Immunology 1997 **90** 95–100

Isoproterenol regulates tumour necrosis factor, interleukin-10, interleukin-6 and nitric oxide production and protects against the development of vascular hyporeactivity in endotoxaemia

C. SZABÓ,* G. HASKÓ,† B. ZINGARELLI,* Z. H. NÉMETH,† A. L. SALZMAN,* V. KVETAN,‡ S. MCCARTHY PASTORES,‡ & E. S. VIZI† **Division of Critical Care Medicine, Children's Hospital Medical Center, Cincinnati, OH, USA, †Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary, and ‡Division of Critical Care Medicine, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY, USA*

SUMMARY

Pro-inflammatory cytokines, such as tumour necrosis factor (TNF) and free radicals, such as nitric oxide (NO), are mediators of endotoxaemia. Catecholamines are in clinical use to treat the haemodynamic consequences of severe septic shock. Beta-adrenergic agonists exert many of their effects by elevation of intracellular cyclic AMP (cAMP) concentration. Cyclic AMP can modulate endotoxin-induced cytokine and NO production. Here we investigate the effect of isoproterenol pretreatment on the cytokine and NO production induced by bacterial lipopolysaccharide (LPS, 4–10 mg/kg). Pretreatment with isoproterenol (10 mg/kg) blunted the LPS-induced TNF response, increased the LPS-induced formation of interleukin-10 and interleukin-6 and reduced the LPS-induced production of NO in conscious mice. In anaesthetized rats, pretreatment with isoproterenol prevented the LPS-induced suppression of vascular contractility to norepinephrine in the thoracic aorta *ex vivo*. The hyporeactivity is due to expression of the inducible isoform of NO synthase (iNOS) and was restored by *in vitro* administration of N^G-methyl-L-arginine (L-NMA), an inhibitor of NO synthase. However, L-NMA did not alter vascular contractility in control vessels or in rings taken from the LPS-treated rats pretreated with isoproterenol. Our findings suggest that, in addition to its haemodynamic actions, isoproterenol may also exert beneficial effects by modulating the endotoxin-induced inflammatory response.

INTRODUCTION

Sepsis and the systemic inflammatory response syndrome are characterized by peripheral vasodilatation, myocardial depression and suppression of cellular metabolic rate.[1] The haemodynamic changes in sepsis are, in a significant part, mediated by the production of pro-inflammatory cytokines [such as interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α)] and by the overproduction of free radical and oxidant mediators of shock [such as superoxide, nitric oxide (NO), peroxynitrite and hydroxyl radical].[1–3]

The clinical strategies to prevent or restore the haemodynamic consequences of septic shock are limited. While clinical trials with inhibitors of NO synthesis suggest a potential future treatment,[2,4] the present clinical practice is restricted to the use of vasopressors and inotropes, both of which lose their effectiveness in severe sepsis.[1,2,5]

Current evidence suggests that many 'classical' pharmacological agents, such as ligands of alpha-adrenergic,[6,7] beta-adrenergic,[8,9] dopamine[10] and adenosine[11–13] receptors, can influence the production of pro- and anti-inflammatory cytokines in response to endotoxin (bacterial lipopolysaccharide, LPS). Some of these effects were proposed to be related to modulation of intracellular cyclic AMP (cAMP) levels.[6,8,11–13]

In the present study, we investigated the effect of the potent, non-selective beta-receptor agonist isoproterenol on the LPS-induced production of TNF-α, IL-10, IL-6 and NO in rodent models. Since vascular hyporeactivity (reduced responsiveness of the blood vessels to contractile agents) is a prominent feature of shock, and it is known to be mediated by overproduction of NO within the vascular smooth muscle,[2] we also studied whether isoproterenol modulates the development of vascular hyporeactivity in endotoxic shock *ex vivo*.

Received 5 September 1996; accepted 29 September 1996.

Abbreviations: iNOS, inducible isoform of nitric oxide synthase; L-NMA: N^G-methyl-L-arginine; NO, nitric oxide; PAF, platelet-activating factor; TNF-α, tumour necrosis factor-α.

Correspondence: Dr C. Szabó, Division of Critical Care Medicine, Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229–3039, USA.

MATERIALS AND METHODS

Animals

Male BALB/c mice (25–35 g) were purchased from Charles River Laboratories (Budapest, Hungary), and male Wistar

rats were purchased from Charles River Laboratories (Wilmington, MA). Animals received food and water *ad libitum*, and lighting was maintained on a 12-hr cycle.

Materials

Isoproterenol was obtained from Research Biochemicals Inc. (Natick, MA). LPS from *Escherichia coli* 055:B5 and all other drugs were obtained from Sigma (St Louis, MO).

Experimental design for plasma TNF-α, IL-10, IL-6 and nitrite/nitrate measurements in mice

Mice were injected intraperitoneally (i.p.) with either drug vehicle or isoproterenol (10 mg/kg) in a volume of 0·1 ml/10 g body weight. Thirty minutes later they were challenged with 4 mg/kg of i.p. LPS. The animals were killed 90 min after LPS treatment. For the measurements of plasma nitrite/nitrate, mice were injected i.p. with either drug vehicle or isoproterenol, followed by an i.p. LPS challenge (10 mg/kg) 30 min later. Blood was taken at 3, 6, 9, 24 and 48 hr after LPS injection. Blood was collected in ice-cold Eppendorf tubes containing ethylene diamine tetraacetic acid (EDTA), and centrifuged for 10 min at 4°. The plasma was stored at −70° until assayed.

Endotoxic shock protocol in the rat

Male Wistar rats (Charles River Laboratories, Wilmington, MA) were anaesthetized with sodium thiopentol (120 mg/kg, i.p.) and instrumented as described.[14] Rats of 280–300 g weight were used in all experiments, and there were no significant differences between the various treatment groups in this respect. The trachea was cannulated to facilitate respiration and temperature was maintained at 37° using a homeothermic blanket. The right carotid artery was cannulated and connected to a pressure transducer for the measurement of phasic and mean arterial blood pressure (MAP) and heart rate which were digitized using a Maclab A/D converter (AD Instruments, Milford, MA) and stored and displayed on a Macintosh personal computer. The left femoral vein was cannulated for the administration of drugs.

The endotoxin shock protocol used here was performed in accordance with National Institutes of Health (NIH) guidelines and with the approval of the institutional review board of the Children's Hospital Research Foundation. Upon completion of the surgical procedure, cardiovascular parameters were allowed to stabilize for 10 min. Then, animals were divided into two groups: the first group received isoproterenol (10 mg/kg) ($n=8$), the second group received vehicle ($n=8$).

After 30 min, the isoproterenol-pretreated and the vehicle-pretreated animals were divided into two groups: one group of animals received LPS [10 mg/kg intravenously (i.v.)], the other group received saline. At 180 min after the injection of LPS, animals in all four groups (control; LPS; isoproterenol and isoproterenol + LPS; $n=4$ rats in each group) were killed, and thoracic aortae and plasma samples obtained for the measurement of contractile function or for the measurement of plasma nitrite/nitrate levels and plasma 6-keto-prostaglandin $F_{1\alpha}$ concentration (see below).

Organ bath experiments

Thoracic aortae from rats were cleared of adhering periadventitial fat and cut into rings of 3–4-mm width. Endothelium was removed by gently rubbing the intimal surface with a thin wooden stick.[15] Lack of a detectable acetylcholine-induced relaxation was taken as evidence that endothelial cells had been removed. The rings were mounted in organ baths (5 ml) filled with warmed (37°), oxygenated (95% O_2 5% CO_2) Krebs' solution (pH 7·4) consisting of (mM): NaCl 118, KCl 4·7, KH_2PO_4 1·2, $MgSO_4$ 1·2, $CaCl_2$ 2·5, $NaHCO_3$ 25 and glucose 11·7, in the presence of indomethacin (10 μM). Isometric force was measured with isometric transducers (Kent Scientific Corp. Litchfield, CT), digitized using a Maclab A/D converter (AD Instruments, Milford, MA). A tension of 1 g was applied and the rings were equilibrated for 60 min, changing the Krebs' solution every 15 min.[15] Indomethacin was used to prevent the production of cyclo-oxygenase metabolites that are predominantly vasoconstrictors in this experimental setting.[16] Concentration–response curves to noradrenaline (10^{-9}–10^{-5} M) in the absence or presence of the NO synthase (NOS) inhibitor N^G-methyl-L-arginine (L-NMA, 100 μM, 30 min treatment) were obtained.

Cytokine assays

Cytokine levels in plasma were determined by enzyme-linked immunosorbent assay (ELISA) kits that are specific against murine cytokines. Plasma levels of IL-10 and IL-6 were measured using ELISA kits purchased from Endogen (Endogen Inc., Boston, MA). Concentrations of TNF-α in the plasma were determined using ELISA kits from Genzyme (Genzyme Corp., Boston, MA). Plasma samples were diluted to 1 : 6; 1 : 2 and 1 : 200 for TNF-α, IL-10 and IL-6, respectively, prior to the cytokine measurements. Assays were performed as described.[7,10,13] Detection limits were <15 pg/ml, <0·15 pg/ml and <5 pg/ml for TNF-α, IL-10 and IL-6, respectively. The baseline values detected in the present study and the magnitude of the LPS-induced increases in the cytokine plasma levels were similar to previously reported values.[7–13]

Measurement of plasma nitrite/nitrate concentration

In plasma samples, nitrate is the major degradation product of NO. Nitrate was converted to nitrite by incubation with 60 mU nitrate reductase and 25 μM nicotinamide adenine dinucleotide phosphate (NADPH) for 180 min. Nitrite was then measured as previously described[17] by adding Griess reagent (1% sulphanilamide and 0·1% naphthylethylenediamine in 5% phosphoric acid) to plasma samples diluted (1 : 10) in phosphate-buffered saline. The optical density at 550 nm (OD_{550}) was measured by using a Spectramax 250 microplate reader (Molecular Devices, Sunnyvale, CA). Nitrite/nitrate concentrations were calculated by comparison with OD_{550} of standard solutions of sodium nitrite and sodium nitrate. The measurements of nitrite/nitrate were performed using reagents free of nitrite and nitrate: no basal or background nitrite or nitrate levels were detected.

Analysis of 6-keto-$PGF_{1\alpha}$ by radioimmunoassay

Plasma samples were diluted 1 : 5 in a buffer containing 0·1% polyvinylpyrrolidone, 0·9% NaCl, 50 mM Tris base, 1·7 mM $MgSO_4$ and 0·16 mM $CaCl_2$ (pH 7·4) before radioimmunoassay. The stable metabolite of prostacyclin, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), was determined by radioimmunoassay as described by Wise *et al.*[18]

Statistical evaluation

Values in the figures and text are expressed as mean ± SEM of n observations. Statistical analysis of the data was performed by one-way analysis of variance followed by Dunnett's test, as appropriate. A P-value less than 0·05 was considered statistically significant.

RESULTS

Effect of isoproterenol on LPS-induced TNF-α, IL-6 and IL-10 plasma levels in mice

Intraperitoneal injection of LPS (4 mg/kg) into mice resulted in an elevation of plasma TNF-α concentrations. Pretreatment i.p. of animals with isoproterenol, 30 min prior to LPS injection resulted in a significant reduction of LPS-induced plasma levels of TNF-α (Fig. 1a). In addition, isoproterenol caused a significant elevation in the plasma levels of IL-6 in response to LPS (Fig. 1b). LPS caused a slight elevation of IL-10 plasma concentrations (Fig. 2). The peak was observed at 90 min, and IL-10 returned to near baseline levels at 6 hr (not shown). Pretreatment of animals with isoproterenol i.p., 30 min prior to LPS injection resulted in a significant augmentation of LPS-induced plasma levels of IL-10 at 90 min (Fig. 2).

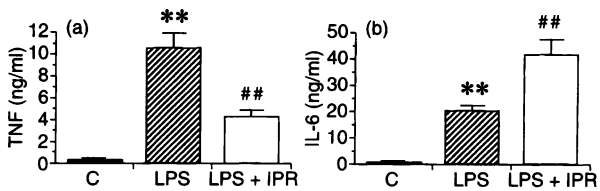

Figure 1. Effect of pretreatment with isoproterenol (10 mg/kg) on LPS-induced plasma TNF-α (a) and IL-6 (b) levels at 90 min after i.p. administration of 4 mg/kg LPS. Data are means ± SEM of six to nine mice per group (**$P<0·01$, from control; ##$P<0·01$ significant effect of isoproterenol in the presence of LPS).

Effect of isoproterenol on NO production and vascular hyporeactivity

In response to injection of LPS at 4 mg/kg (i.p.) into mice, a marked increase in plasma nitrite/nitrate concentrations was observed at 3–48 hr (Fig. 3). Pretreatment of animals with isoproterenol significantly suppressed this LPS-induced NO response at 6, 9 and 24 hr (Fig. 3).

In anaesthetized rats, isoproterenol caused a rapid increase in heart rate (from 370 ± 27 to 529 ± 12 beats/min, $P<0·01$, $n=8$) and a rapid fall in blood pressure from 113 ± 13 to 85 ± 14 mmHg ($P<0·01$, $n=8$). Subsequent administration of LPS, however, did not alter blood pressure in these animals over 180 min (Fig. 4b). In contrast, in vehicle-pretreated rats injected with LPS, there was a rapid fall in MAP, which was sustained over 180 min (Fig. 4a). In the vehicle-treated control animals, MAP was stable (initial MAP: 119 ± 13 mmHg, MAP at 180 min: 120 ± 6 mmHg), whereas in the animals that received isoproterenol only, the hypotension was maintained for 180 min (MAP 5 min after isoproterenol injection: 79 ± 5 mmHg, MAP 205 min later: 89 ± 14 mmHg, $n=4$). The initial tachycardia, in response to isoproterenol gradually returned to basal levels over 180 min. Thus, at the end of the experiments (i.e. at 180 min after LPS or vehicle injection), heart rate was similar in all four groups: 388 ± 19; 454 ± 17; 420 ± 26 and 421 ± 50 beats/min in the vehicle-treated, isoproterenol-treated, vehicle + LPS-treated and isoproterenol-treated rats, respectively ($n=4$ in each group).

LPS also caused a significant depression of the contractile ability of the thoracic aorta to norepinephrine (1 nM–10 μM) *ex vivo*, an effect which was prevented by *in vivo* isoproterenol treatment (Fig. 5). This improvement was comparable to the one seen by a 30-min *in vitro* incubation with the NOS inhibitor L-NMA (100 μM) to rings obtained from LPS-treated animals (Fig. 6). Interestingly, incubation of rings obtained from isoproterenol-treated LPS rats with L-NMA did not result in additional improvement in the contractile ability of the vessels (Fig. 6). *In vivo* isoproterenol treatment did not affect the contractile ability of control rings (Figs. 5 and 6).

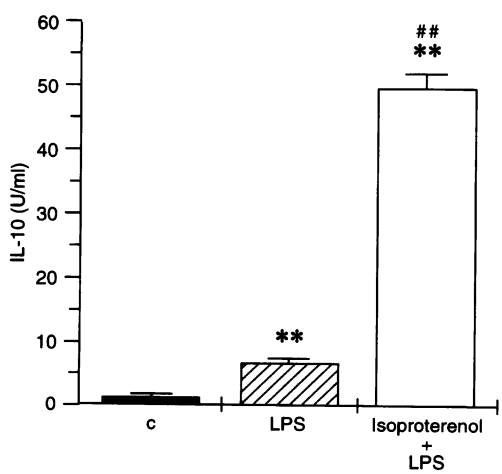

Figure 2. Effect of pretreatment with isoproterenol (10 mg/kg) on LPS-induced plasma IL-10 levels at 90 min after i.p. administration of 4 mg/kg LPS. Data are means ± SEM of six to nine mice per group (**$P<0·01$, from control; ##$P<0·05$ significant effect of isoproterenol in the presence of LPS).

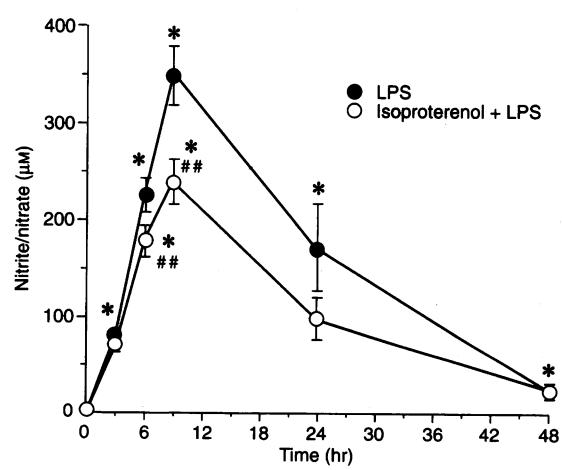

Figure 3. Effect of pretreatment with isoproterenol (10 mg/kg) on LPS-induced plasma nitrite/nitrate levels at various time points after i.p. administration of 10 mg/kg LPS. Data are means ± SEM of six to nine mice per group (*$P<0·01$, from control; ##$P<0·01$ significant effect of isoproterenol in the presence of LPS).

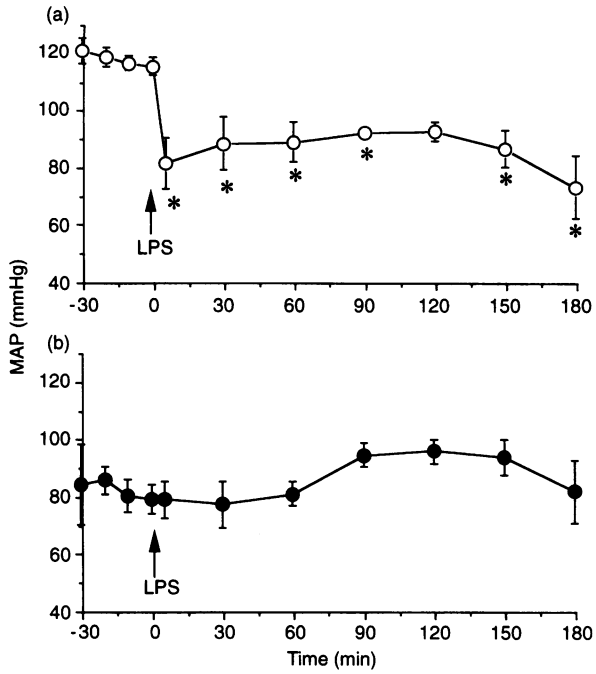

Figure 4. Effect of LPS (10 mg/kg i.v.) on MAP in vehicle-treated (a) and in isoproterenol-pretreated (b) anaesthetized rats. Rats were treated with LPS at time 0 (arrow). Data are expressed as means ± SEM of $n=4$ animals in each group. $^*P<0{\cdot}01$ represents significant hypotensive effect of LPS.

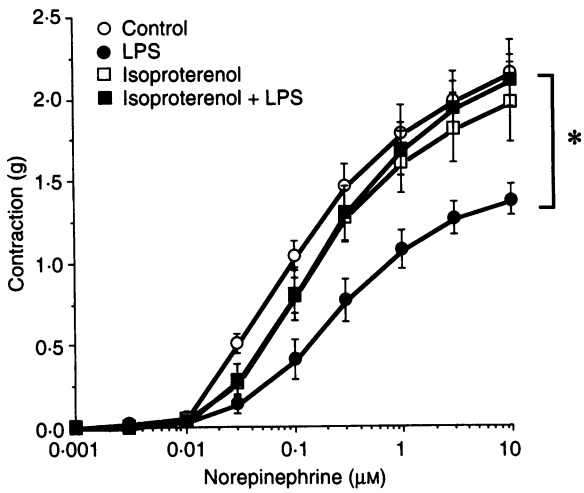

Figure 5. Concentration–response curves for noradrenaline (10^{-9}–10^{-5} M) in endothelium-denuded aortic rings. Open circles, control rings; open squares, rings from rats treated with isoproterenol without LPS challenge; closed circles, rings from rats treated with LPS; closed squares, rings from rats treated with isoproterenol and LPS. There was a significant decrease in the contractions in response to LPS ($P<0{\cdot}01$). $^*P<0{\cdot}01$ represents significant protective effects of isoproterenol in rings from LPS-treated rats when compared to LPS alone. In each group $n=8$ rings.

Similar to the effects of isoproterenol in mice at 3 hr after LPS injection, there was no significant reduction in the plasma nitrite/nitrate levels by isoproterenol in the LPS-treated rats, although a tendency for reduction was observed (Fig. 7). Moreover, there was a tendency for an increase in plasma

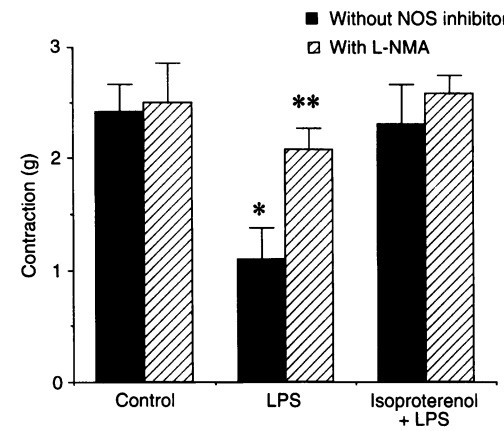

Figure 6. Contractions in response to noradrenaline (10^{-5} M) in endothelium-denuded aortic rings. Closed columns represent contractions in control rings (left), rings from LPS-treated rats (middle) and rings from the LPS-treated rats pretreated with isoproterenol (right). $^*P<0{\cdot}01$ represents significant decrease in the contractions in response to LPS, $^{**}P<0{\cdot}01$ represents significant increase in contractions in response to L-NMA treatment. In each group $n=8$ rings.

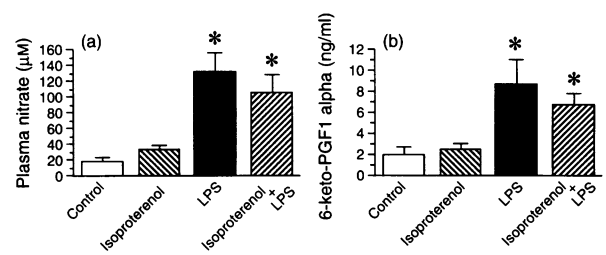

Figure 7. Plasma levels of nitrite/nitrate (a) and 6-keto-prostaglandin $F_{1\alpha}$ (b) in control animals, in animals treated with isoproterenol alone, in animals treated with LPS and in animals treated with isoproterenol and LPS. Plasma samples were taken at 180 min after injection of LPS or vehicle. $^*P<0{\cdot}01$ represents significant increase in the plasma levels of nitrite/nitrate or 6-keto-prostaglandin $F_{1\alpha}$.

nitrite/nitrate in the isoproterenol-treated animals without LPS injection ($P=0{\cdot}07$). No significant effect of isoproterenol was seen on the LPS-induced production of prostaglandin metabolites (Fig. 7).

DISCUSSION

Isoproterenol (*d,l-β*[3,4-dihydroxyphenyl]-α-isopropylaminoethanol) is a potent, non-selective beta-adrenergic agonist with very low affinity for alpha-adrenoceptors. Isoproterenol elicits its pharmacological actions by stimulation of the adenylate cyclase–cAMP system.[19] In the present study, we investigated the effects of isoproterenol treatment on the production of various pro-inflammatory (TNF-α, IL-6, NO) and anti-inflammatory (IL-10) mediators in response to LPS *in vivo*.

The major findings of the current study are the following: isoproterenol reduces the LPS-induced TNF-α response; isoproterenol increases the LPS-induced formation of IL-10 and IL-6; isoproterenol reduced the LPS-induced production of NO in conscious mice; and isoproterenol prevents the LPS-induced suppression of vascular contractility to norepinephrine in the thoracic aorta *ex vivo*. Overall, these actions point towards an anti-inflammatory and anti-shock effect of

isoproterenol. The suppression of LPS-induced TNF-α plasma levels by isoproterenol and the parallel increase in IL-10 is not unique: similar effects were observed with prostaglandin E,[20] with epinephrine[21] and with adenosine receptor agonists.[13] All of these agents are known to modulate intracellular cAMP levels. For example, PGE_2 enhances production of LPS-induced IL-10 and inhibits release of LPS-induced TNF *in vivo* and *in vitro*, and that the effect of PGE_2 can be mimicked by agents that similarly elevate intracellular cAMP levels.[22] Similar findings were reported with dibutyryl cAMP, the stable analogue of cAMP *in vitro* and *in vivo*.[23,24] Thus, it is generally assumed that increased intracellular cAMP levels are responsible for both increased IL-10 production and decreased TNF-α release observed with the administration of these agents.

Nitrite and nitrate, the breakdown products of NO in plasma, were used in this study to assess LPS-induced NO production. Two different experimental protocols were used in rats and mice (anaesthetized versus conscious; i.v. LPS versus i.p. LPS), which may have influenced the findings when the two rodent models are compared. The use of the two species was, however, necessary, for two reasons: first, methodological limitations make the measurements of vascular contractility in blood vessels from mice extremely difficult, and second, the availability and specificity of cytokine ELISA kits are better for mice than for rats. Our study in rats and the study in mice, however, resulted in similar findings in that isoproterenol treatment caused a partial suppression of NO production at later time-points after LPS injection (6–12 hr), whereas no significant suppression was seen at the early time-points (3 hr). Perhaps somewhat surprisingly, isoproterenol pretreatment completely abolished the development of the vascular hyporeactivity of the thoracic aorta *ex vivo*. In the present model of endotoxic shock, the inducible NO synthase (iNOS) plays a key role in the development of vascular failure and multiple organ dysfunction.[2,15,25] In this model, iNOS is expressed in various organs and in the vasculature after several hours of endotoxic shock, and agents that inhibit the induction or the activity of iNOS are able to prevent or reverse the delayed hypotension and the *in vivo* or *ex vivo* vascular hyporeactivity.[2] The present data demonstrate that isoproterenol has pronounced beneficial effects against the LPS-induced hypotension and vascular hyporeactivity in this experimental model in which injury is clearly driven by iNOS. Thus, we suggest that isoproterenol inhibits the development of the delayed vascular failure by inhibiting the expression of iNOS or the production of NO in this model. Inhibition of iNOS activity with L-NMA in the same model resulted in restoration of the endotoxin-induced vascular hyporeactivity, while L-NMA did not alter contractility in isoproterenol-pretreated LPS rats (Fig. 6). The non-additivity of the effect of isoproterenol (*in vivo*) and L-NMA (*in vitro*) strengthens our point that the protection against the suppression of vascular hyporeactivity by isoproterenol is due to inhibition of the expression of iNOS in the vessels.

The effects of isoproterenol on the *ex vivo* vascular reactivity were paralleled by the effects of the drug on the LPS-induced changes in the blood pressure: in the isoproterenol-pretreated animals, both the acute and the delayed hypotension induced by LPS were absent (Fig. 4). In this respect, however, it is noteworthy that the interpretation of the *in vivo* cardiovascular data may be confounded by the different cardiovascular status (isoproterenol-treated animals have increased heart rate and cardiac output and lower peripheral vascular resistance and blood pressure). In this model, the immediate hypotensive effect of LPS is related to the acute production of platelet-activating factor.[26] Although it is beyond the scope of the present investigation to establish whether the inhibition by isoproterenol of the early hypotensive response is related to inhibition of the production of platelet-activating factor, it is noteworthy that an increase in cAMP by isoproterenol reduces the release of platelet-activating factor from human neutrophils *in vitro*.[27]

How can we reconcile the relatively modest effect of isoproterenol on plasma nitrite/nitrate with the marked protection against the vascular hyporeactivity *in vivo*? *In vitro* studies have presented conflicting evidence on the role of cAMP and the effect of cAMP-elevating agents in the induction of iNOS. While in some studies elevation in cAMP caused a reduction in the induction of iNOS,[28,29] in other studies, elevation of cAMP caused induction of iNOS or enhancement of the induction of iNOS in response to additional stimulants.[30,31] It is conceivable that the regulation of the induction of iNOS *in vivo* is cell-specific: while in certain cells and tissues cAMP up-regulates the induction of iNOS, in other cells, it may be inhibitory. Moreover, the time–course of the intervention may be an important factor: the suppression of iNOS induction by cAMP elevating agents may require more prolonged pretreatment. Assuming that the regulation by isoproterenol of iNOS induction *in vivo* is cell-specific, it is not surprising that the overall effect of isoproterenol on plasma nitrite/nitrate levels (which represents the total NO output) are relatively modest: it is conceivable that NO production from non-vascular sources (macrophages, epithelial cells, etc.) importantly contributes to the whole body NO production.

There are numerous reports demonstrating the beneficial effects of isoproterenol and other adrenergic agents in various models of circulatory shock.[32,33] A recent report demonstrates marked additional improvement in the cardiovascular parameters in endotoxemia by combined treatment with the NOS inhibitor L-NMA with dobutamine.[33] In these studies, it was assumed that all of the effects of dobutamine were direct consequences of the inotropic effects of the drug. In light of the present study, the question arises as to whether the marked additional effects of dobutamine may be, in part, related to suppression of iNOS induction.

In summary, the data presented here provide functional evidence that isoproterenol exerts marked anti-inflammatory effects by suppressing LPS-induced TNF-α and NO production, while simultaneously increasing LPS-induced IL-10 production. Regarding the mechanism of action, several possibilities should be considered. Some of the effects may be related to the primary, cAMP-increasing effect of the drug (effects on IL-10, TNF-α, and, possibly, IL-6). The reduction of NO production may be a cAMP-mediated effect, or may be secondary to the suppression of TNF-α production, since TNF-α is known to be involved in the process of iNOS induction in response to LPS.[25] While the mechanism and the sequence of isoproterenol's effects on the LPS-induced inflammatory response requires further studies, the present study, coupled with other current observations[34] suggests that beta-adrenergic receptor agonists, in addition to their cardiovascular effects, also have multiple anti-inflammatory effects

in endotoxaemia, via modulation of the production of key inflammatory mediators.

ACKNOWLEDGMENTS

The expert technical assistance of Mrs F. Tóth, Miss J. Benkö, Mrs L. Fong and Mr P. Hake is gratefully acknowledged. This work was supported by grants from OTKA (Nos. T 017567 and T013024, Hungarian Research Fund) and by a grant from the Foundation for Hungarian Higher Education and Research (144/94).

REFERENCES

1. Schlag G. & Redl H. (eds.) (1993) *Pathophysiology of Shock, Sepsis and Organ Failure*. Springer-Verlag, Berlin.
2. Szabó C. (1995) Alterations in nitric oxide production in various forms of circulatory shock. *New Horizons* **3,** 2.
3. Szabó C. (1996) The role of peroxynitrite in the pathophysiology of shock and inflammation. *Shock* **6,** 79.
4. Petros A., Lamb G., Leone A., Moncada S., Bennett D. & Vallance P. (1994) Effects of a nitric oxide synthase inhibitor in humans with septic shock. *Cardiovasc Res* **28,** 34.
5. Rudis M.I., Basha M.A. & Zarowitz B.J. (1996) Is it time to reposition vasopressors and inotropes in sepsis? *Critical Care Med* **24,** 525.
6. Ignatowski T.A. & Spengler R.N. (1995) Regulation of macrophage-derived tumor necrosis factor production by modification of adrenergic receptor sensitivity. *J Neuroimmunol* **61,** 61.
7. Haskó G., Elenkov I.J., Kvetan V. & Vizi E.S. (1995) Differential effect of selective block of alpha-2 adrenoceptors on plasma levels of tumour necrosis factor-alpha, interleukin-6 and corticosterone induced by bacterial lipopolysaccharide in mice. *J Endocrinol* **144,** 457.
8. Severn A., Rapson N.T., Hunter C.A. & Liew F.Y. (1992) Regulation of tumor necrosis factor production by adrenaline and beta-adrenergic agonists. *J Immunol* **148,** 3441.
9. Elenkov I.J., Haskó G., Kovács K. & Vizi E.S. (1995) Modulation of lipopolysaccharide-induced tumor necrosis factor-alpha production by selective alpha- and beta-adrenergic drugs in mice. *J Neuroimmunol* **61,** 123.
10. Haskó G., Szabó C., Merkel K. *et al.* (1996) Modulation of lipopolysaccharide-induced tumor necrosis factor and nitric oxide production by dopamine receptor agonists and antagonists in mice. *Immunology Lett* **49,** 143.
11. Bouma M.G., Stad R.K., van den Wildenberg F.A. & Buurman W.A. (1994) Differential regulatory effects of adenosine on cytokine release by activated human monocytes. *J Immunol* **153,** 4159.
12. Parmely M.J., Zhou W.W., Edwards C.K., Borcherding D.R., Silverstein R. & Morrison D.C. (1993) Adenosine and a related carbocyclic nucleoside analogue selectively inhibit tumor necrosis factor-alpha production and protect mice against endotoxin challenge. *J Immunol* **151,** 389.
13. Haskó G., Szabó C., Németh Z.H., Kvetan V., Pastores S.M. & Vizi E.S. (1996) Adenosine receptor agonists differentially regulate IL-10 and TNF production in endotoxemic mice. *J Immunol* **157,** 4634.
14. Szabó C., Salzman A.L. & Ischiropoulos H. (1995) Peroxynitrite-mediated oxidation of dihydrorhodamine 123 occurs in early stages of endotoxic and hemorrhagic shock and ischemia-reperfusion injury. *FEBS Lett* **372,** 229.
15. Southan G.J., Zingarelli B., O'Connor M., Salzman A.L. & Szabó C. (1996) Spontaneous rearrangement of aminoalkylguanidines into mercaptoalkylguanidines – a novel class of nitric oxide synthase inhibitors with selectivity towards the inducible isoform. *Br J Pharmacol* **117,** 619.
16. Wu C.C., Szabó C., Chen S.J., Thiemermann C. & Vane J.R. (1994) Activation of soluble guanylyl cyclase by a factor other than nitric oxide or carbon monoxide contributes to the vascular hyporeactivity to vasoconstrictor agents in the aorta of rats treated with endotoxin. *Biochem Biophys Res Comm* **201,** 436.
17. Zingarelli B., O'Connor M., Wong H., Salzman A.L. & Szabó C. (1996) Peroxynitrite-mediated DNA strand breakage activates poly-ADP ribosyl synthetase and causes cellular energy depletion in macrophages stimulated with bacterial lipopolysaccharide. *J Immunol* **156,** 350.
18. Wise W.C., Cook J.A. & Halushka P.V. (1983) Arachidonic acid metabolism in endotoxin tolerance. *Adv Shock Res* **10,** 131.
19. Nelson H.S. (1982) Beta-adrenergic agonists. *Chest* **82,** 33S.
20. Strassmann G., Patil-Koota V., Finkelman F., Fong M. & Kambayashi T. (1994) Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E_2. *J Exp Med* **180,** 2365.
21. Van der Poll T., Coyle S.M., Barbosa K., Braxton C.C. & Lowry S.F. (1996) Epinephrine inhibits tumor necrosis factor-and potentiates interleukin 10 production during human endotoxemia. *J Clin Invest* **97,** 713.
22. Strassmann G., Patil-Koota V., Finkelman F., Fong M. & Kambayashi T. (1994) Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandine E_2. *J Exp Med* **180,** 2365.
23. Platzer C., Meisel Ch., Vogt K., Platzer M. & Volk H.-D. (1994) Up-regulation of monocytic IL-10 by tumor necrosis factor- and cAMP elevating drugs. *Int Immunol* **7,** 517.
24. Arai T., Hiromatsu K., Kobayashi N. *et al.* (1995) IL-10 is involved in the protective effect of dibutyryl cyclic adenosine monophosphate on endotoxin-induced inflammatory liver injury. *J Immunol* **155,** 5743.
25. Thiemermann C., Wu C.C., Szabó C., Perretti M. & Vane J.R. (1993) Tumour necrosis factor is an endogenous mediator of the induction of nitric oxide synthase in endotoxin shock in the rat. *Br J Pharmacol* **110,** 177.
26. Szabó C., Wu C.C., Mitchell J.A., Gross S.S., Thiemermann C. & Vane J.R. (1993) Platelet-activating factor contributes to the induction of nitric oxide synthase by bacterial lipopolysaccharide. *Circ Res* **73,** 991.
27. Fonteh A.N., Winkler J.D., Torphy T.J., Heravi J., Undem B.J. & Chilton F.H. (1993) Influence of isoproterenol and phosphodiesterase inhibitors on platelet-activating factor biosynthesis in the human neutrophil. *J Immunol* **151,** 339.
28. Bulut V., Severn A. & Liew F.Y. (1993) Nitric oxide production by murine macrophages is inhibited by prolonged elevation of cyclic AMP. *Biochem Biophys Res Comm* **195,** 1134.
29. Feinstein D.L., Galea E. & Reis D.J. (1993) Norepinephrine suppresses inducible nitric oxide synthase activity in rat astroglial cultures. *J Neurochem* **60,** 1945.
30. Koide M., Kawahara Y., Nakayama I., Tsuda T. & Yokoyama M. (1993) Cyclic AMP-elevating agents induce an inducible type of nitric oxide synthase in cultured vascular smooth muscle cells. Synergism with the induction elicited by inflammatory cytokines. *J Biol Chem* **268,** 24959.
31. Imai T., Hirata Y., Kanno K. & Marumo F. (1994) Induction of nitric oxide synthase by cyclic AMP in rat vascular smooth muscle cells. *Clin Invest* **93,** 543.
32. Worthley L.I., Tyler P. & Moran J.L. (1985) A comparison of dopamine, dobutamine and isoproterenol in the treatment of shock. *Int Care Med* **11,** 13.
33. Kilbourn R.G., Cromeens D.M., Chelly F.D. & Griffith O.W. (1994) N^G-methyl-L-arginine, an inhibitor of nitric oxide formation, acts synergistically with dobutamine to improve cardiovascular performance in endotoxemic dogs. *Critical Care Med* **22,** 1835.
34. Pastores S.M., Haskó G., Vizi E.S. & Kvetan V. (1996) The role of vasoactive drugs in the regulation of cytokine production in sepsis. *New Horizons* **4,** 252.