The Influence of Phosphodiesterase Inhibitor, Rolipram, on Hemodynamics in Lipopolysaccharide-Treated Rats

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ABSTRACT—Administration of bacterial endotoxin (lipopolysaccharide, LPS) intravenously has been noted to produce a shock state, which is characterized by hypotension and multi-organ system failure. The aim of the present investigation was to (a) examine the influence of rolipram on hemodynamics, plasma levels of tumor necrosis factor- α (TNF- α) levels, and production of inducible nitric oxide synthase (iNOS) in the lungs, *ex vivo*, in LPS-treated rats, and (b) determine the cardiovascular effects of a selective α_1 -adrenoceptor agonist, methoxamine, in the absence or presence of rolipram in rats treated with LPS. Blood pressure, cardiac index, heart rate and arterial resistance were assessed in Long-Evans rats anesthetized with thiobutabarbital. Administration of LPS to animals resulted in a significant reduction in cardiac index over time. The administration of LPS to rats resulted in a substantial rise in the plasma levels of TNF- α . Furthermore, the injection of LPS resulted in a significant increase in the iNOS activity in the lungs. Pre-treatment with rolipram prevented the decline in cardiac index in animals that received LPS. Infusion of methoxamine into animals injected with rolipram and pre-treated with LPS did not result in significant changes in cardiac index. Pre-treatment with rolipram or dexamethasone in animals injected with LPS significantly prevented the rise in TNF- α when compared to the respective values in vehicle-treated animals. Our present observations support the view that the cardiac index can be maintained in animals treated with LPS independent of iNOS inhibition.

Keywords: Lipopolysaccharide, Hemodynamics, Tumor necrosis factor- α , Nitric oxide synthase, α_1 -Adrenoceptor stimulation

The administration of lipopolysaccharide (LPS) under experimental conditions has been reported to produce hypotension (1). It has also been reported that a reduction in cardiac output is associated with the fall in blood pressure (2, 3). It has been suggested that changes in hemodynamics resulting from the administration of LPS is the result of an over-production of nitric oxide (NO) (4). Evidence in the literature indicates that the administration of LPS does result in the induction of nitric oxide synthase (iNOS) (5, 6). This, reportedly, is responsible for the over-production of NO within the system (1). This increase in NO production has been suggested to result in vascular hyporeactivity, ultimately producing loss of vascular tone and cardiovascular collapse (7–9).

There is evidence in the current literature indicating that administration of LPS results in the release of cytokines such as tumor necrosis factor- α (TNF- α) (6, 10). In addition,

it is believed that TNF- α is one of the factors that activates the process responsible for iNOS, ultimately leading to an over-production of NO in the body (11). Rolipram, a putative selective inhibitor of phosphodiesterase type IV (12), reportedly was able to reduce TNF- α production *in vitro* (13, 14) and *in vivo* (15). Collectively, published data in the literature seems to support the view that in septic shock, cardiovascular collapse results from an increase in plasma TNF- α that eventually results in an over-production of NO which produces vascular hyporeactivity and collapse of the cardiovascular system (9, 16). Recently, it has been suggested that phosphodiesterase type IV inhibitors are capable of inhibiting the expression, and thus reducing the production and release, of this cytokine (17). Thus, the purposes of the present investigation were two-fold: a) to examine the influence of inhibitor of phosphodiesterase type IV, rolipram, on hemodynamics, plasma TNF- α levels and production of iNOS in the lungs, *ex vivo*, in LPS-treated rats, and b) to determine the impact of the administration of a vasoconstrictor, methoxamine (selective

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α_1 -adrenoceptor agonist), on hemodynamics in LPS-treated rats in the absence or presence of rolipram. Furthermore, in an additional series of experiments, a parallel comparison was made between the effects of dexamethasone, a selective inhibitor of iNOS (18), and rolipram on the cardiovascular system in rats treated with LPS.

MATERIALS AND METHODS

Surgical preparation of animals

Male Long-Evans rats (330–360 g) were anesthetized with thiobutabarbital (100 mg/kg) i.p. Catheters (polyethylene tubing; I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the left and right iliac arteries and veins. The catheters inserted into left iliac artery and vein were used for the measurement of blood pressure, and drug/vehicle administrations, respectively, while the catheters inserted into right iliac artery and vein were used for blood withdrawal of radiolabeled microspheres and return of blood samples after each cardiac output measurement, respectively. An additional catheter was inserted into the left ventricle via the right carotid artery for injection of radiolabeled microspheres. The animals were tracheotomized and allowed to stabilize for a period of 1 h while arterial pressure and heart rate were monitored continuously.

All catheters were filled with heparinized saline (25 iu/ml). Body temperature was maintained at $37 \pm 1^\circ\text{C}$ using a heating lamp and monitored using a rectal thermometer. Arterial blood pressure was recorded with a pressure transducer (Model PD23B; Gould Statham, Oxnard, CA, USA) connected to a Gould Universal amplifier and recorder (Model 8188-2202-00; Gould, Ballainvilliers, France). Heart rate was calculated from the blood pressure tracing.

Measurement of cardiac output

This technique has been described in detail elsewhere (19). Briefly, suspensions of microspheres (15- μm -diameter; Mandel, Guelph, Ontario, Canada) labeled with ^{57}Co (20,000–22,000 in 150 μl) were injected into the left ventricle over a period of 10 s. Blood was withdrawn from the right femoral artery at the rate of 0.35 ml/min starting 15 s before microsphere injection using an infusion/withdrawal pump (Model 120; Kd Scientific, Boston, MA, USA) for 1 min. The blood sample and syringes used for injection of microspheres or withdrawal of blood were counted for radioactivity at 80–160 Kev using a dual channel automatic gamma counter (Model 1272; LKB Wallac, Clinic Gamma Counter, Turku, Finland). The withdrawn blood sample was slowly injected back into the animals immediately after counting of radioactivity.

Experimental protocol

Series I: Animals were randomly assigned to two

groups ($n = 5$): saline-treated (Group I: 0.8 ml/kg bolus) and LPS (Group II: 0.8 mg/kg). After the completion of surgery, blood pressure and heart rate were continuously monitored for 60 min, after which each animal received either saline or LPS. Five blood samples (120 μl each) were collected into a pre-chilled syringe containing EDTA to yield a final concentration of 1 mg/ml. After centrifugation, the plasma was frozen and stored at -80°C until it was assayed for TNF- α . The first blood sample was taken just before the administration of saline or LPS and the other four samples were collected at 30, 60, 120 and 180 min post saline or LPS administration. Cardiac output was also measured five times in these groups of animals, the first measurement being just before the administration of saline or LPS and four other measurements thereafter every hour. At the end of each experiment, the lungs were quickly excised, placed in liquid nitrogen and stored at -80°C (Fig. 1A).

Series II: Animals were randomly assigned to four groups ($n = 5$): vehicle-treated (Group III: 2-hydroxypropyl- β -cyclodextrin, 2.0 ml/kg), rolipram-treated (Group IV: 3 mg/kg and Group V: 10 mg/kg), and dexamethasone-treated (Group VI: 5 mg/kg). After the stabilization period, each animal was treated with vehicle or drugs. At 15–20 min post treatment with vehicle/drugs, a blood sample (120 μl) was collected for plasma TNF- α measurements as previously described, and cardiac output was measured. Each animal (Groups III–VI) was then treated with a bolus dose of LPS (0.8 mg/kg). Two more blood samples (120 μl each time) were subsequently collected for plasma TNF- α assessment at 60 and 120 min post-LPS treatment. At 4 h after the administration of LPS, a second cardiac output measurement was made. Subsequently, methoxamine (100 or 300 $\mu\text{g/kg}$ per min) was infused and cardiac output was measured 14–16 min after the start of infusion. In each animal, repeated cardiac output measurements were made during the infusion of each dose of methoxamine. The time allowed between each dose of methoxamine was 15–16 min. At the end of each experiment, the lungs were quickly excised, placed in liquid nitrogen and stored at -80°C (Fig. 1B).

TNF- α assay in plasma

The total TNF- α in plasma was determined by a commercially available colorimetric enzyme linked immunosorbent assay kit (R&D Systems; Minneapolis, MN, USA) for rat TNF- α . The sensitivity of the assay was 6 pg/ml.

NOS assay in lungs

NOS was assessed by measuring the conversion of [^3H]L-arginine to [^3H]L-citrulline as described by Thiernemann et al. (6), with slight modifications. Frozen lungs were homogenized on ice in buffer composed of: 50 mM Tris-HCl, 0.1 mM EDTA, 0.1 mM EGTA, 12 mM

Series I (Groups I and II)

Saline/LPS						
1 st BS CO1	2 nd BS	3 rd BS CO2	4 th BS CO3	5 th BS CO4	CO5	lungs removed
0	30 min	60 min	120 min	180 min	240 min	

Series II (Groups III to VI)

Vehicle/Drugs	LPS			Methoxamine (D1) Methoxamine (D2)		
	1 st BS CO1	2 nd BS	3 rd BS	CO2	CO3	CO4 lungs removed
0	15-20 min	60 min	120 min	240 min		

Fig. 1. Schematic time-bar for experimental protocols in series I and II. Blood sample (BS), cardiac output (CO), methoxamine (D1: 100 $\mu\text{g/kg}$ per min), methoxamine (D2: 300 $\mu\text{g/kg}$ per min).

2-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride (pH 7.4). A 50- μl aliquot of homogenate was incubated in the presence of [^3H]L-arginine/L-arginine (10 μM), NADPH (1.0 mM), calmodulin (10 $\mu\text{g/ml}$), tetrahydrobiopterin (5.0 μM) and Ca^{2+} (2.0 mM) (total volume of 200 μl) at 37°C for 30 min. The reaction was stopped using stop buffer (1.0 ml) of the following composition: 20 mM HEPES, 2.0 mM EDTA and 2.0 mM EGTA (pH 5.5). Each sample was applied to a 2-ml column of Dowex AG 50W-X8 (sodium form) (Bio-Rad Laboratory, Mississauga, Ontario, Canada) and eluted four times with 1.0 ml of stop buffer. Radioactivity in each sample was measured using a scintillation counter (Model LS 3801; Beckman, Irvine, CA, USA). Assays were performed in duplicate in the presence of NADPH to determine constitutive nitric oxide synthase activity, in the absence of NADPH to determine the extent of [^3H]L-citrulline formation independent of NOS, and in a Ca^{2+} -free buffer containing NADPH and EGTA (5 mM) to determine Ca^{2+} -independent (induced) NOS activity. Protein concentration was measured using Bradford's method (20).

Chemicals

Rolipram, thiobutabarbital, L-arginine and 2-hydroxypropyl- β -cyclodextrin were purchased from Research Biochemical International (Natick, MA, USA). All other fine chemicals were purchased from Sigma Chemical Company (Oakville, Ontario, Canada). Rolipram was dissolved in 2-hydroxypropyl- β -cyclodextrin and this was the vehicle

used in the experiments.

Analyses of data

Mean arterial blood pressure (mmHg) is reported as diastolic blood pressure plus one third of the difference between systolic and diastolic blood pressures. Cardiac output (ml/min) was calculated as the rate of withdrawal of blood multiplied by total injected cpm divided by cpm in withdrawn blood. The cardiac index is the cardiac output divided by body weight. Arterial resistance (mmHg $\cdot$ min/ml per kg) was obtained by dividing the mean blood pressure by the cardiac index.

The data were analyzed by one-way analysis of variance with repeated measures for comparison. Duncan's multiple range test was used for comparison between means. In all cases, a probability of error of less than 0.05 was selected as the criterion for statistical significance.

RESULTS

There were no significant changes in hemodynamic values (cardiac index, mean blood pressure, arterial resistance and heart rate) over time after the administration of saline (Figs. 2 and 3). In addition, plasma levels of TNF- α did not change following the administration of saline (Fig. 4).

The administration of LPS to animals resulted in a significant reduction in cardiac index over time (Fig. 2A). At 4 h post-LPS treatment, the cardiac index was reduced by over 27% when compared to the cardiac index measured

Fig. 2. The effects of treatment of anesthetized rats with saline (0.8 ml/kg) (closed circles) or LPS (0.8 mg/kg) (opened circles) on cardiac index (A) and heart rate (B) overtime. First measurements taken at time zero before the administrations of either saline or LPS. Data represent the mean of five experiments $\pm$ S.E.M. *Significantly different from the respective values in saline-treated animals, $P < 0.05$.

prior to the administration of LPS. Although arterial resistance and heart rate did increase over time in LPS-treated animals, these changes were found to be insignificant (Figs. 2B and 3B). There were no appreciable changes in mean blood pressure in animals treated with LPS over time (Fig. 3A). The administration of LPS to rats resulted in a substantial rise in the plasma levels of TNF- α (Fig. 4). The time course for the peak and subsequent decline in plasma concentrations of TNF- α was within the 180 min time frame after the injection of LPS (Fig. 4). In the present investigation, the peak concentration of TNF- α detected in the plasma at 120 min post-LPS injection was 190 times that of control level prior to LPS administration. Furthermore, the injection of LPS resulted in a significant increase in the iNOS activity in the lungs of animals ex vivo (Table 1).

Fig. 3. The effects of treatment of anesthetized rats with saline (0.8 ml/kg) (closed circles) or LPS (0.8 mg/kg) (opened circles) on blood pressure (A) and arterial resistance (B) overtime. First measurements taken at time zero before the administrations of either saline or LPS. Data represent the mean of five experiments $\pm$ S.E.M.

Fig. 4. The effects of treatment of anesthetized rats with saline (0.8 ml/kg) (closed bars) or LPS (0.8 mg/kg) (cross-hatched bars) on plasma concentration of TNF- α overtime. First measurements were taken at time zero before the administrations of either saline or LPS. Data represent the mean of five experiments $\pm$ S.E.M. *Significantly different from the respective values in saline-treated animals, $P < 0.05$.

Table 1. Values of enzymatic activity of inducible nitric oxide synthase (iNOS) and constitutive (cNOS) forms of nitric oxide synthase (pmol/mg protein per min) in lungs of various groups of animals treated with saline (Group I: 0.8 ml/kg), LPS (Group II: 0.8 mg/kg), and vehicle (Group III: 2 ml/kg), rolipram (Group IV: 3 mg/kg or Group V: 10 mg/kg) and dexamethasone (Group VI: 5 mg/kg) prior to treatment with LPS (0.8 mg/kg)

Groups	I	II	III	IV	V	VI
iNOS	0.3 ± 0.04	15.0 ± 3.0 ^{ab}	13.2 ± 1.6 ^{ab}	19.0 ± 3.0 ^{ab}	17.0 ± 4.0 ^{ab}	4.30 ± 0.7 ^a
cNOS	0.4 ± 0.1	2.8 ± 0.8 ^a	1.8 ± 0.6 ^a	1.8 ± 0.6 ^a	2.6 ± 0.8 ^a	0.80 ± 0.2

Each value represents the mean of five experiments ± S.E.M. ^aSignificantly different from group I, $P < 0.05$. ^bSignificantly different from group VI, $P < 0.05$.

Effects of rolipram and dexamethasone on hemodynamics in LPS-treated rats

Prior to the administration of LPS, pre-treatment of animals with rolipram (3 and 10 mg/kg) or dexamethasone (5 mg/kg) did not result in significant changes to cardiac index, mean blood pressure, arterial resistance or heart rate when compared to animals that were treated with vehicle (Table 2). There were no significant changes detected in

mean blood pressure, arterial resistance or heart rate in animals that were pretreated with vehicle, rolipram or dexamethasone after LPS injection (Table 2). In animals that were pre-treated with vehicle, LPS-treatment reduced cardiac index by over 25% when compared to cardiac index measured prior to the administration of LPS (Table 2). In contrast, pre-treatment with rolipram (10 mg/kg) and dexamethasone prevented the decline in cardiac index in ani-

Table 2. Hemodynamic changes in various groups of animals treated with LPS (0.8 mg/kg) in the absence or presence of vehicle (2 ml/kg), rolipram (3 or 10 mg/kg) or dexamethasone (Dexa, 5 mg/kg)

(Groups)	Pre-LPS	Post-LPS (+ 4 h)	Methoxamine (100 µg/kg per min)	Methoxamine (300 µg/kg per min)
Cardiac index (ml/min per kg)				
Vehicle (III)	295 ± 22.0	220 ± 10.0 ^a	236 ± 11.0	200 ± 8.0
Rolipram (IV)	334 ± 17.0	258 ± 12.0	258 ± 20.0	226 ± 7.0
Rolipram (V)	342 ± 15.0	280 ± 21.0 ^c	288 ± 29.0 ^d	290 ± 27.0 ^{cd}
Dexa (VI)	302 ± 27.0	318 ± 36.0 ^c	192 ± 10.0 ^b	216 ± 20.0 ^b
Mean blood pressure (mmHg)				
Vehicle (III)	102 ± 2.0	89 ± 6.0	99 ± 4.0 ^d	107 ± 3.0 ^b
Rolipram (IV)	96 ± 3.0	81 ± 6.0	84 ± 6.0 ^d	85 ± 6.0 ^d
Rolipram (V)	103 ± 5.0	83 ± 6.0	88 ± 5.0 ^d	92 ± 4.0 ^d
Dexa (VI)	117 ± 5.0	107 ± 7.0	114 ± 7.0 ^b	116 ± 6.0 ^b
A _R (mmHg · min/ml per kg)				
Vehicle (III)	0.38 ± 0.03	0.39 ± 0.03	0.42 ± 0.02 ^d	0.51 ± 0.02 ^{bd}
Rolipram (IV)	0.29 ± 0.01	0.31 ± 0.02	0.33 ± 0.03 ^d	0.38 ± 0.04 ^d
Rolipram (V)	0.30 ± 0.02	0.30 ± 0.03	0.31 ± 0.02 ^d	0.33 ± 0.02 ^d
Dexa (VI)	0.40 ± 0.03	0.35 ± 0.02	0.61 ± 0.07 ^b	0.56 ± 0.05 ^b
Heart rate (beats/min)				
Vehicle (III)	344 ± 11	430 ± 28	392 ± 23	382 ± 19
Rolipram (IV)	424 ± 13	462 ± 13	460 ± 12	456 ± 17
Rolipram (V)	398 ± 19	445 ± 16	428 ± 16	430 ± 24
Dexa (VI)	332 ± 14	380 ± 12	388 ± 24	372 ± 20

The values represents the mean of five experiments ± S.E.M. ^aSignificantly different from pre-LPS within the same group, $P < 0.05$. ^bSignificantly different from post-LPS-treatment (+ 4 h) within the same group, $P < 0.05$. ^cSignificantly different from the respective values in the vehicle-treated group, $P < 0.05$. ^dSignificantly different from the respective values in the dexamethasone-treated group, $P < 0.05$.

mals that received LPS (Table 2). Cardiac index was significantly higher in rolipram (10 mg/kg) and dexamethasone treated animals when compared to vehicle-treated animals injected with LPS (Table 2).

Effects of α_1 -adrenoceptor stimulation on hemodynamics in LPS-treated rats

Infusion of methoxamine (100 and 300 μ g/kg per min) did not appear to have any significant effects on cardiac index in either vehicle-treated or rolipram-treated rats when compared to the respective values within each group prior to infusion of the α_1 -adrenoceptor agonist 4-h post-LPS treatment (Table 2). In contrast, the administration of methoxamine (100 and 300 μ g/kg per min) to dexamethasone-treated rats produced a significant reduction in cardiac index when compared to the respective values prior to methoxamine infusion in dexamethasone-treated rats 4-h post-LPS treatment (Table 2). Administration of methoxamine did not significantly affect mean blood pressure and arterial resistance in rolipram treated animals when compared to respective values prior to infusion of methoxamine 4-h post-LPS treatment. However, in vehicle-treated animals, infusion of methoxamine significantly increased blood pressure and arterial resistance at the higher but not lower dose when compared to the respective values prior to the administration of α_1 -adrenoceptor agonist 4-h post-LPS treatment (Table 2). Moreover, administration of methoxamine also significantly increased mean blood pressure and arterial resistance at both dose levels in dexamethasone-treated rats when compared to the respective values prior to the infusion of methoxamine 4-h post-LPS treatment (Table 2). Stimulation of α_1 -adrenoceptors did not result in any significant changes in heart rate in any group of animals (Table 2).

Effects of rolipram and dexamethasone on plasma levels of TNF- α

Plasma concentrations of the cytokine TNF- α were significantly elevated in all groups subsequent to injection

of LPS (Table 3). In animals that had received rolipram (10 mg/kg) or dexamethasone, significantly lower plasma levels of TNF- α were detected at both 60- and 120-min post-LPS treatment when compared to the respective values in vehicle-treated animals that had also received LPS (Table 3). However, even though plasma levels of TNF- α were lower in LPS-treated rats administered the lower dose of rolipram (3 mg/kg) when compared to vehicle-treated animals, differences in plasma levels of TNF- α were not found to be significant (Table 3).

Effects of rolipram and dexamethasone on NOS activity in lungs in LPS-treated animals

The activity of NOS was elevated significantly following the treatment of animals with LPS (Table 1). Pre-treatment of animals with rolipram or vehicle did not affect NOS activity in animals that had received LPS. However, dexamethasone pre-treatment significantly reduced iNOS activity in the lungs of animals that had received LPS when compared to vehicle-treated animals that had also received LPS (Table 1).

DISCUSSION

In the present study, we found that treatment of animals with LPS results in a progressive decline in the cardiac index over time. There was also an increase in circulating levels of TNF- α in plasma, as well as an induction of NOS activity in lungs ex vivo. Pre-treatment of animals with a putative selective phosphodiesterase type IV inhibitor, rolipram, or synthetic glucocorticoid, dexamethasone, prevented the decline in cardiac output due to LPS. Moreover, both rolipram and dexamethasone also significantly reduced the rise in plasma levels of TNF- α that had resulted from injection of LPS. However, it would seem that only dexamethasone but not rolipram was able to significantly inhibit iNOS activity in lungs of animals that had received LPS.

Administration of LPS to animals reportedly produces

Table 3. Plasma TNF- α values in various groups of animals treated with LPS (0.8 mg/kg) in the absence or presence of vehicle (2 ml/kg), rolipram (3 or 10 mg/kg) or dexamethasone (5 mg/kg)

(Groups)	TNF- α (ng/ml)		
	Pre-LPS treatment	1-h Post-LPS	2-h Post-LPS
Vehicle (III)	0.004 $\pm$ 0.001	6.0 $\pm$ 2.1 ^a	14.8 $\pm$ 3.8 ^a
Rolipram (IV)	0.008 $\pm$ 0.004	3.4 $\pm$ 0.9 ^a	9.6 $\pm$ 3.5 ^a
Rolipram (V)	0.003 $\pm$ 0.001	3.1 $\pm$ 1.0 ^{ab}	4.0 $\pm$ 1.2 ^{ab}
Dexamethasone (VI)	0.008 $\pm$ 0.005	0.8 $\pm$ 0.2 ^{ab}	0.9 $\pm$ 0.3 ^{ab}

The values represents the mean of five experiments $\pm$ S.E.M. ^aSignificantly different from the respective values pre-LPS treatment, $P < 0.05$. ^bSignificantly different from the respective values in group III, $P < 0.05$.

hypotension (1). More recently, it was reported that a single bolus injection of LPS to the rat resulted in a progressive reduction in cardiac output over time. Associated with this reduction in cardiac output was a reduction in mean circulatory filling pressure, an index of total body's venous tone (2). Reportedly, treatment of rats with LPS did not appear to significantly affect arterial resistance or resistance to venous return (2). However, endotoxic shock has been reported to lead to an impairment of portal venous flow with increased portal venous resistance causing an increase in splanchnic blood pooling and subsequent decrease in venous return and thus cardiac output in anesthetized pigs (21). Taken together, the evidence would suggest that the reduction in cardiac output in endotoxic shock is, in part, due to a reduction in venomotor tone. In the present investigation, we find that pretreatment with LPS resulted in a reduction in cardiac output without any significant change in arterial resistance. In addition, there were no significant changes in heart rate. Thus, it is possible that the reduction in cardiac output observed in animals treated with LPS in the present investigation was the result of a reduction in venous return (2, 21). Moreover, it is possible that treatment with rolipram and dexamethasone augmented venous return, thereby resulting in the maintenance of cardiac output in LPS-treated rats. This idea, however, remains to be tested.

It is evident from the present investigation that pretreatment with rolipram in LPS-treated rats did not result in inhibition of iNOS but that cardiac index in these animals was maintained. This could be indicative of the fact that the reduction in cardiac index following the administration of LPS is not necessarily due to an over-production of NO. Moreover, there is evidence to indicate that treatment of patients in a state of septic shock with a non-selective inhibitor of NOS, N^G -nitro-L-arginine methyl ester, results in increased mean arterial pressure and arterial resistance with concomitant reduction in cardiac output (16). Furthermore, under experimental conditions, treatment of rats with methylene blue in endotoxaemia results in increased arterial resistance and reduction in cardiac output (3). Collectively, the evidence in the literature seems to suggest that inhibition of NOS and/or NO pathways *per se* does not improve cardiac output in endotoxemia and that cardiac output can be maintained independently of iNOS inhibition in endotoxemia.

It is also evident from the present investigation that infusion of methoxamine into animals injected with rolipram and pre-treated with LPS did not result in a significant increase or decrease in either cardiac index or heart rate. In contrast, in animals treated with dexamethasone and pre-treated with LPS, infusion of methoxamine significantly reduced cardiac index. This was predominantly due to substantial increases in arterial resistance. Since, dexameth-

asone did inhibit iNOS, vascular reactivity to methoxamine was not reduced and thus an increase in arterial resistance occurred following infusion with methoxamine. It is evident that the reduction in cardiac output in dexamethasone treated rats was not the result of significant changes in the heart rate. We observed a similar pattern with infusion of noradrenaline in animals that were hemorrhaged but were pre-treated with the same dose of dexamethasone (22). In our previous investigation, dexamethasone also inhibited iNOS following hemorrhage in thiobutabarbital-anesthetized rats (22). It is recognized that under normal circumstances an increase in arterial resistance (afterload) can result in a reduction in cardiac output which occurs due to increased impedance to flow (23). Moreover, a reduction in arterial resistance can result in the opposite effect and thus increase cardiac output under normal, as well as, pathophysiological conditions (24, 25).

Certainly, previous investigators have reported that treatment of animals with LPS resulting in endotoxemia leads to an induction of NOS secondary to an elevation of circulating levels of TNF- α . However, the decline in cardiac output following administration of LPS does not correlate well with induction of iNOS. In the present investigation, we find that cardiac index is reduced within an hour following injection of LPS. Similar observations were made by other investigators (2, 4). There is clearly an induction of iNOS in a time-dependent manner in many organs such as the liver, spleen, kidney and heart in endotoxemia (1). Moreover, this time-dependent increase in calcium-independent NOS activity appears to be most pronounced in the lungs (1). The role of nitric oxide in lung injury remains unclear, and both beneficial and detrimental roles have been described. However, it has been reported that mice deficient in the iNOS gene are more resistant to LPS-induced acute lung injury than the wild-type mice (26).

It seems that iNOS begins to have a substantial impact on the cardiovascular system within approximately 3 h post-LPS injection (1). It is evident from the present investigation that the peak concentration of TNF- α in plasma does not occur until at least 120 min post-LPS injection. Such an observation is supported by other reports (27, 28). However, it is also apparent that the concentration of TNF- α is significantly elevated 1 h post-LPS, and certainly this could trigger other processes that may have an immediate impact on cardiac output and the circulatory system. Collectively, evidence in the literature and our present findings indicate that the initial negative impact of LPS on the cardiovascular system, and especially on cardiac output, may be independent of iNOS activity.

The possibility that LPS can produce induction of NOS independent of TNF- α needs to be considered. Recently, it was reported that *in vitro* exposure of human colon epithelial cells to *Escherichia coli* results in induction of NOS

(29). In addition, there is evidence in the literature suggesting that LPS is able to induce NOS in cytokine receptor-deficient mice (30). Moreover, in a recent report, it was demonstrated that endogenous TNF- α is not required for LPS-mediated induction of NO in rats (31). In contrast to these observations, earlier studies had indicated that pre-treatment with TNF monoclonal antibodies prevented symptoms associated with shock in anesthetized baboons (32). Recently, Thiernemann and associates (6) had reported that pretreatment of rats with monoclonal antibody for TNF- α prevented the induction of NOS activity in the lung of animals that were subsequently treated with LPS. However, in the present investigation, we found that a significant reduction in the circulating levels of TNF- α in animals treated with rolipram did not prevent induction of NOS in the lungs of animals treated with LPS. Our present findings support the view that perhaps mediators other than TNF- α may contribute to induction of NOS in animals treated with LPS (11). Certainly, the possibility that LPS may directly activate pathways that result in induction of NOS cannot be ruled out at present.

In summary, the findings of the present study indicate that treatment of animals with LPS results in a reduction in cardiac index, elevated plasma levels of TNF- α , as well as, induction of NOS in lungs. Moreover, pre-treatment with rolipram and dexamethasone prevents the decline in cardiac index and significantly reduces the rise in plasma levels of TNF- α as a result of LPS. However, dexamethasone but not rolipram inhibits induction of NOS. The results appear to support the view that induction of NOS may occur, in part, independent of TNF- α . Furthermore, circulating TNF- α may affect cardiac output independent of iNOS.

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