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L-Canavanine and dexamethasone attenuate endotoxin-induced suppression of ischaemia-reperfusion arrhythmias

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Abstract

The role of inducible nitric oxide synthase in the antiarrhythmic effects of *Escherichia coli* endotoxin was examined in an anaesthetised rat model of myocardial ischaemia (7 min occlusion) and reperfusion (7 min) arrhythmias by using its specific blocker L-canavanine (100 mg/kg) and dexamethasone (5 mg/kg), which inhibits its expression. Endotoxin (1 mg/kg) or its solvent saline was administered intraperitoneally 4 h before the occlusion of the left coronary artery and L-canavanine or dexamethasone was administered 1 h before endotoxin or saline injection. The mean arterial blood pressure of rats receiving endotoxin was significantly lower than that of saline-treated controls, and neither L-canavanine nor dexamethasone prevented the hypotension exerted by endotoxin. However, during both the occlusion and reperfusion periods, endotoxin significantly reduced the total number of ectopic beats (e.g., during reperfusion, saline: 1177 ± 183, $n = 11$; endotoxin: 248 ± 91, $n = 9$; $P < 0.005$) and the duration of ventricular tachycardia (e.g., during occlusion, saline: 30.9 ± 5.7 s; endotoxin: 1.8 ± 0.9 s; $P < 0.0001$) while L-canavanine or dexamethasone treatment abolished the reduction exerted by endotoxin. Therefore we conclude that endotoxin possesses significant antiarrhythmic (protectant) effects in this rat model of ischaemia-reperfusion arrhythmias, and that its mechanism appears to involve the inducible nitric oxide synthase since both L-canavanine and dexamethasone inhibited this phenomenon.

Keywords: Ischemia-reperfusion arrhythmia; Endotoxin; Dexamethasone; L-Canavanine; Nitric oxide (NO) synthase, inducible

1. Introduction

Sublethal doses of Gram-negative bacterial endotoxins (lipopolysaccharides) have been shown to increase the tolerance of the myocardium to a subsequent challenge hours later with ischaemia and reperfusion (Brown et al., 1989; reviewed in Parratt and Szekeres, 1995). Similarly, a non-pyrogenic derivative of *Salmonella* lipopolysaccharide, monophosphoryl lipid A, has been shown to reduce infarct size in a canine model of ischaemia (Yao et al., 1993) while producing delayed anti-ischaemic effects in isolated rat (Nelson et al., 1991) and rabbit (Baxter et al., 1996) hearts. This effect was associated with increased myocardial catalase activity only if monophosphoryl lipid A was administered 24 h but not 1 h prior to coronary artery occlusion of canine hearts (Yao et al., 1993).

Although the responses to endotoxin administration involve a multiplicity of factors (Abel, 1989), particular interest has focused on the possibility that nitric oxide ($^{\cdot}NO$) might have a major role. Recently, $^{\cdot}NO$ has been proposed as a candidate for the putative endogenous (antiarrhythmic) protectant in ischaemic preconditioning (Vegh et al., 1992) and $^{\cdot}NO$-involved action is attenuated by dexamethasone (Vegh et al., 1994), which is an inhibitor of the expression of inducible cyclooxygenase (cyclooxygenase-2) (Masferrer et al., 1992) and inducible nitric oxide synthase in endothelial cells (Radomski et al., 1990), in vascular smooth muscle cells (Fleming et al., 1990) and also in myocardium (Salter et al., 1991; Schulz et al., 1992). The ability of dexamethasone to attenuate the antiarrhythmic effects of endotoxin in Langendorff perfused isolated rat hearts (Song et al., 1994) and in anaesthetized rats (Song et al., 1996) further increases the possibility that inducible nitric oxide synthase and/or cyclooxygenase-2 products are heavily involved in endotoxin-induced antiarrhythmic effects. However, the relative contribution of inducible nitric oxide synthase and cyclooxygenase-2 to this phenomenon remains to be established by studies which use the selective inhibitors/activators of each com-

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ponent.

There is no uniform agreement on the antiarrhythmic property of ·NO. A ·NO-donor substance, 3-morpholino-sydnonimine-*N*-ethylcarbamide (SIN-1), is reported to be ineffective in attenuating ischaemia-induced arrhythmias (Coker and Barnes, 1993) while N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of both the constitutive and inducible forms of nitric oxide synthase (Gross et al., 1990), has failed to modify the number or severity of either ischaemia- or reperfusion-induced arrhythmias in anaesthetised rats (Iskit and Guc, 1996a).

Therefore, the aim of the present study was to investigate the effects of L-canavanine, a selective inhibitor of inducible nitric oxide synthase (Iyengar et al., 1987; Umans and Samsel, 1992), in comparison to dexamethasone, on endotoxin-induced alterations of both ischaemia and reperfusion arrhythmias in anaesthetised rats.

A preliminary account of our data was presented in abstract form at the XIIIth Biannual Meeting of the Turkish Pharmacological Society in November 1996.

2. Materials and methods

2.1. General procedures

Male Sprague-Dawley rats (250–350 g, obtained from Hacettepe University's Laboratory Animals Breeding Unit) housed under conventional conditions (ambient temperature) with ad libitum access to food (pellet) and water (drinking bottle) were used in adherence to all of the Guiding Principles in the Care and Use of Animals together with the recommendations from the declaration of Helsinki.

Endotoxin derived from *Escherichia coli* (O55:B5) or an equivalent volume (0.1 ml/kg) of non-pyrogenic sterile saline (NaCl 0.9%, w/v, dissolved in pyrogen-free distilled water) was given by intraperitoneal injection to animals 4 h before they went under coronary artery ligation by surgery. Therefore, time 0 was considered as the initiation of the coronary artery occlusion. The timing of endotoxin administration was chosen on the basis of previous studies which reported that inducible nitric oxide synthase activity in myocardium reaches its maximum within 2–6 h after endotoxin administration (Schulz et al., 1992; Gardiner et al., 1995; Bateson et al., 1996).

In preliminary experiments, four different doses of endotoxin (0.1, 0.5, 1 or 4 mg/kg) were tested and the maximum possible dose that yielded a mean arterial blood pressure over 60 mmHg (i.e., 1 mg/kg) at the end of the equilibration period was used for the rest of the study.

2.2. Surgical procedure

Fundamental surgical procedures used in the present study were described previously by Clark et al. (1980). Briefly, male Sprague-Dawley rats (250–350 g) were anaesthetised with sodium pentobarbitone (60 mg/kg, i.p.) and placed on a heat-insulated cork sheet-covered operating table. The trachea and left jugular vein were cannulated for artificial respiration and drug administration respectively. Systolic and diastolic oscillations in arterial blood pressure were monitored from the left carotid artery by using a Statham pressure transducer (PX23L) and displayed on a Harvard Oscillograph pen recorder together with a standard lead-1 electrocardiogram. The chest was opened by a left thoracotomy, followed by sectioning of the fourth and fifth ribs, 2 mm to the left of the sternum. Positive-pressure artificial respiration (Cole-Palmer Small Animal Respirator, volumetrically calibrated according to the displacement of water column inside an inverted measure immersed in a water tank) was started immediately with room air, using a volume of 1.5 ml per 100 g body weight at a rate of 60 strokes/min. Body temperature (37°C) was stabilised within 0.1°C by a rectal thermistor probe-controlled incandescent lamp placed 25 cm above the abdominal region.

After the pericardium was incised, the heart was eased out of the chest by gentle pressure on the rib cage. A 6/0 silk suture, attached to a 10-mm micropoint reverse-cutting needle, was placed under the left main coronary artery. The heart was replaced in the chest, attention being paid to the proper reinflation of the lungs. The animal was allowed to recover for an equilibration period of 15 min.

Just before the occlusion of the left coronary artery, 0.1 ml of arterial blood was withdrawn with a heparin-coated (flushed twice with 100 IU/ml of heparin) 1 ml syringe, and in saline-treated animals blood gas analyses were performed by using an AVL Type 995 (Graz, Austria) blood gas autoanalyser at 37°C. The results were corrected automatically according to the air pressure (around 690 mmHg in the laboratory) at the time of the analysis. The artificial respiration parameters mentioned above yielded arterial PaO_2 values always greater than 70 mmHg, $PaCO_2$ values always lower than 40 mmHg, pH values between 7.35–7.45, oxygen saturation around 95% and HCO_3 values around 20 mmol/l.

Any animal in which this surgical procedure produced arrhythmias or a sustained decrease in mean arterial blood pressure below 60 mmHg was discarded. A small plastic snare was threaded through the ligature and placed in contact with the heart. The artery could be occluded by applying tension to the ligature, and reperfusion was achieved by releasing the tension. After the equilibration period of 15 min, the artery was occluded for 7 min and reperfused for another 7 min. Successful occlusion of the coronary artery was judged visually by the development of pallor of the exposed myocardium, ST-segment elevation, increased R wave amplitude and occurrence of arrhythmias while successful reperfusion was judged by the reversal of ischaemia-induced ST-segment changes as outlined previously (Barnes and Coker, 1995). This experimental proto-

col was especially chosen since we are familiar with its outcome (Guc et al., 1993; Iskit and Guc, 1996a,b).

2.3. Drug administration

Animals were randomly allocated to each of the six treatment groups and dexamethasone (5 mg/kg) or L-canavanine (100 mg/kg) was administered in a total volume of 1 ml/kg by intraperitoneal injection 1 h before the administration of endotoxin or saline. During the planning of the experiments, the doses of the drugs were chosen on the basis of the available information in the literature. The doses used in our study are either equal (in the case of L-canavanine, Teale and Atkinson, 1996) or slightly greater (in the case of dexamethasone, Song et al. (1996) used 3 mg/kg) than those of previous studies which reported efficient inhibition of the effects of endotoxin administration in rats.

All drugs were prepared daily, dissolved in non-pyrogenic sterile saline and appropriately warmed to body temperature (37°C) before injection. Drug solutions were kept in dark containers until injected in order to protect them from light. Control animals received an equal volume (1 ml/kg, i.p.) of saline.

2.4. Evaluation of arrhythmias

Electrocardiogram and arterial blood pressure recordings were monitored throughout the occlusion and reperfusion periods and the heart rate was derived from these recordings. Ventricular ectopic activity was assessed according to the diagnostic and analytic criteria advocated in the Lambeth Conventions (Walker et al., 1988). No effort was made to reanimate or to defibrillate any animal which exhibited fatal arrhythmias. The number of ventricular ectopic beats and the duration (in seconds) of ventricular tachycardia were quantified only in survivors (i.e., if the animal died during the reperfusion period then the number of ventricular ectopic beats observed in that experiment was excluded from the final sum for the reperfusion period). The incidence of ventricular tachycardia, ventricular fibrillation and mortality was quantified for both periods.

2.5. Drugs used

Sodium chloride (Merck, USA), sodium pentobarbitone (Abbott, USA), heparin sodium (Roche, Switzerland), L-canavanine (Sigma, USA), lipopolysaccharide (*E. coli* endotoxin, serotype O55:B5, Sigma, USA), dexamethasone (Hoechst, Germany).

2.6. Statistics

Data are expressed as arithmetic means ± standard error of the mean (S.E.M.) of the number (n) of experiments. Differences between the medians of groups were analysed by using the Mann-Whitney U-test (e.g., total number of ectopic beats) or one-way or two-way analysis of variance (ANOVA) followed by the Student-Newman Keuls' post-hoc test where appropriate. Two-way ANOVA for repeated measurements was performed by using a computer program written by one of us (Guc, 1992) to analyse the differences between the mean arterial blood pressure curves plotted over time (Winer, 1972), and the rest of the statistical procedures were performed by using the Instat Statistical Software Package, GraphPAD. Fisher's exact test was used to analyse the differences between the incidences of arrhythmias and mortality. When $P < 0.05$, the differences were accepted as being statistically significant.

3. Results

Satisfactory anaesthesia was achieved as judged by the attenuation of responses to a painful stimulus (i.e., pinching of the skin of the foot with tweezers). 79 rats underwent surgical preparation for the experiments included in this study; 3 of them exhibited sustained arrhythmias or mean arterial blood pressure values lower than 60 mmHg at the end of the equilibration period. These animals were excluded from the study. None of the drugs significantly modified the heart rate (around 420 beats per minute) of the rats and in preliminary experiments endotoxin at 0.1 and 0.5 mg/kg doses did not significantly modify any of the parameters. However, at the 4 mg/kg dose, endotoxin treatment always yielded mean arterial blood pressure values less than 60 mmHg (data not shown), therefore a 1 mg/kg dose was used throughout the rest of the study.

3.1. The effects of drugs on the blood pressure

Fig. 1 shows the mean arterial blood pressure curves obtained from saline-treated control, endotoxin (1 mg/kg), dexamethasone (5 mg/kg) and endotoxin plus dexamethasone-treated rats. The initial mean arterial blood pressure of endotoxin-treated rats was significantly lower than that of controls (at 0 min, control: 104.8 ± 6.0 mmHg, $n = 14$; endotoxin: 66.5 ± 2.9 mmHg, $n = 14$, $P < 0.001$) while dexamethasone neither significantly altered the mean arterial blood pressure per se (105.3 ± 4.2 mmHg, $n = 12$) nor significantly attenuated the mean arterial blood pressure decrease produced by endotoxin at 0 min (dexamethasone + endotoxin: 78.8 ± 2.6 mmHg, $n = 12$) (Fig. 1).

Mean arterial blood pressure curves of L-canavanine (100 mg/kg) and L-canavanine plus endotoxin-treated rats are given in Fig. 2 together with the curves obtained from saline-treated control and endotoxin-treated animals. The latter two curves also appear in Fig. 1. In contrast to dexamethasone, the mean arterial blood pressure of the

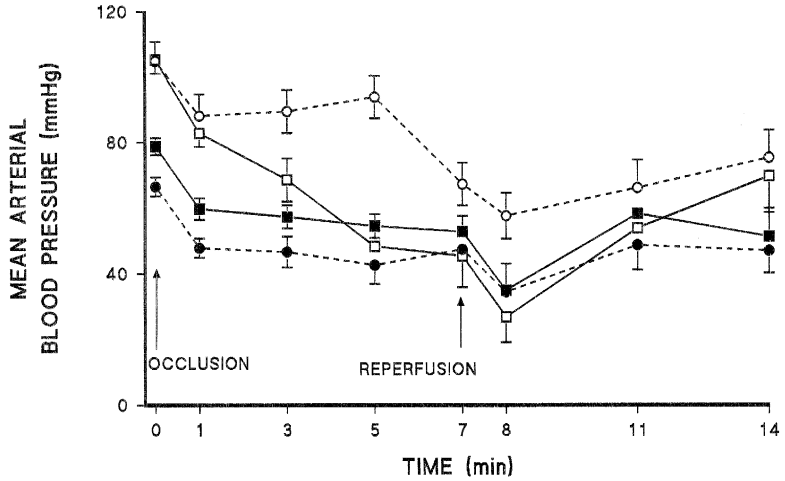

Fig. 1. Mean arterial blood pressure (mmHg) plotted against time in saline- (○), endotoxin- (1 mg/kg, ●), dexamethasone- (5 mg/kg, □) or dexamethasone + endotoxin- (■) treated rats under sodium pentobarbitone (60 mg/kg, i.p.) anaesthesia undergoing coronary artery occlusion and reperfusion at time points indicated by the arrows. Vertical bars indicate the standard error of the arithmetic mean of the number of data points included in that observation. Some standard error bars have been omitted for the sake of clarity. $n = 9–14$ for each data point obtained from the survivors for that period. Two-way ANOVA for repeated measures applied to all four curves indicated that: (i) the effect of endotoxin administration: $P < 0.00001$; (ii) the effect of dexamethasone administration: $P = 0.3298$; (iii) interaction between endotoxin and dexamethasone: $P = 0.06551$.

L-canavanine group was significantly lower than that of controls at 0 min (84.0 ± 3.2 mmHg, $n = 12$, $P < 0.01$ vs. controls). However, like dexamethasone, L-canavanine treatment did not reverse the endotoxin-induced mean arterial blood pressure decrease observed at 0 min (L-canavanine + endotoxin: 75.7 ± 2.3 mmHg, $n = 12$, $P > 0.05$ vs. endotoxin) (Fig. 2).

In all groups, occlusion of the coronary artery produced a sustained decrease in the mean arterial blood pressure and this was not significantly modified by reperfusion, except in L-canavanine- and L-canavanine plus endotoxin-treated animals (Figs. 1 and 2). In contrast to dexamethasone-treated animals, the mean arterial blood pressure of L-canavanine-treated animals was not significantly different from the initial value at 0 min (mean arterial blood pressure at 14 min, L-canavanine: 78.1 ± 8.5 mmHg, $n = 8$; L-canavanine + endotoxin: 70.5 ± 6.7 mmHg, $n = 7$) (Fig. 2).

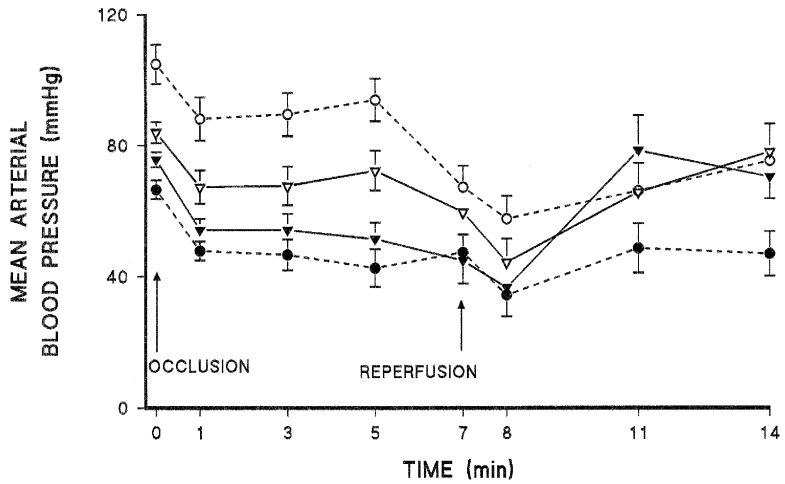

Fig. 2. Mean arterial blood pressure (mmHg) plotted against time in saline- (○), endotoxin- (1 mg/kg, ●), L-canavanine- (100 mg/kg, ▽) or L-canavanine + endotoxin- (▼) treated rats under sodium pentobarbitone (60 mg/kg, i.p.) anaesthesia undergoing coronary artery occlusion and reperfusion at time points indicated by the arrows. Vertical bars indicate the standard error of the arithmetic mean of the number of data points included in that observation. Some standard error bars have been omitted for the sake of clarity. $n = 7–14$ for each data point obtained from the survivors for that period. Two-way ANOVA for repeated measures applied to all four curves indicated that: (i) the effect of endotoxin administration: $P < 0.00001$; (ii) the effect of L-canavanine administration: $P = 0.0546$; (iii) interaction between endotoxin and L-canavanine: $P = 0.0001$.

Table 1
Duration of ventricular tachycardia (VT) together with the incidence of ventricular fibrillation (VF) and mortality in saline- or endotoxin-treated rats subjected to coronary artery occlusion and reperfusion which also received pretreatment with dexamethasone, L-canavanine or their solvent saline

Groups	Occlusion			Reperfusion		
	VT duration (s)	VF incidence (%)	Mortality (%)	VT duration (s)	VF incidence (%)	Mortality (%)
Saline						
Saline	30.9 ± 5.7 ($n = 14$)	1/14 (7.1%)	0/14 (0%)	97.3 ± 16.7 ($n = 11$)	8/14 (57.1%)	3/14 (21.4%)
Endotoxin	1.8 ± 0.9 ($n = 14$) [a]	0/14 (0%)	0/14 (0%)	20.0 ± 8.5 ($n = 9$) [b]	8/14 (57.1%)	5/14 (35.7%)
Dexamethasone						
Saline	48.8 ± 12.6 ($n = 12$)	4/12 (33.3%)	0/12 (0%)	85.4 ± 23.1 ($n = 9$)	8/12 (66.7%)	3/12 (25.0%)
Endotoxin	21.9 ± 5.7 ($n = 12$)	0/12 (0%)	0/12 (0%)	51.4 ± 13.4 ($n = 9$)	8/12 (66.7%)	3/12 (25.0%)
L-Canavanine						
Saline	22.7 ± 5.7 ($n = 12$)	3/12 (25%)	0/12 (0%)	88.4 ± 16.4 ($n = 8$)	8/12 (66.7%)	4/12 (33.3%)
Endotoxin	15.8 ± 3.1 ($n = 12$)	1/12 (8.3%)	0/12 (0%)	66.4 ± 15.9 ($n = 7$)	11/12 (91.7%)	5/12 (41.7%)

n = number of experiments. [a] $P < 0.0001$ and [b] $P < 0.005$ vs. saline, Mann-Whitney U-test.

3.2. The effects of drugs on ventricular tachycardia duration, ventricular fibrillation incidence and mortality

Table 1 summarizes the duration of ventricular tachycardia, the incidence of ventricular fibrillation and total mortality observed in saline or endotoxin-administered rats which also received saline, dexamethasone or L-canavanine treatments. In saline-treated rats (first two rows), the duration of ventricular tachycardia was significantly shorter in endotoxin-treated rats during both periods. However, this kind of difference was absent in dexamethasone- (third and fourth rows) or L-canavanine-treated (fifth and sixth rows) rats. The incidence of ventricular fibrillation or mortality was not significantly different from that of the corresponding control for the same period (Table 1).

3.3. The effects of drugs on the number of ectopic beats

All animals exhibited arrhythmias after coronary artery occlusion-reperfusion and the total number of ectopic beats was significantly greater during the reperfusion period (Fig. 3). The number of ectopic beats in saline-administered rats which also received saline, dexamethasone or L-canavanine pretreatments was not significantly different within a particular period (Fig. 3). However, endotoxin treatment significantly reduced the number of ectopic beats

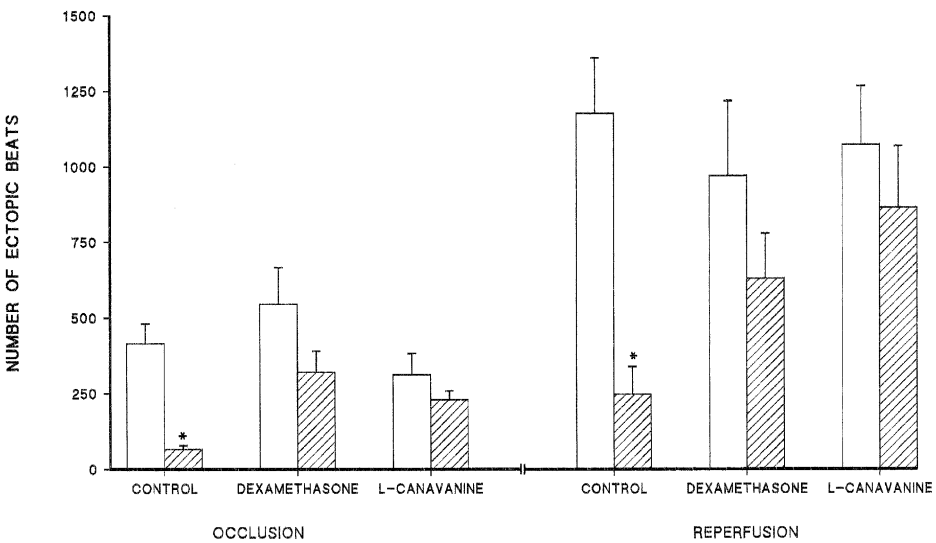

Fig. 3. Total number of ventricular ectopic beats observed during coronary artery occlusion and reperfusion periods in saline- (open columns) or endotoxin- (1 mg/kg, hatched columns) treated groups which also received dexamethasone (5 mg/kg) or L-canavanine (100 mg/kg) pretreatment before receiving endotoxin or its solvent saline. The solvent of L-canavanine and dexamethasone was also saline and the results from these saline- (1 ml/kg) pretreated 'control' groups are given separately. Vertical bars indicate the standard error of the arithmetic mean of the number of data points for that column. $n = 7–14$ for each data point obtained from the survivors for that period. * Significant difference ($P < 0.0005$) from its adjacent column.

during the occlusion and the reperfusion periods. During both periods, there was no significant difference between the number of total ectopic beats obtained from animals which received dexamethasone or L-canavanine pretreatment. In other words, dexamethasone or L-canavanine pretreatment attenuated the decrease in ectopic activity exerted by endotoxin (Fig. 3).

4. Discussion

The present results constitute the first account of the effects of a specific inducible nitric oxide synthase inhibitor, L-canavanine, on *Escherichia coli* endotoxin-induced attenuation of ischaemia and reperfusion arrhythmias in vivo. Briefly, endotoxin administration produced hypotension, markedly reduced the number of ectopic beats and ventricular tachycardia duration but had no significant effect on the incidence of ventricular fibrillation or mortality whereas dexamethasone and L-canavanine significantly attenuated the antiarrhythmic effects of endotoxin without modifying its hypotensive effect in this anaesthetised rat model of myocardial ischaemia and reperfusion.

It has been known for many years that endotoxin has profound effects on the heart such as depression of myocardial contractility (Solis and Downing, 1966) and cardiac responses to exogenous catecholamines in vivo (Parratt, 1973). Although the exact mechanisms underlying such effects are not well understood, extensive evidence shows that they occur as the consequence of numerous processes. It is now generally accepted that there are at least two phases of cardiovascular hyporesponsiveness to catecholamines during experimental endotoxaemia. The first involves prostaglandins (Gray et al., 1990), constitutively formed $^{\cdot}$NO (Szabo et al., 1993) and bradykinin (Fleming et al., 1992). Since prostaglandins and constitutively formed $^{\cdot}$NO are released by bradykinin, the involvement of both mediators can be unified (Fleming et al., 1992). The second phase of impairment in the responsiveness to catecholamines is due to the formation of large amounts of $^{\cdot}$NO as a consequence of endotoxin-induced nitric oxide synthase in various tissues (Moncada et al., 1991).

An enhanced antioxidant status with increased endogenous myocardial catalase activity and overexpression of superoxide dismutase after endotoxin exposure is reported to decrease ischemia-reperfusion injury of isolated rat hearts (Brown et al., 1989). Additionally, various cardioprotective mediators, like heat-shock proteins, some phosphodiesterase isoforms and Na^+/K^+ ATPase, are also triggered by endotoxin (for a review see Parratt and Szekeres, 1995). Among various mediators, particular interest has focused on $^{\cdot}$NO, which has also been implicated in the antiarrhythmic and cardioprotective effects of preconditioning against ischaemia (Vegh et al., 1992), effects which can also be inhibited by dexamethasone (Vegh et al., 1994).

Endotoxin administration leads to the induction of calcium-independent inducible nitric oxide synthase in blood vessels (Fleming et al., 1990) and in the heart (Salter et al., 1991; Schulz et al., 1992) within 30 min and peaks at 2–6 h (Bateson et al., 1996). Therefore, the timing of endotoxin injection in our experimental protocol, 4 h before the occlusion of the coronary artery, most probably means that our arrhythmia observation period coincided with maximum $^{\cdot}$NO production in rats (Tracey et al., 1995).

In general, our results are in agreement with those of previous studies which reported that endotoxin (Song et al., 1994, 1996) or its derivative monophosphoryl lipid A (Baxter et al., 1996) exert antiarrhythmic actions while dexamethasone at lower doses (3 mg/kg) than we used in this study can effectively inhibit its effects. Since dexamethasone is an inhibitor of the expression of inducible nitric oxide synthase (Radomski et al., 1990) and cyclooxygenase-2 (Masferrer et al., 1992), the mediators produced by these enzymes are expected to have important roles in the endotoxin-induced antiarrhythmic effects. However, the limited importance of cyclooxygenase products in modifying arrhythmogenesis in this experimental model (Parratt, 1989) increases the relative importance of inducible nitric oxide synthase in the effects of endotoxin. The attenuation of the antiarrhythmic effects of endotoxin by L-canavanine, which is a selective inhibitor of inducible nitric oxide synthase (Iyengar et al., 1987), in the present study further supports the hypothesis that cyclooxygenase-2 products have limited importance in this experimental model. In contrast to previous studies (Teale and Atkinson, 1996), L-canavanine failed to prevent the hypotension produced by endotoxin. This can be explained by the difference between the administration route of L-canavanine together with the dose of endotoxin used in our study and that of Teale and Atkinson (1996). In that study, L-canavanine was administered via intravenous infusion at a dose (100 mg/kg) which we administered intraperitoneally. Since we have no information on the pharmacokinetic properties of L-canavanine in anaesthetised rats, it could be suggested that the final concentration of L-canavanine at its site of action was lower in comparison, and was thus insufficient to restore the mean arterial blood pressure. Since we did not have the facilities for measuring the activity of inducible nitric oxide synthase in the myocardium, the prevention of the steady fall in mean arterial blood pressure due to the occlusion and reperfusion of the coronary artery by L-canavanine can be taken as indirect evidence that the dose used was sufficient. However, there is no clear-cut agreement in the literature on the protocol to be used in experiments with L-canavanine to block various effects of endotoxin. In a recent study, an admittedly arbitrarily chosen administration protocol for L-canavanine (Fatehi-Hassanabad et al., 1996) was found to be useful in preventing endotoxin-induced organ damage

and it was also found efficient by another group (Cai et al., 1996) in preventing endotoxin-induced elevations in nitrosylhaemoglobin. The protocol used in those studies (10 mg/kg, i.v. bolus, every hour) yielded a total dose of 40 mg/kg in 4 h, which was less than we used (100 mg/kg) in the present study. Biochemical methods indicated that the former protocol produced sufficient inhibition of the activity of inducible nitric oxide synthase (Fatehi-Hassanabad et al., 1996).

The experimental protocol used in this study can be criticised from the point that the use of 7 min of occlusion followed by 7 min of reperfusion may have failed to produce a sufficient amount of ectopic activity and mortality to be able to detect a possible protective effect of endotoxin against a more serious form of arrhythmias. However, by using the same protocol, we were able to demonstrate the antiarrhythmic properties of diltiazem, siratiazem (Guc et al., 1993) and thimerosal, an inhibitor of acyl-coenzyme A:lysolecithin acyltransferase which in turn activates $^{\cdot}$NO production (Iskit and Guc, 1996b), while others have shown the antiarrhythmic effects of verapamil and nicardipine (Swies et al., 1990). Also there are experimental protocols which use 5 min of occlusion duration followed by a 10-min follow-up period during reperfusion for investigation of the pathophysiological basis of reperfusion-induced arrhythmia generation in this rat model (Barnes and Coker, 1995). Our results are similar in broad terms to those of the latter study, in which 8 out of 12 rats (66.7%) were reported to display reperfusion-induced ventricular fibrillation in the control group, compared to 8 out of 14 rats (57.1%) in the present study. Moreover, the duration of ventricular tachycardia did not appear to be significantly different (i.e., 179 ± 64 s, $n = 12$ in the former study and 97.3 ± 16.7 s, $n = 11$ in the present study). However, the incidence of mortality in the former study was apparently higher compared to that of the present study.

Our results can be regarded as complementary to the clear-cut results of the thorough studies of Song et al. (1994, 1996) in the sense that a selective inhibitor of inducible nitric oxide synthase was found to attenuate the antiarrhythmic effects of endotoxin as well as dexamethasone. Another interesting finding of this study is the failure of endotoxin to alter the incidence of ventricular fibrillation during reperfusion, whereas the work of Song et al. (1996) suggested the attenuation of ischaemia-induced ventricular fibrillation by endotoxin. Taken together, these observations imply that endotoxin can only afford protection against less severe forms of arrhythmias and that the underlying mechanism is yet to be established.

Therefore, in conclusion, we demonstrated that endotoxin attenuates ischaemia-reperfusion arrhythmias in anaesthetised rats and that the likely mechanism appears to involve $^{\cdot}$NO production through the activation of inducible nitric oxide synthase, because dexamethasone and L-canavanine inhibited this phenomenon. However, our results do not rule out the possibility of a contribution from cyclooxygenase products, and this remains to be further investigated by using selective blockers of this pathway.

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