed after 30 min ischemia followed by 120 min of reperfusion in hearts developed VF or did not show VF. Cardiac function was also monitored. ATP synthase subunit 6 and cytochrome oxidase III showed an expression after 30 min ischemia in both nondiabetics and diabetic myocardium. Upon reperfusion, the down-regulation of these genes was only observed in fibrillated hearts. A reduction in the expression of mRNAs was not seen in nonfibrillated myocardium. Cardiac function showed no correlation between the up- or down-regulation of these mitochondrial genes in nondiabetic and diabetic ischemic/reperfused hearts. Northern blotting and RT-PCR confirm an increase in mRNA levels of the genes studied after 30 min ischemia, and a decline throughout an additional two-hour period of reperfusion in both fibrillated nondiabetic and diabetic myocardium. Our data suggest that ATP synthase subunit 6 and cytochrome oxidase III may play a critical role in arrhythmogenesis and the stimulation of the expression of ATP synthase subunit 6 or cytochrome oxidase III may prevent the development of VF in both diabetic and nondiabetic myocardium.

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A "DELAYED RECTIFIER" KÁLIUMÁRAM GYORS ÉS LASSÚ KOMPONENSE EGÉSZSÉGES HUMÁN KAMRAI SZÍVIZOMSEJTEKEN

Iost Norbert, Virág László, Varró András, Opincariu Miklós, Szécsi János, Papp Gyula
Farmakológiai és Farmakoterápiai Intézet és Szívsebészeti Önálló Osztály, Szent-Györgyi Albert Orvostudományi Egyetem, Szeged
Elektrofiziológia, szívizom alapkutatás

Jelentős eltérések tapasztalhatók a különböző emlősök szívéből izolált "delayed rectifier" káliumáramokban (I_K). Az eddig ismert humán adatok olyan beteg szívekből származnak, ahol a mérések során az I_K tulajdonságait jelentősen megváltoztató Cd^{2+}-t és Ba^{2+}-t használtak. Ezért kísérleteink célja az I_K aktivációs és deaktivációs kinetikai tulajdonságainak vizsgálata volt 16 egészséges humán bal kamrából izolált 32 szívizomsejten a patch-clamp technika egészsejtes konfigurációja segítségével 35 °C-on. A kalcium áram (I_{Ca}) gátlásához nifedipint használtunk.
Minden sejtben regisztráltunk egy E-4031 szenzitív farokáramot, amit az I_K-áram gyors komponensének (I_{Kr}) tekinthetünk. Ezen áram feszültség-áram karakterisztikája magasabb feszültségeknél már nem emelkedett tovább ("inward rektifikáció"). Az áram -10 mV-nál kezdett aktiválódni, maximumát (0.27±0.07 pA/pF, n=10) +20 mV-nál érte el. Az I_{Kr}-áram aktivációs kinetikája nagyon gyors volt (t = 36.6 ± 3.2 ms, 30 mV-on, n=6), a deaktivációs görbe pedig viszonylag lassú időállandójú kétexponenciális függvénnyel volt illeszthető (t_1 = 600.0 ± 53.9 ms és t_1 =6792 ± 875.7 ms, -40 mV-on, n=6). Forskolin jelenlétében egy E-4031 inszenzitív és chromanol 293B szenzitív áramot figyeltünk meg. Ezt az áramot tekintjük az I_K-áram lassú komponensének (I_{Ks}). Az I_{Ks} áram maximális értéke 0.206±0.035 pA/pF (amit 50 mV-nál ért el, n=6), ez az I_{Kr}-áram 75%-nak felel meg. Az áram aktivációs időállandója viszonylag lassú (t=1096.8±126.5, 50 mV-on, n=6), deaktivációs időállandója viszonylag gyors volt (t=109.9±11.6 ms, -40 mV-on, n=6).
Eredményeink arra utalnak, hogy az egészséges humán kamrában a "delayed rectifier" káliumáramnak mind a gyors, mind a lassú komponense jelen van. A mért I_K legjobban a kutyaszíven meghatározott I_K-áramhoz hasonlít, viszont jelentősen eltér a tengeri malacban meghatározott I_K-áramtól. Valószínűsíthető, hogy az I_K fontos szerepet játszik a humán kamra repolarizációjának frekvenciafüggő modulálásában.
A munka az OTKA T 020604 és az FKFP 1025/97 támogatásával készült.

FAST AND SLOW DELAYED RECTIFIER POTASSIUM CURRENTS IN UNDISEASED HUMAN VENTRICULAR MYOCYTES

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There are large inter-species variations in the cardiac delayed rectifier potassium current (I_K). The very few available data regarding I_K in man originate from diseased hearts in the presence of Cd^{2+} and Ba^{2+}, which cations fundamentally alter the properties of I_K. Therefore, the characteristics of I_K (including activation and deactivation kinetics) were studied in 32 myocytes isolated from 16 undiseased human left ventricle, by applying the whole cell configuration of the patch-clamp technique at 37 C°. Nifedipine was used to block I_{Ca}.
The E-4031 sensitive rapid component of I_K (I_{Kr}) was found to be present in all myocytes. The current-voltage relationship of the I_{Kr} tail current showed apparent inward rectification. The activation of the current started at -10 mV and reached its maximal amplitude (0.27±0.07 pA/pF, n=10) at 20 mV. The activation of the I_{Kr} was fast (t=36.6±3.2 ms, at 30 mV, n=6) with relatively slow bi-exponential deactivation kinetics (t_1=600.0±53.9 ms and t_2=6792±875.7 ms, at -40 mV, n=6). In the presence of forskolin an E-4031 insensitive and chromanol 293B sensitive tail current, which represented the slow component of I_K (I_{Ks}), was also observed. The maximal amplitude of I_{Ks} tail was 0.206±0.035 pA/pF at 50 mV, n=6, ie. 75 % of the amplitude of I_{Kr}. The time constant for activation of I_{Ks} was rather slow (t=1096.8±126.5 ms, n=6) and for deactivation it was relatively rapid (t=109.9±11.6 ms, n=6).
It is concluded that both I_{Kr} and I_{Ks} exist in undiseased human ventricle. The recorded I_K resembled I_K detected in dog ventricular myocytes but substantially differed from I_K measured in myocytes from guinea pig ventricle. I_{Kr} and I_{Ks} probably play an important role in the frequency dependent and pharmacological modulation of repolarization in human ventricle.
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A PITVARI NÁTRIURETIKUS PEPTID (ANP) HATÁSA A KORONÁRIA OKKLÚZIÓ ÉS REPERFÚZIÓ OKOZTA KAMRAI ARITMIÁKRA ALTATOTT KUTYÁKBAN

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Jelen kísérleteink célja az volt, hogy megvizsgáljuk vajon az ANP milyen módon befolyásolja az iszkémia-reperfúzió során megjelenő kamrai aritmiák súlyosságát altatott kutyákban. Kloralóz-uretán keverékével altatott kutyákban a mellkast megnyitottuk, majd a bal coronaria arteria descendens anterior ágának (LAD) 25 perces okklúziója előtt 30 perccel, ill. azt követően 10 perccel, intravénásan szintetikus humán ANP-t (10 µg kg^{-1} + 0.1µg kg^{-1} min^{-1}, n=11) infundáltunk. A kontroll állatokban csak 25 perces LAD okklúziót ill. azt követően reperfúziót végeztünk. Az ANP infúzió az artériás középnyomást (-17±2 mmHg, P < 0.05) és a diasztolés koronária rezisztenciát (-0.11±0.06 mmHg ml^{-1} min, P > 0.05) mérsékelten csökkentette. Az ANP-vel kezelt kutyákban a koszorúér okklúzió hatására bekövetkező balkamrai végdiasztolés nyomás emelkedés kisebb volt, mint a kontroll csoport állataiban (9.0±0.9 v. 12.7 ± 0.6 mmHg, P < 0.05). Összehasonlítva a kontroll csuporttal, az ANP-vel kezelt állatokban az okklúzió alatt kevesebb kamrai extrasystole (26±12 v. 390±74, P < 0.05), kevesebb kamrai tachikardiás epizód (0.7±0.3 v. 9.0±1.9, P < 0.05) jelent meg. Az ANP-vel kezelt állatokban ugyancsak számottevően csökkent a kamrai tachikardia (45% v. 100%, P < 0.05) valamint a kamrafibrilláció (VF; 18% v. 40%, P < 0.05) okklúzió alatti gyakorisága, és szignifikánsan emelkedett a túlélés aránya (64 % v. 0 %, P < 0.05). Kísérleteinkben az iszkémia súlyosságát két paraméterrel jellemeztük; az epikardiális ST-szakasz emelkedés valamint az elektromos aktiváció inhomogenitásának iszkémia hatására bekövetkező változásával. Mindkét paraméternek a koszorúér okklúzióra bekövetkező változása szignifikánsan kissebb volt az ANP-vel kezelt kutyákban, mint a kontroll állatokban. Eredményeinkből arra következtetünk, hogy az ANP jelentős mértékben csökkenti a koszorúér okklúzió illetve reperfúzió során megjelenő kamrai aritmiák súlyosságát.
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ATRIAL NATRIURETIC PEPTIDE (ANP) REDUCES THE SEVERITY OF VENTRICULAR ARRHYTHMIAS RESULTING FROM CORONARY ARTERY OCCLUSION AND REPERFUSION IN ANAESTHETISED DOGS

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Arrhythmias, ischaemia, myocardial protection

The aim of the present study was to examine whether atrial natriuretic peptide (ANP) reduces the severity of ventricular arrhythmias in a canine model of ischaemia-reperfusion. In chloralose-urethane anaesthetised mongrel dogs, after thoracotomy, human synthetic ANP was infused intravenously (10µg kg^{-1} + 0.1µg kg^{-1} min^{-1} , n=11), 30 min before and 10 min after the 25 min occlusion of the left anterior descending coronary artery (LAD). Control dogs (n=20) were simply subjected to a 25 min occlusion of the LAD, followed by reperfusion. ANP infusion resulted in a fall in mean arterial blood pressure (-17±2 mmHg, P<0.05) and a moderate reduction in diastolic coronary resistance (-0.11±0.06 mmHg ml^{-1} min, P>0.05). During ischaemia, the increase in left ventricular end-diastolic pressure was less marked in the ANP treated than in the control dogs (9.0 ± 0.9 vs 12.7 ± 0.6 mmHg, P <0.05). Compared to the controls, in the presence of ANP, there was a reduced number of ventricular premature beats (26±12 vs 390±74, P<0.05) and episodes of ventricular tachicardia (0.73±0.3 vs 9.0±1.9, P <0.05), lower incidences of VT (45% vs 100%, P< 0.05) and VF (18% vs 40%, P<0.05) during occlusion. Survival from the combined ischaemia-reperfusion insult was also increased (64% vs 0%, P<0.05). The severity of myocardial ischaemia, assessed from changes in epicardial ST-segment and degree of inhomogeneity, were significantly less marked in dogs given ANP. We conclude that ANP may protect the myocardium against the severe consequences of myocardial ischaemia, resulting from coronary artery occlusion and reperfusion.
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ABSTRACT BOOK

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