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Delayed protection against ischaemia-induced ventricular arrhythmias and infarct size limitation by the prior administration of *Escherichia coli* endotoxin

Wu Song, Brian L. Furman & [1]James. R. Parratt

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, G1 1XW, Scotland

1 Bacterial endotoxin (lipopolysaccharide derived from *Escherichia coli*) was injected intraperitoneally in conscious rats in doses ranging from 0.5 to 2.5 mg kg^{-1}. At various times afterwards the animals were anaesthetized and subjected to a 30 min period of left coronary artery occlusion.

2 Under these conditions the severity of ventricular arrhythmias was markedly suppressed, in comparison with saline-injected controls, but this was particularly marked with the higher doses (1.5 and 2.5 mg kg^{-1}); the number of ventricular premature beats was reduced from 1687 ± 227 over the 0.5 h coronary artery occlusion period to 190 ± 46 in those rats administered 2.5 mg kg^{-1} endotoxin 8 h previously ($P<0.05$). The duration of ventricular tachycardia was also significantly reduced (138 ± 26 s to 8.9 ± 4.2 s; $P<0.01$) and there was a reduction in the incidence of ventricular fibrillation (from 56% to 10%).

3 The time course of this protection was studied following the administration of a single dose of 2.5 mg kg^{-1} of endotoxin by anaesthetizing rats 4, 8 or 24 h later. Protection was apparent at each time but was particularly marked at 8 h.

4 No rat given the highest dose of endotoxin (32 in all) died as a result of ventricular fibrillation, or from any other cause, during an occlusion, in contrast to a 26% mortality in the controls ($P<0.01$).

5 Infarct size, measured following a 30 min period of coronary artery occlusion followed by a 3 h reperfusion period, was reduced both 8 and 24 h after the administration of 2.5 mg kg^{-1} endotoxin (reductions of 24.3 and 23.1% respectively; $P<0.05$). Endotoxin had no significant effect on the area at risk.

6 The beneficial effects of endotoxin on infarct size and on ventricular arrhythmias were markedly attenuated by the prior administration of dexamethasone, 3 mg kg^{-1} given 1 h prior to endotoxin administration. Dexamethasone itself reduced infarct size ($P<0.05$) but had no direct effect on arrhythmia severity following coronary artery occlusion.

7 The mechanisms of this 'cross-tolerance' induced by bacterial endotoxin against ischaemia-reperfusion injury remain to be elucidated but the most likely mechanisms appear to be the induction of protective enzymes or proteins (e.g. nitric oxide synthase, cyclo-oxygenase (COX) 2) probably mediated by cytokine release.

Keywords: Endotoxin; cardiac arrhythmias; infarct size limitation; dexamethasone; myocardial ischaemia

Introduction

There has been considerable recent interest in the possibility that the heart can be protected against the consequences of ischaemia by a number of procedures given hours, or even days previously. Such 'delayed cardioprotection' can result from ischaemic preconditioning (Kuzuya *et al.*, 1993; Marber *et al.*, 1993 and reviewed by Yellon & Baxter, 1995), heat stress (Marber *et al.*, 1993; 1994), cardiac pacing (Szekeres *et al.*, 1993; Vegh *et al.*, 1994) and by the administration of certain derivatives of prostacyclin (e.g. 7-oxo-prostacyclin, Szekeres *et al.*, 1989). This concept of delayed cardioprotection has been recently reviewed (Parratt & Szekeres, 1995).

Nitric oxide is probably involved in the antiarrhythmic effects of ischaemic preconditioning (Vegh *et al.*, 1992) and of cardiac pacing (Vegh *et al.*, 1994). The finding that nitric oxide is responsible for the loss of vascular reactivity which occurs in endotoxaemia (Julou-Schaeffer *et al.*, 1990; Stoclet *et al.*, 1993; and reviewed by Parratt & Stoclet, 1995), as a result of the induction of nitric oxide synthase (Rees *et al.*, 1990; and reviewed by Thiemermann, 1994), prompted us to examine whether endotoxin administration also results in delayed protection of the heart against certain consequences of ischaemia. Our initial studies (Wu Song *et al.*, 1994) demonstrated that hearts removed from rats previously given *Escherichia coli* endotoxin, and then perfused through the aorta and subjected to coronary artery occlusion had fewer ventricular ectopic beats and a lower incidence of ventricular fibrillation during ischaemia. The present studies were designed to determine whether this protection also occurs *in vivo*, whether it is dose- and time-dependent and whether it is modified by dexamethasone. A preliminary account was presented to the Fifth Vienna Shock Forum in Vienna in May 1995 (Wu Song *et al.*, 1995). Since this manuscript was submitted it has been shown that rats made tolerant to bacterial endotoxin, by repeated daily injections, also have limitation of myocardial ischaemic damage (infarct size) after coronary artery occlusion and reperfusion (Eising *et al.*, 1996). Arrhythmias were not assessed in this study.

Methods

Dose-dependency of cardioprotection by bacterial endotoxin

Male rats weighing between 250 and 330 g (but with the weights closely matched within each particular group) were given, by intraperitoneal injection, either *Escherichia coli* endotoxin (055:B5, Boivin preparation, Difco Laboratories) in

[1] Author for correspondence.

doses of 0.5, 1.5 or 2.5 mg kg^{-1} (9 or 10 animals in each group), or an equivalent volume (0.5 ml) of 0.9% w/v normal saline (also 9 or 10 animals in each group) 4 or 8 h prior to anaesthesia with sodium pentobarbitone (60 mg kg^{-1} by intraperitoneal injection). The rats were then respired with room air through an intratracheal cannula at a rate and volume sufficient to maintain blood gases, after thoracotomy, within the normal range (Clark *et al.*, 1980). Blood pressure was measured from a carotid artery with a Statham transducer (Gould, U.S.A.), and the electrocardiogram recorded from standard limb leads; the heart rate was calculated from the electrocardiogram. Blood pressure and the electrocardiogram were recorded on a Grass polygraph (model 7D, Grass Instruments, Quincy, Mass., U.S.A.). After a left thoracotomy and a 20 min stabilization period, the left main coronary artery was occluded for a period of 30 min and the resultant ventricular arrhythmias assessed as previously described (Clark *et al.*, 1980) and according to the Lambeth Conventions (Walker *et al.*, 1988). We measured the total number of ventricular premature complexes (or beats; VPBs), the incidence and total duration throughout the occlusion period of ventricular tachycardia (VT), the total number of VPBs during ventricular tachycardia and the incidence of ventricular fibrillation (VF), which in this model is often reversible. Non-reversible VF (mortality) was also recorded.

Time course of cardioprotection by bacterial endotoxin

A second series of experiments was designed to explore the time course of protection against arrhythmias using a single dose of 2.5 mg kg^{-1} of endotoxin. Either endotoxin or an equivalent volume of saline, was given to groups of 9–12 rats and 4, 8 and 24 h later the animals were anaesthetized and subjected to coronary artery occlusion as outlined above. The severity of ventricular arrhythmias in these rats following coronary artery occlusion was assessed as described above. Further groups of 9 or 10 rats were given either saline, endotoxin (2.5 mg kg^{-1}) or dexamethasone (3 mg kg^{-1}, i.p., 1 h prior to injection of either saline or endotoxin) and the animals anaesthetized 8 h later, ventilated, thoracotomized and subjected to coronary artery occlusion.

It should be noted that each endotoxin group was compared with an equivalent randomly distributed control group injected with saline at the same time. We did not assess reperfusion-induced arrhythmias in any of the groups; after a 0.5 h occlusion period such arrhythmias are not severe (Kane *et al.*, 1984).

Infarct size limitation by bacterial endotoxin

A third series of experiments was designed to explore the possibility that endotoxin reduces myocardial ischaemic damage (infarct size). Endotoxin (2.5 mg kg^{-1}) or saline (0.5 ml) was given to groups of 8–9 rats (mean body weight 301±7 and 292±11 g respectively) either 8 or 24 h prior to anaesthesia and coronary artery occlusion. Two additional groups of rats ($n=9$ in each group; mean body weights of 303±7 g and 294±8 g) were given dexamethasone (3 mg kg^{-1}) by intraperitoneal injection 1 h prior to either endotoxin or saline administration. In these rats the occlusion time was also 0.5 h and the ischaemic myocardium was then reperfused for 3 h prior to assessment of the area at risk (R) and infarct size (I) with 3% Evans blue dye and 1% 2,3,5-triphenyltetrazolium chloride respectively (Clark *et al.*, 1980). In brief, at the end of the 3 h reperfusion period, the coronary artery was re-occluded and 0.5 ml Evans blue (Sigma, Poole, Dorset; 3% w/v) injected into the jugular venous catheter to delineate the area at risk. The animals were then killed with an overdose of pentobarbitone, the hearts rapidly removed, rinsed, blotted dry and frozen until further processing, usually the next day. The heart was sectioned from base to apex into five or six sections of 2 mm thickness. The slices were incubated for 15 min at 37°C in 20 ml of 0.1 M phosphate buffer, pH 7.4, containing 0.2 g of 2,3,5-triphenyltetrazolium dichloride (TTZ; (Sigma, Poole, Dorset, 1% w/v) to delineate the infarcted region. The areas of nonischaemic (Evans blue stained), viable (TTZ positive, brick red) and infarct (TTZ negative, pale) tissue were determined in each slice by a gravimetric method. Infarct size was expressed as a percentage of the area at risk.

Data evaluation and statistics

Except for the incidence of VT and VF, all values are expressed as means±s.e.mean. Differences between means were compared by Student's two-tailed unpaired *t*-test with, when appropriate, a Dunnet's multiple comparison procedure with an experiment-wise error rate of 0.05. Incidences of ventricular tachycardia and fibrillation were compared by Fisher-Irwin (chi-squared with Yates correction) test. Body weight, heart weight, area at risk and infarct size were compared with a one way ANOVA followed, when ANOVA was significant, by a Tukey test for multiple comparisons. A value of $P<0.05$ was considered significant.

Results

Effect of various doses of E. coli *endotoxin on the severity of arrhythmias resulting from coronary artery occlusion*

Prior to coronary artery occlusion, the mean arterial pressure was usually lower 8 h after endotoxin doses of 0.5, 1.5 and 2.5 mg kg^{-1} ($P<0.05$) than in the controls (e.g. 92±4 ($P<0.05$), 103±9 mmHg (NS) and 86+2 mmHg ($P<0.01$) respectively compared to the control value of 106±5 mmHg). Blood pressure fell on occlusion by 20–30 mmHg (see Figure 1) with little recovery during the occlusion period. Heart rate prior to occlusion was not significantly influenced by endotoxin pretreatment at any time after administration except with the highest (2.5 mg kg^{-1}) dose at 8 h (363±12 beats min^{-1} compared to 391±7 beats min^{-1} in the controls; $P<0.05$). Again, except at the highest dose at 8 h, heart rate was unchanged after coronary artery occlusion (data not shown).

There was a dose-dependent reduction in arrhythmia severity especially at 8 h and this is shown in Table 1. The dis-

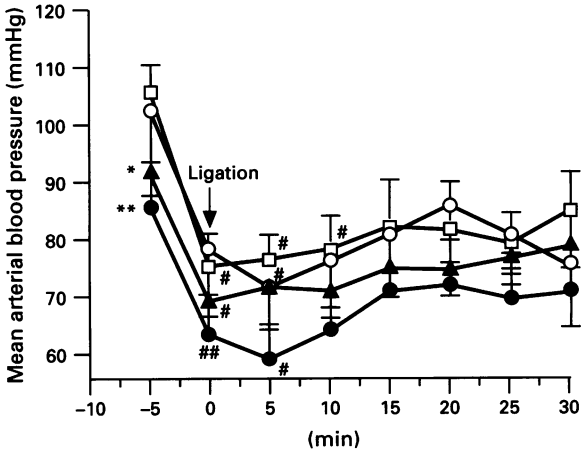

Figure 1 Changes in blood pressure (mmHg) following the administration of *E. coli* endotoxin (LPS) in doses of 2.5 mg kg^{-1} (●), 1.5 mg kg^{-1} (○) and 0.5 mg kg^{-1} (▲) or saline (□) given 8 h previously. Resting blood pressure prior to occlusion was significantly less in those rats given endotoxin in doses of 2.5 and 0.5 mg kg^{-1}. Coronary artery occlusion (at the arrow) resulted in an immediate reduction in arterial blood pressure with little recovery during the 30 min occlusion period. *$P<0.05$; **$P<0.01$, compared to saline control at the same time points; #$P<0.05$ and ##$P<0.01$, compared to the pre-occlusion blood pressure in the same group.

tribution of VPBs during the 30 min occlusion period is illustrated in Figure 2; with the higher doses (1.5 and 2.5 mg kg^{-1}) there was almost no ventricular ectopic activity after 15 min of ischaemia.

Effect of varying the time of the prior administration of E. coli *endotoxin on the severity of arrhythmias resulting from coronary artery occlusion*

In view of the dose study described above we used the highest dose (2.5 mg kg^{-1}) to evaluate the time course of protection against ischaemia-induced arrhythmias. The results are illustrated in Figure 3. There was a clear reduction in arrhythmia severity (total VPBs, number of VPBs as VT, duration of VT, incidence of VF) when the coronary artery was occluded at 4, 8 or 24 h after endotoxin administration. The maximum protection was at 8 h. The arrhythmias in the saline control groups were remarkably constant (Figure 3). No rat given the highest dose of endotoxin (32 in total) died as a result of VF, or from any other cause, during occlusion (Figure 4).

The effect of coronary artery occlusion on blood pressure is illustrated in Figure 1; the effect of endotoxin on arterial blood pressure, never pronounced with this dose, was also maximal at 8 h (94±5 mmHg at 4 h, 86±2 mmHg at 8 h ($P<0.05$) and 101±7 mmHg at 24 h (compared with control values at these times of 112±7, 106±5 and 109±5 mmHg respectively).

Effect of E. coli *endotoxin on myocardial infarct size*

In a dose of 2.5 mg kg^{-1} endotoxin significantly ($P<0.05$) reduced infarct size when this was assessed both 24 h and 8 h after administration (Figure 5). In the controls, the weight of the infarcted zone was a mean of 109±5 mg (at 8 h) and 117±8 mg (at 24 h; NS) which is 32.3±2.8% and 36.1±3.0% of the area at risk (9.0±0.5% and 9.8±0.7% of the total heart weights, which were 1214±25 and 1208±36 mg respectively in the 8 and 24 h groups). There were 24.3% and 23.1% reductions in infarct size at 8 and 24 h after endotoxin administration. There was no significant difference in the area at risk between any of the four groups (345±15 mg, 353±20 mg, 333±27 mg, 374±24 mg).

Effect of dexamethasone on the reduction by endotoxin of arrhythmia severity and infarct size

The beneficial effects of endotoxin on the consequences of coronary artery occlusion in this model were markedly reduced by the prior administration of dexamethasone (3 mg kg^{-1}) 1 h prior to the endotoxin challenge. This is illustrated for infarct size in Figure 5 and for arrhythmias in Figure 6. Dexamethasone itself had no direct effect on arrhythmia severity (Table 2) but, as is well documented, (e.g. Spath *et al.*, 1975; Hillis & Braunwald, 1977; Maclean *et al.*, 1978) did itself reduce infarct size, without influencing the area at risk (339±19 mg compared to 359±70 mg).

Discussion

These results demonstrate that the administration of sublethal doses of endotoxin are effective in reducing the major consequences of coronary artery occlusion, that is arrhythmia severity and the extent of myocardial necrosis. These doses (maximum of 2.5 mg kg^{-1}) are much lower than those required to induce shock in this species; in our hands, a dose of 2.5 mg kg^{-1} h^{-1} is required to lower substantially systemic arterial blood pressure. This dose (total of 20 mg kg^{-1}) results in an almost 100% mortality after around 8 h.

Several studies have demonstrated that bacterial endotoxin

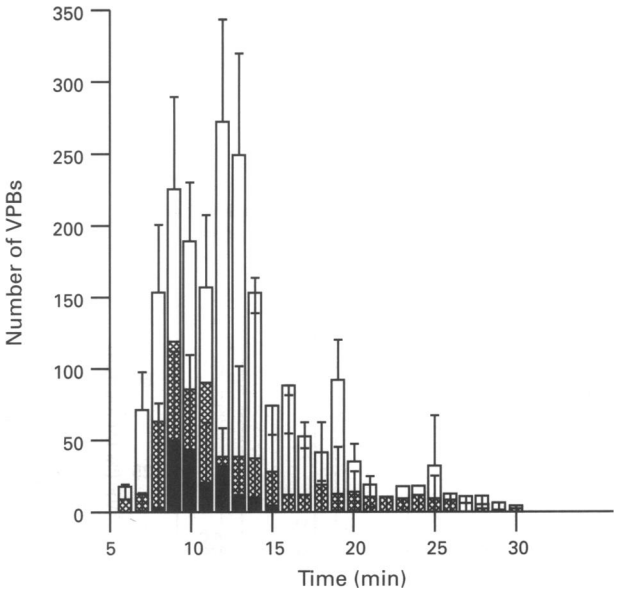

Figure 2 The distribution of ventricular premature beats (VPBs) during a 30 min occlusion of the left main coronary artery in anaesthetized rats which had been previously given *E. coli* bacterial endotoxin (lipopolysaccharide, LPS) in doses of 0.5 (vertically lined columns), 1.5 (cross hatched columns) or 2.5 (solid columns) mg kg^{-1}, or saline (open columns) 8 h previously. The values are means±s.e.mean ($n=9$ or 10) at 1 min intervals during the occlusion period. Ectopic activity commences around 6 or 7 min but virtually disappears by 21 or 22 min. The pronounced ectopic activity seen in normal rats is markedly reduced, in a dose-dependent manner, by *E. coli* endotoxin. The error bars have been omitted from the LPS-treated groups for purposes of clarity.

Table 1 Effect of *E coli* endotoxin pretreatment on the severity of ischaemia-induced arrhythmias during a 30 min coronary artery occlusion in anaesthetized rats

Endotoxin dose (mg kg^{-1})	*Ventricular tachycardia*				*Ventricular fibrillation*	
	VPBs	*Incidence* (%)	*VPBs*	*Duration (s)*	*Incidence* (%)	*Mortality*
Pretreatment time 8 h:						
Saline	1687±227	100	1425±221	137.6±26.0	56	33
0.5 mg kg^{-1}	966±278	100	796±242	86.5±27.1	60	20
1.5 mg kg^{-1}	710±107*	90	351±103**	36.2±10.5**	30	10
2.5 mg kg^{-1}	190±46**	80	89±37**	8.9±4.2**	10	0
Pretreatment time 4 h						
Saline	1486±207	100	1101±159	97.5±18.9	70	20
0.5 mg kg^{-1}	1269±195	100	1090±167	108.8±17.5	67	22
2.5 mg kg^{-1}	632±115*	80	384±116*	39.4±11.2*	20	0

Significantly different from saline. *$P<0.05$; **$P<0.01$. Endotoxin given by intraperitoneal injection either 4 or 8 h prior to occlusion. Values are given as mean±s.e.means from either 9 or 10 rats per group. VPBs = ventricular premature (ectopic) beats.

protects the heart against various aspects of ischaemia-reperfusion injury. These studies have been concerned with recovery of contractile function during reperfusion following periods of ischaemia. For example, this recovery is enhanced following a 35 or 50 min period of ischaemia in hearts isolated from rats (Brown *et al.*, 1989; McDonough & Causey, 1993; 1994) and guinea-pigs (McDonough *et al.*, 1995) on the day following endotoxin administration. This protection afforded by endotoxin is presumably cytokine-mediated because similar protection, with the same time course has been demonstrated following the administration of tumour necrosis factor (TNF-α) and interleukin-1 (IL-1), both of which reduce the release of cytoplasmic enzymes such as creatine kinase (CK) and lactate

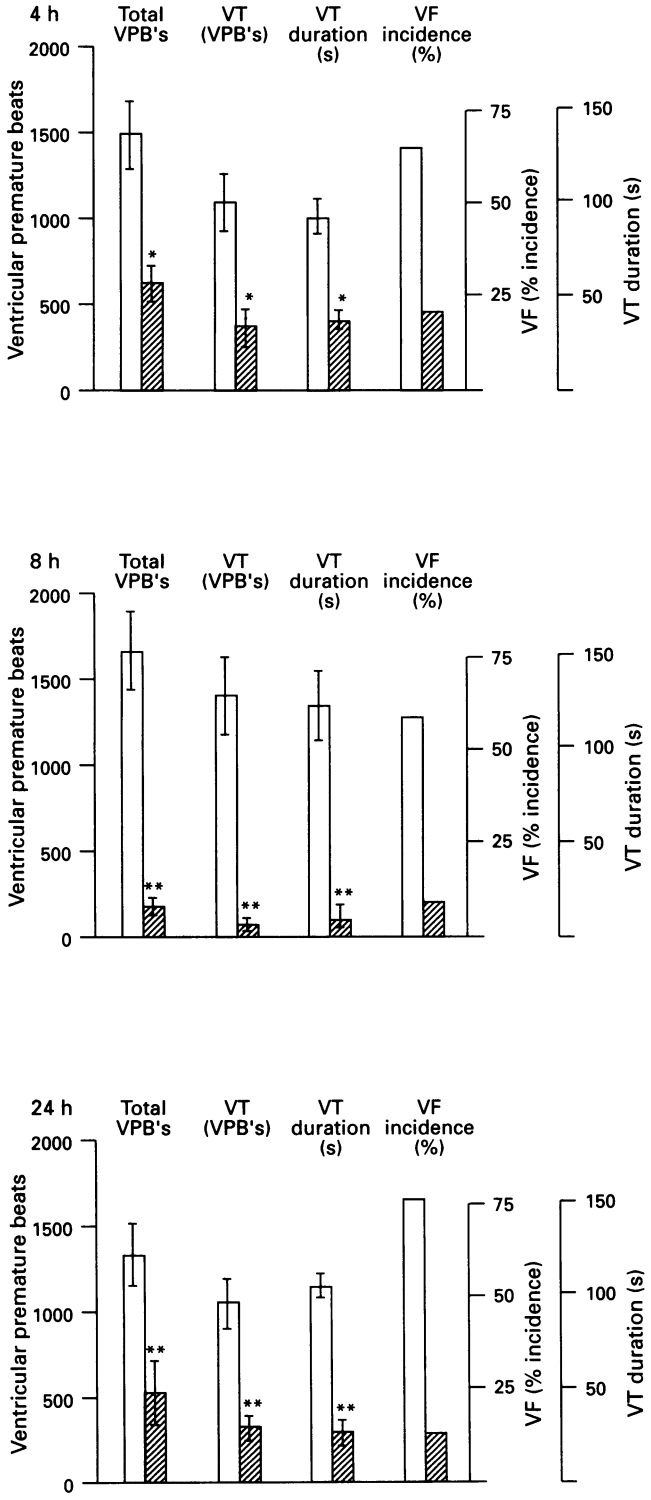

Figure 3 The effect of bacterial endotoxin ($2.5\,mg\,kg^{-1}$) on arrhythmia severity during coronary artery occlusion in anaesthetized male rats, 4, 8 or 24 h after administration. The results from endotoxin-treated rats are shown by the hatched columns and for an equivalent volume of normal saline, given at the same time, is shown by the open columns. Arrhythmia severity is expressed as the total number of ventricular premature beats (VPBs) over a 30 min coronary artery occlusion period, as the number of VPBs occurring as ventricular tachycardia (VT), as the duration of ventricular tachycardia (in seconds) and as the incidence of ventricular fibrillation (VF). Values are means ± s.e.mean of 9 or 10 animals in each group. $*P<0.05$; $**P<0.01$.

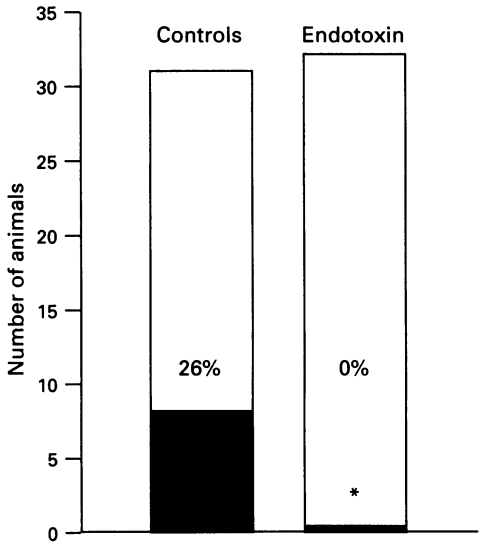

Figure 4 Mortality (solid area) from coronary artery occlusion in rats administered endotoxin ($2.5\,mg\,kg^{-1}$), 4, 8 or 24 h prior to occlusion, in comparison with saline-treated controls: No animal administered endotoxin died during the occlusion period compared with 8 out of 31 in the saline controls. All the control rats that died during the occlusion period succumbed in non-reversible ventricular fibrillation.

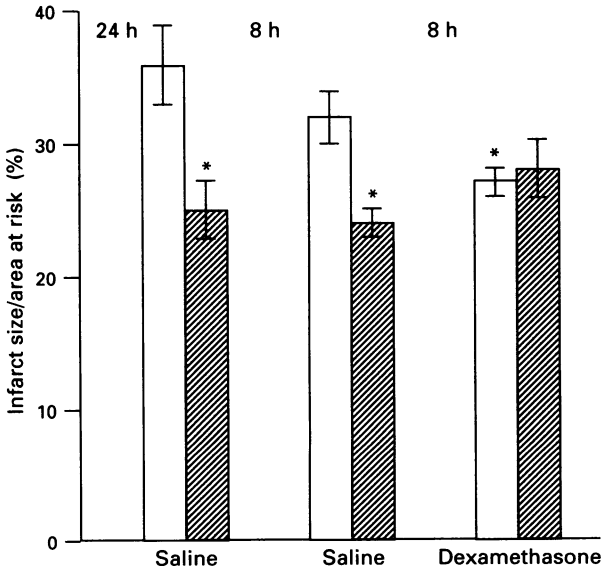

Figure 5 The effect of endotoxin (hatched columns) on infarct size in a model of coronary artery occlusion and reperfusion. Infarct size was measured, as a percentage of the area at risk, in rats given endotoxin either 8 or 24 h previously. The reduction in infarct size by endotoxin was not seen if dexamethasone ($3\,mg\,kg^{-1}$) had been given 1 h prior to endotoxin administration. Dexamethasone itself also significantly reduced infarct size. Values given are means ± s.e.mean ($n=8$ for each group). $*P<0.05$.

dehydrogenase (LDH) from rat isolated hearts subjected to a 20 min period of global ischaemia (Eddy *et al.*, 1992). These enzymes leave the cell and appear in the circulation, or perfusion fluid, following the development of irreversible ischaemic injury.

There are several possible explanations for the cardioprotective effects of endotoxin and of cytokines such as TNF-α and IL-1. These explanations depend upon the induction of various protective proteins. Thus, there is evidence for enhanced antioxidant status with increased levels of catalase (Brown *et al.*, 1989) and of superoxide dismutase (Maulik *et al.*, 1992) the over-expression of which can protect cells from insults associated with the production of oxygen free radicals, which are known to be generated following reperfusion. There is also some evidence that elevation of heat shock (stress) proteins, as a consequence of other insults to the myocardium (elevated whole body temperature, ischaemia) also protect the heart against subsequent periods of ischaemia and reperfusion (Marber *et al.*, 1993).

A second possibility, and the one which stimulated the present experiments, is that endotoxin and cytokines are known to induce a nitric oxide synthase (NOS) in a variety of cells and that this is responsible for the loss of vascular reactivity to noradrenaline that occurs during endotoxaemia in this species (Julou-Schaeffer *et al.*, 1990; Stoclet *et al.*, 1993 and reviewed by Thiemermann, 1994 and by Parratt & Stoclet, 1995). In the heart, this NOS induction, which we (Fatehi-Hassanabad, unpublished) and others (Szabo *et al.*, 1993) have shown, is cytokine-dependent (Schulz *et al.*, 1995) is partially responsible for the myocardial depression that is a feature of both endotoxaemia and clinical sepsis (Finkel *et al.*, 1992; and reviewed by Kumar & Parrillo, 1995). This depression is mainly direct (due to overproduction of NO in cardiac myocytes; Schulz *et al.*, 1992) but inhibition of noradrenaline release from sympathetic nerves (Fatehi-Hassanabad *et al.*, 1995) and a reduced responsiveness of cardiac myocytes to β-adrenoceptor agonists such as noradrenaline (Gulick *et al.*, 1989) may also contribute.

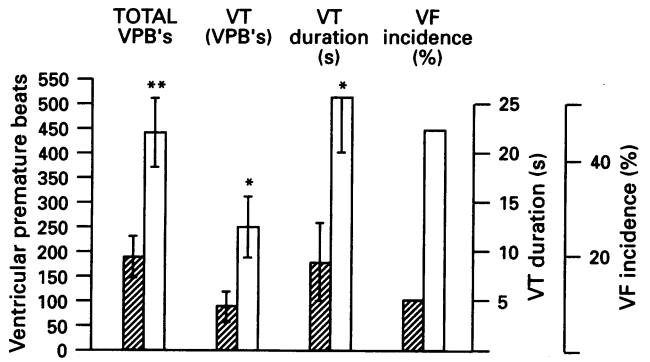

Figure 6 Attenuation of the antiarrhythmic effects of endotoxin by the prior administration of dexamethasone (3 mg kg^{-1} given 1 h prior to the endotoxin challenge; open columns). Endotoxin (2.5 mg kg^{-1}; hatched columns) was given 8 h prior to coronary artery occlusion. Values are means ± s.e.mean (n = 8–10 in each group). *P<0.05; **P<0.01.

There is good evidence for a cardioprotective effect of nitric oxide under conditions of ischaemia and reperfusion (Maulik *et al.*, 1995; Pabla & Curtis, 1995; Williams *et al.*, 1995; Beresewicz *et al.*, 1995). The mechanisms include a reduction in myocardial oxygen demands (Shen *et al.*, 1994), an effect mediated by cyclic GMP (Weiss *et al.*, 1994). The time course of the protection against arrhythmias, certainly *in vitro* (Wu Song *et al.*, 1994), matches closely that of NOS induction and the attenuation of this protection by dexamethasone, seen both in the present study (Figures 5 and 6) and in the previous *in vitro* study, might also suggest the involvement of NO since NOS induction by endotoxin is prevented by this steroid (Rees *et al.*, 1990). Support for a role for cytokines in mediating the protective effect of endotoxin comes from the recent findings that dexamethasone not only inhibits the generation of cytokines following endotoxin administration (van der Poll & Lowry, 1995) but also reduces the cardiovascular responses to interleukin-1 (Watanabe *et al.*, 1996). Part of the attenuation of the protective effect of endotoxin by dexamethasone might also result from its ability to prevent the induction by endotoxin of a nitric oxide synthase (Rees *et al.*, 1990). A nitric oxide hypothesis would also accord with the most likely mechanism of the antiarrhythmic effects of ischaemic preconditioning (Vegh *et al.*, 1992; Parratt & Vegh, 1994); these depend upon bradykinin-stimulated nitric oxide generation from endothelial cells (Parratt & Vegh, 1996) with a subsequent elevation of myocardial cyclic GMP.

A third possibility for the protection afforded by endotoxin could be the induction of cyclo-oxygenase (COX) 2 which is also inhibited by dexamethasone (Masferrer *et al.*, 1992). There is good evidence that cyclo-oxygenase (COX 1) products such as prostaglandin E_2 (PGE_2) and prostacyclin reduce myocardial ischaemic damage following coronary artery occlusion and reperfusion (Ogletree *et al.*, 1979; Ribeiro *et al.*, 1981) and are able to suppress early ischaemia-induced ventricular arrhythmias in anaesthetized rats (Coker & Parratt, 1981) and in other species (reviewed by Parratt, 1989). Indeed, the suggestion has been made (Parratt, 1993) that prostacyclin should be considered an 'endogenous myocardial protective (antiarrhythmic) substance' and that its generation during ischaemia modulates the severity of the early ischaemia-induced arrhythmias such as those studied in the present experiments. However, it is of some interest that whereas dexamethasone completely prevented the reduction in infarct size that resulted from endotoxin administration, it only attenuated the pronounced antiarrhythmic effects of this procedure. This suggests that some dexamethasone (and thus perhaps NO and PG) -insensitive component of the protection against arrhythmias remains. Some of our own, as yet unpublished, *in vitro* studies, using a model similar to that previously described (Wu Song *et al.*, 1994) also revealed that not all the antiarrhythmic effect of endotoxin is abolished by a combination of N^G-nitro-L-arginine methyl ester (L-NAME) and indomethacin in concentrations as high as 10^{-2} and 10^{-4} M respectively. We do not know what this additional component of the protection is.

Protection against ischaemia-reperfusion injury by the administration of bacterial endotoxin is an example of 'cross-tolerance'. Other examples include protection of animals by TNF-α against radiation, hyperoxia and various chemother-

Table 2 Effect of pretreatment (9 h previously) with dexamethasone (3 mg kg^{-1}) on the severity of ischaemia-induced arrhythmias during a 30 min coronary artery occlusion in anaesthetized rats

		Ventricular tachycardia			*Ventricular fibrillation*	
Treatment	*VPBs*	*Incidence (%)*	*VPBs*	*Duration (s)*	*Incidence (%)*	*Mortality*
Saline (control)	1687±227	100	1425±221	137.6±26.0	56	33
Dexamethasone + saline	1774±245	100	1488±208	162.4±18.6	78	33

VPBs = ventricular premature (ectopic) beats.

apeutic drugs (Eddy *et al.*, 1992). The cardioprotective effect of bacterial endotoxin has led to the recent development of non-toxic derivatives of the lipid A component of the endotoxin molecule such as monophosphoryl lipid-A (MPL-C). This compound has been shown to enhance recovery of contractile function in rat isolated hearts subjected to global ischaemia (Nelson *et al.*, 1991) reduce myocardial infarct size (Yao *et al.*, 1993) and ventricular arrhythmias (Vegh *et al.*, 1996) in dogs subjected to ischaemia and also, although in considerable larger doses, in rats (Wu Song, Furman & Parratt, unpublished observations). This had led to the suggestion that this might offer an interesting alternative approach to protecting the myocardium against ischaemia and reperfusion by 'mimicking' the effects of the protection afforded by ischaemic pre-conditioning (Przyklenk & Kloner, 1995).

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