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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 μg 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 μg 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 μg 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

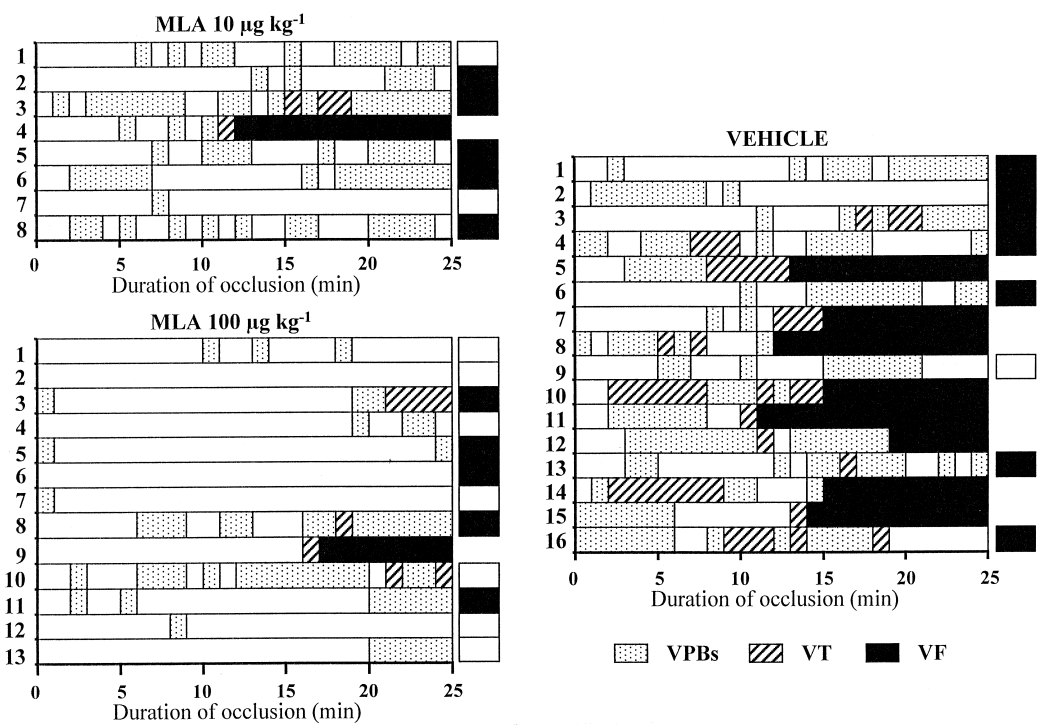

Fig. 1. The distribution of ventricular arrhythmias during a 25-min occlusion of the left anterior descending coronary artery (LAD) in anaesthetised dogs treated with monophosphoryl lipid-A (10 or 100 μg kg^{-1}, 24 h prior to occlusion) or the appropriate vehicle controls. The figures show the time 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.

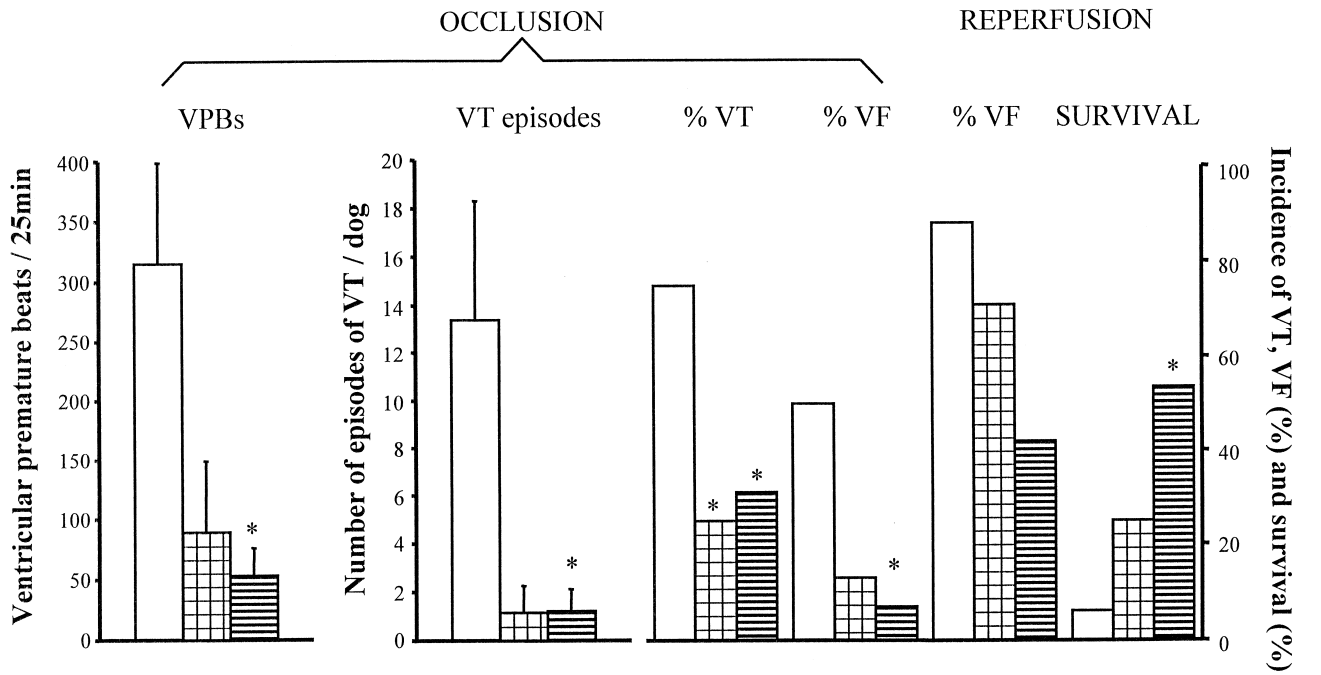

Fig. 2. Ventricular arrhythmias in anaesthetised dogs subjected to coronary artery occlusion after treatment with monophosphoryl lipid-A 10 μg kg^{-1} (cross-shaded histograms), 100 μg kg^{-1} (vertically shaded histograms) or the appropriate vehicle (open histograms). VPBs are the total number of ventricular 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

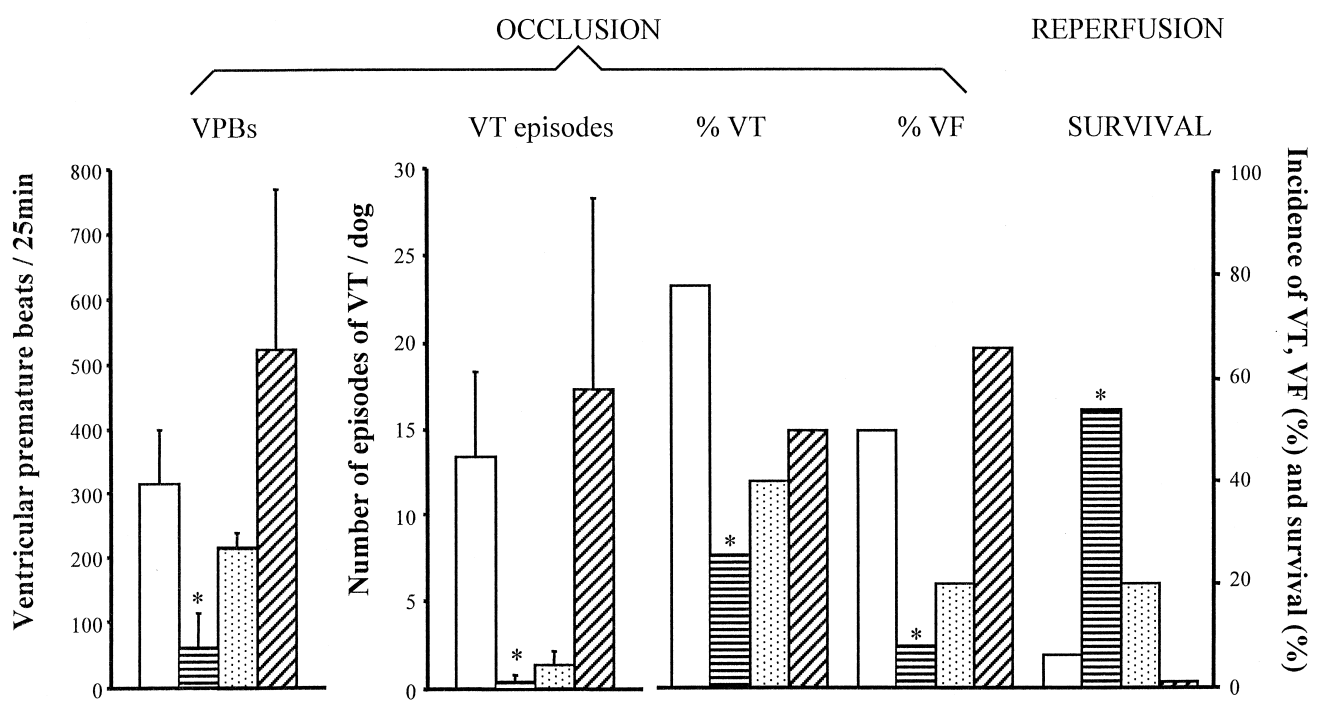

Fig. 3. Ventricular arrhythmias in anaesthetised dogs subjected to coronary artery occlusion after treatment with monophosphoryl lipid-A 100 μg kg^{-1} (vertically shaded histograms), and then given either aminoguanidine (stippled histograms) or aminoguanidine and meclofenamate (hatched histograms), 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-

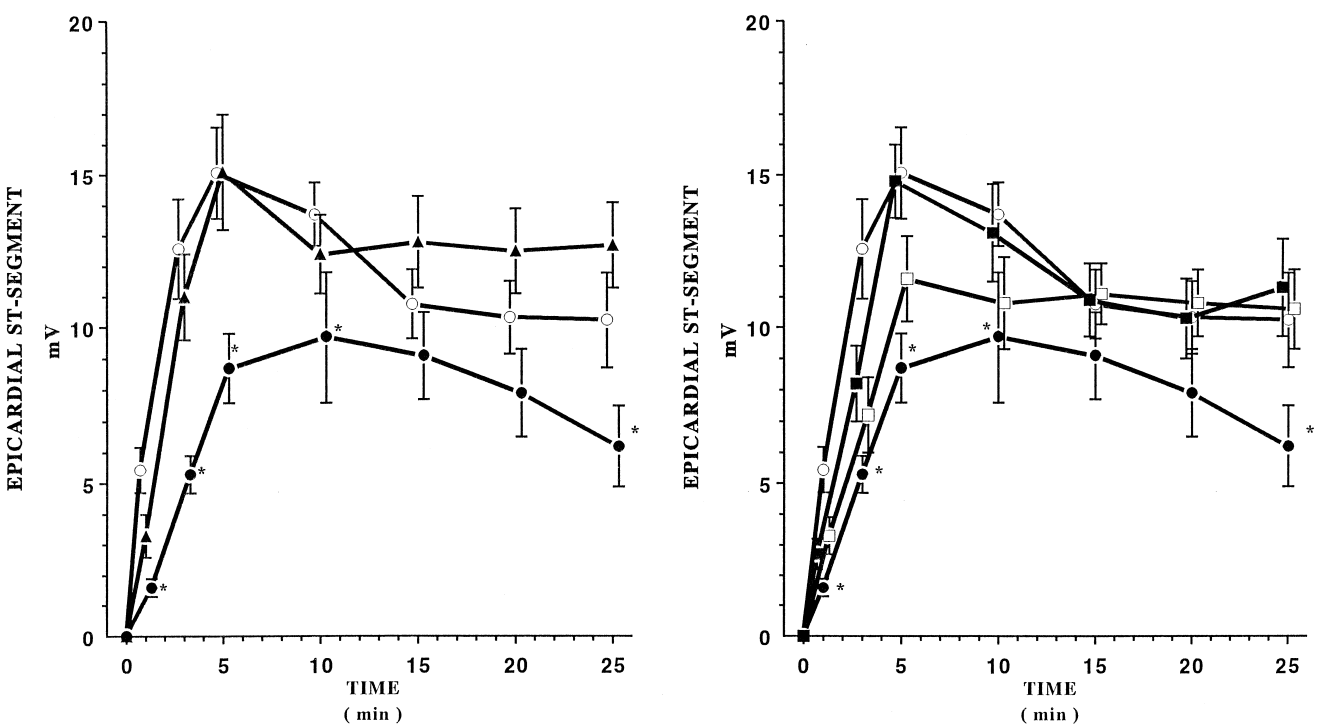

Fig. 4. Changes in the epicardial ST-segment (mV) during a 25-min coronary artery occlusion in dogs given 24 h previously, monophosphoryl lipid-A 100 μg kg^{-1} (filled circles), 10 μg kg^{-1} (filled triangles) or 100 μg kg^{-1} monophosphoryl lipid-A and then either aminoguanidine (open squares) or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

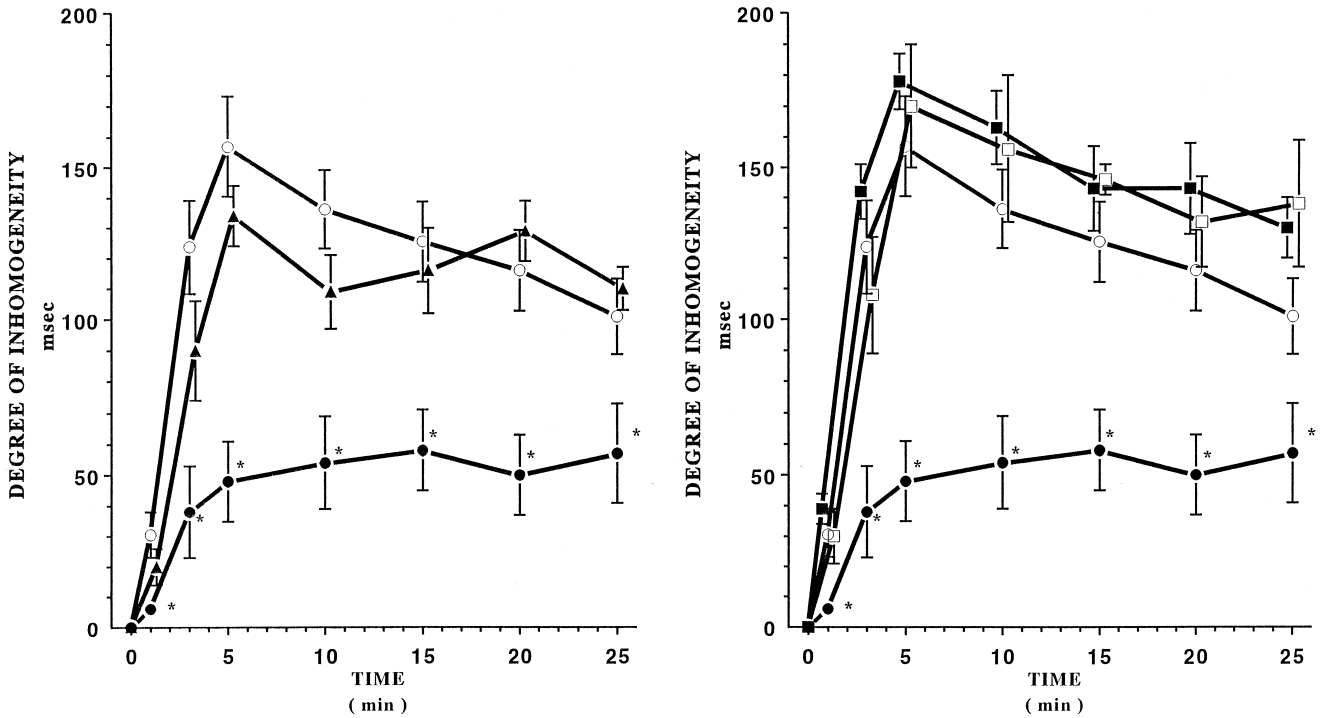

Fig. 5. Changes in the degree of inhomogeneity of electrical activation (ms) during a 25-min coronary artery occlusion in dogs given 24 h previously, monophosphoryl lipid-A 100 μg kg^{-1} (filled circles), 10 μg kg^{-1} (filled triangles) or 100 μg kg^{-1} monophosphoryl lipid-A and then either aminoguanidine (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 ($40.1 \pm 1.3\%$ in the dogs given the higher dose of monophosphoryl lipid-A, $39.1 \pm 1.7\%$ in those given the lower dose of monophosphoryl lipid-A, $40.7 \pm 0.7\%$ in the vehicle controls, $41.2 \pm 1.1\%$ 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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