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Different mechanism of LPS-induced vasodilation in resistance and conductance arteries from SHR and normotensive rats

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1 The direct and endothelium-dependent effects of lipopolysaccharide (LPS) were investigated on resistance and conductance arteries from normotensive Wistar (NWR) and spontaneously hypertensive (SHR) rats.

2 In both NWR and SHR, LPS induced dose-dependent relaxations of the mesenteric vascular bed, which were inhibited by L-NNA in SHR but not in NWR. Iberiotoxin (IBTX) inhibited the responses to LPS in both groups, indicating the participation of high conductance Ca^{2+}-dependent K^+ channels.

3 In mesenteric artery rings, the resting membrane potentials and the hyperpolarizing responses of NWR to LPS did not differ in endothelized and denuded preparations but L-NNA inhibited the responses only in endothelized rings. These responses were reduced by bosentan, suggesting that endothelin release may mask a possible hyperpolarizing response to LPS. The hyperpolarizing responses to LPS were blocked by IBTX in both endothelized and de-endothelized NWR rings. In the SHR only intact rings showed hyperpolarization to LPS, which was inhibited by IBTX and by L-NNA.

4 In SHR aortic endothelized or denuded rings, LPS induced hyperpolarizing responses which, in endothelized rings, were partially blocked by L-NNA, by IBTX or by glibenclamide, but totally abolished by IBTX plus glibenclamide. No response to LPS was observed in NWR aortic rings.

5 Our results indicate that LPS activates large conductance Ca^{2+}-sensitive K^+ channels located in the smooth muscle cell membrane both directly and indirectly, through NO release from the endothelium in NWR, whereas NO is the major mediator of the LPS responses in SHR resistance vessels.

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Abbreviations: EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; IBTX, iberiotoxin; L-NNA, N^{ω}-nitro-L-arginine; LPS, lipopolysaccharide; NO, nitric oxide; NWR, normotensive Wistar rat; ODQ, 1H-(1,2,4)oxadiazolo(4,3-a) quinoxalin-1-one; RMP, resting membrane potential; SHR, spontaneously hypertensive rat

Introduction

Sepsis and septic shock are very serious clinical problems, frequently associated with a high mortality. The hypotension and vascular hyporeactivity that occur during sepsis are responsible for the progressive reduction in tissue perfusion, which often culminates in the multiple organ distress syndrome and death (Balk, 2000).

Lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria (Bone, 1994), also known as endotoxin, induces vasorelaxation and when injected in experimental animals. These responses are thought to be mediated by several substances that are released into the circulating blood after LPS administration, such as interleukins, tumour necrosis factor, leukotrienes, thromboxane A_2, platelet activating factor and bacterial exo- or endotoxins (Bone, 1991). However, the overproduction of nitric oxide (NO) is generally believed to be the most important factor responsible for the systemic vasodilatation induced by LPS administration (Mitolo-Chieppa *et al*., 1996; Yen *et al*., 1997; Hoang & Mathers, 1998; Chen *et al*., 1999). Furthermore, nitrovasodilators are potent activators of Ca^{2+}-dependent K^+ channels in vascular smooth muscle cells, which contribute to endotoxin-induced hypotension (Hall *et al*., 1996; Chen *et al*., 2000; Taguchi *et al*., 1996; Bolotina *et al*., 1994).

The spontaneously hypertensive rat (SHR) may be a good model to study endotoxin-induced septic shock, since it should be more resistant to the hypotensive effect induced by the toxin. However controversial results were obtained with this model. Bernard *et al*. (1998) found that the SHR has a greater ability to resist endotoxin shock than normotensive controls, which was not attributed to the hypertensive state, since this strain seems to have its own genetically determined immunoinflammatory response. On the other hand, Yen *et al*. (1997) observed a shorter survival time in SHR after LPS injection in comparison with normotensive rats. The higher

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mortality of SHR due to LPS was associated with overproduction of inducible NO synthase, which was harmful to these animals, since they already have higher levels of plasma nitrites (Wu & Yen, 1999).

The SHR was shown to present altered vascular responses to various stimuli. For example, in the normotensive rat mesenteric artery apamin-sensitive Ca^{2+}-dependent K^+ channels are the main ion channels activated by the endothelium-dependent hyperpolarizing factor (EDHF) in response to acetylcholine (Chen & Cheung, 1997), whereas these channels seemed to be impaired in mesenteric vessels from SHR (Borges *et al*., 1999). This is probably responsible for the enhanced response to vasoconstrictor stimuli observed in this strain (Feres *et al*., 1998).

On the other hand, England *et al*. (1993) and Liu *et al*. (1997) reported an increase in large-conductance Ca^{2+}-sensitive K^+ channels in aortic muscle cells from SHR, when compared with their normotensive controls. In contrast, in aorta from normotensive rats, ATP-sensitive potassium channels seem to be the only mediators of the relaxant response to α_2-adrenoceptors (Fauaz *et al*., 2000).

Few studies have investigated the direct effect of LPS in SHR vascular smooth muscles (Szabó *et al*., 1997; Wu *et al*., 1994). Therefore, the aim of this study was to analyse the direct effects of LPS on isolated SHR and NWR vascular smooth muscles, with or without endothelium, and in the perfused mesenteric bed, in the absence of plasma substances known to be released *in vivo* by LPS in experimental animals. In this study we used preparations representative of resistance (mesenteric vascular bed), conductance (aortic artery rings) and intermediate (mesenteric artery rings) vessels.

Methods

Animals

Experiments were carried out using male Okamoto & Aoki (1963) spontaneously hypertensive rats (SHR) and normotensive Wistar rats (NWR) from the Wistar Institute, Philadelphia, PA, U.S.A., inbred at Escola Paulista de Medicina, SP, Brazil. The rats were 20–30 weeks old and weighed 250–350 g. Some animals were decapitated to remove their mesenteric vascular bed, which was dissected away from the intestine for perfusion pressure measurements. Others were decapitated to remove the superior mesenteric arteries and the thoracic aorta, which were cleaned of adherent connective tissue and cut into rings (3–4 mm length), for tension and electrophysiological measurements. Care was taken to ensure that the endothelial layer was not damaged during tissue preparation. All procedures complied with the norms of the Ethics Committee for Research of the São Paulo Hospital, Federal University of São Paulo.

Mesenteric vascular bed

Mesenteric vascular bed preparations were set up as previously described (McGregor, 1965; Ross, 1972). Under ether anaesthesia, the abdomen was opened and the pancreatic-duodenal, ileo-cholic and cholic branches of the superior mesenteric artery were tied. The superior mesenteric artery was separated from surrounding tissues in the region of the aorta and a polyethylene cannula (PE 50) was inserted distally into the artery at its origin from the abdominal aorta. The connections of the superior mesenteric plexus to the coeliac ganglia were severed and the intestine was removed by cutting close to the intestinal border of the mesentery. The mesenteric bed was perfused at a constant flow of 4.0 ml min^{-1}, using a peristaltic pump (Model 2115, LKB-Multiperpex), with Krebs solution of the following composition (mM): NaCl 118; KCl 5.0; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $NaHCO_3$ 15.5; $CaCl_2$ 2.0; glucose 11.0. The solution was bubbled with a 5%CO_2–95%O_2 gas mixture and maintained at pH 7.4 and 37°C. The perfusion pressure was monitored with pressure transducers (P-1000, Narco Bio-Systems) connected to a physiograph (DMP-4B, Narco Bio-Systems) and the pH was monitored continuously with a pH meter (E350B, Metrohm) by means of a glass electrode inserted in the perfusion system. After a 20-min period of stabilization, perfusion pressure was raised to *c.a*. 150 mmHg by addition of noradrenaline (6 μM) to the Krebs solution, in the absence or in the presence of the inhibitor of nitric oxide synthase N^{ω}-nitro-L-arginine (L-NNA, 50 or 500 μM), or in the presence of iberiotoxin (IBTX 10 nM), an inhibitor of the large conductance calcium sensitive potassium channel. Experiments were also conducted in the presence of the NO-sensitive guanylyl cyclase inhibitor 1H-(1,2,4)oxadiazol(4,3-a) quinoxalin-1-one (ODQ 1 μM). After the pressure reached a steady level (140–160 mmHg), in either NWR or SHR preparations, concentration-response curves for the vasorelaxant effect of LPS (0.1–1.0 mg) were obtained by injecting 0.1 ml of solutions with the appropriate concentrations, at 15-min intervals, during the noradrenaline-induced tone. Acetylcholine (2 μg) typically reduced perfusion pressure, indicating the preservation of the endothelial cells (Furchgott & Cherry, 1984).

Membrane potential

The superior mesenteric arterial rings and thoracic aorta were placed in a 2-ml perfusion chamber and superfused at a rate of 3 ml min^{-1}, with the respectives Krebs solutions (in mM): NaCl 118; KCl 5.0; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $NaHCO_3$ 15.5; $CaCl_2$ 2.0; glucose 11.0 (for the mesenteric rings) and NaCl 122; KCl 5.9; $MgCl_2$ 1.25; $NaHCO_3$ 15; glucose 11; $CaCl_2$ 1.25 (for the aortic rings), pH 7.4, 37°C, bubbled with the mixture 5% CO_2–95%O_2. Micropipettes (borosilicate glass capillaries 1B120F-6, World Precision Instruments), were made by means of a horizontal puller (Model PN-3, Narishige, Tokio, Japan) and filled with 2 M KCl (tip resistance 20–40 MΩ and tip potential <6 mV). The microelectrodes were mounted in Ag/AgCl half-cells on a micromanipulator (Leitz, Leica) and connected to an electrometer (Intra 767, WPI). The superior mesenteric arterial rings and thoracic aorta, with and without endothelium, were initially equilibrated for 2 h under an optimal resting tension of 1.0 g, and the impalements of the smooth muscle cells were made from the adventitial side. The electrical signals were continuously monitored on an oscilloscope (Model 54645A, Hewlett Packard) and recorded in a potentiometric chart recorder (Model 2210, LKB-Produkter AB). The successful implantation of the electrode was evidenced by a sharp drop in voltage upon entry into a cell, a stable potential ($\pm$3 mV) for at least 1 min after

impalement, a sharp return to zero upon exit, and minimal change (<10%) in microelectrode resistance after impalement.

Measurements of membrane potential of mesenteric rings and thoracic aorta were obtained in Krebs solution before and after stimulation of the vessels with LPS (10 μg ml^{-1}), in the presence or in the absence of L-NNA (50 μM), or IBTX (10 nM), or glibenclamide (1 μM) or bosentan (1 or 3 μM). The time of contact of the drugs with the preparations before the impalements was 10 min. Cumulative dose-response curves were obtained by measuring the membrane potential during a 30-min interval after each stepwise increase in the LPS concentration and 10-min incubation at the new concentration.

The presence of a functional endothelium was tested in all preparations by checking whether acetylcholine induced hyperpolarization of the preparations, a response which is characteristic for vessels with an intact endothelium (Furchgott, 1981).

Drugs

Noradrenaline hydrochloride, L-NNA, IBTX, acetylcholine chloride (ACh), glibenclamide, lipopolysaccharide (LPS, from Escherichia coli Serotype 0111:B4) and ODQ were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Bosentan was a gift from Dr Martine Clozel, Actelion, Basel, Switzerland. The inorganic salts were products of the highest analytical grade from Merck Darmstadt.

Statistical analysis

All data are expressed as means ± s.e.mean with the number of animals in parentheses. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test in the case of pairwise comparisons between groups. When the data consisted of repeated observations at successive time points, ANOVA for repeated measurements was applied to determine differences between groups. Where more than one impalement was made on the same ring from the same rat, the measurements were averaged and considered as $n=1$. Differences were considered significant when $P<0.05$.

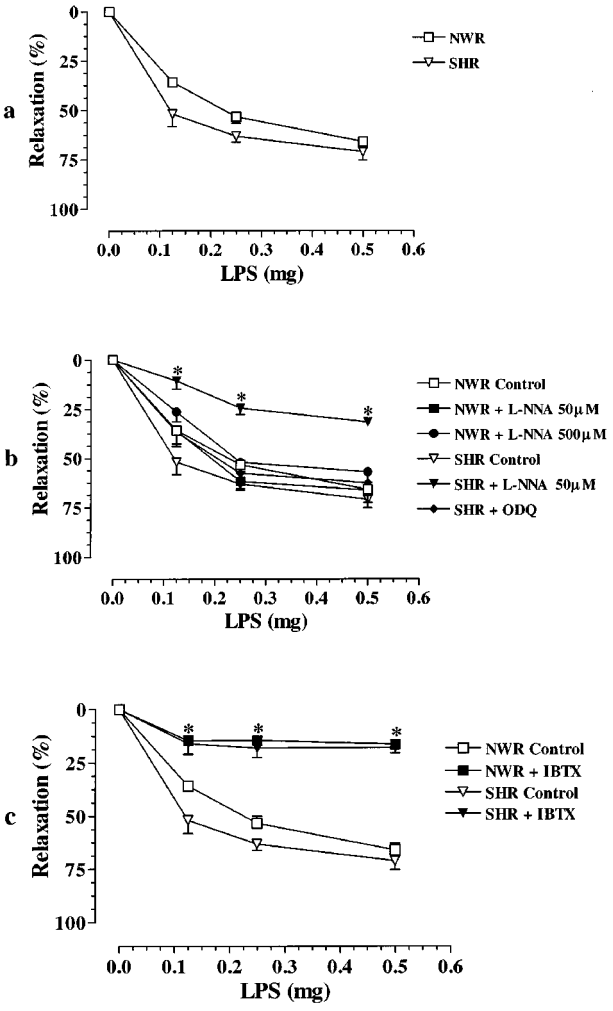

Figure 1 Dose-response curves for the vasorelaxation induced by LPS in the perfused mesenteric vascular bed from NWR and SHR in the absence (a) and in the presence of 50 μM or 500 μM L-NNA or 1 μM ODQ (b), or 10 nM IBTX (c). The relaxations are expressed as percentages of the initial tone maintained by perfusion with 6 μM noradrenaline. Symbols and error bars indicate means and s.e.means; $n=7-10$ in each group. *$P<0.05$ versus respectives controls (Newman-Keuls test).

Results

Relaxant responses of the mesenteric vascular bed to LPS

To study the direct effect of LPS on resistance vessels, we chose the isolated mesenteric vascular bed, which plays an important role in blood pressure maintenance (Cristensen & Mulvany, 1993).

LPS induced similar concentration-dependent relaxant responses in NWR and in SHR preparations pre-contracted by noradrenaline, and no significant difference was observed in the amplitude of the responses in the two strains (Figure 1a).

To evaluate the participation of NO in the responses to LPS, dose-response curves were performed in the presence of 50 μM L-NNA, in both strains. A significant inhibition was observed only in SHR preparations (Figure 1b), indicating the participation of the endothelium-derived relaxing factor (EDRF) in the relaxant response of SHR but not NWR preparations. To further ascertain that NO is not involved in the response to LPS in NWR, the dose-response curves to LPS were also done in the presence of 500 μM L-NNA. No inhibition was observed (Figure 1b), ruling out the participation of NO in the NWR response. To verify whether the relaxant response to LPS in SHR is induced by NO or through the guanylate cyclase pathway, dose-response curves to LPS in SHR preparations were done in the presence of 1 μM ODQ. No inhibition was observed (Figure 1b), which indicates that NO is the mediator of this response.

The role of potassium channels was investigated by performing LPS dose-response curves in the presence of potassium channel inhibitors. IBTX, an inhibitor of large-conductance Ca^{2+}-dependent K^+ channels, blocked the

responses in NWR as well as in SHR vascular beds (Figure 1c). Inhibitors of low-conductance Ca^{2+}-dependent (apamin) or ATP-dependent (glibenclamide) K^+ channels did not affect the responses in preparations of either strain (results not shown).

Membrane potential responses to LPS in mesenteric artery rings

Intracellular recordings were used to measure the membrane potential of smooth muscle cells through impalements of microelectrodes from the adventitial side of rings from superior mesenteric arteries. The resting membrane potential of NWR rings with or without endothelium did not differ, and similar hyperpolarizing responses to LPS were observed in the two cases (Table 1). To determine whether these responses could be due to the release of EDRF, the effect of L-NNA on the hyperpolarizing response to LPS was tested. L-NNA inhibited the responses in NWR rings with endothelium (Figure 2a), but not in de-endothelized rings (Figure 2b). However, the hyperpolarizations induced in NWR rings, with or without endothelium, were both blocked by 10 nM IBTX (Figure 2a,b), suggesting that LPS may act by opening Ca^{2+}-dependent K^+ channels in the smooth muscle.

The unexpected lack of hyperpolarization response to LPS in the presence of L-NNA in endothelized NWR rings might be due to endothelin release by LPS. To investigate this possibility, the effect of bosentan, an ETA/ETB antagonist, was studied. Figure 3 shows that the inhibitory effect of L-NNA on the response to LPS is reduced by bosentan, suggesting that the release of endothelin by LPS may mask a possible hyperpolarizing response to the toxin.

Although the resting membrane potential of SHR preparations was not significantly different in rings with or without endothelium (Table 1), the hyperpolarizing responses to LPS were only observed in endothelized rings (Figure 4a), being inhibited both by L-NNA and by IBTX (Figure 4b).

Membrane potential responses to LPS in aortic rings

It has been shown that SHR aortic smooth muscle cell membranes present an increased number of Ca^{2+}-sensitive K^+ channels when compared to normotensive controls (Liu *et al*., 1997; England *et al*., 1993). We used this conductance artery as a model for comparison with the results obtained in mesenteric arteries.

LPS showed no hyperpolarizing effect in NWR rings, with or without endothelium (Figure 5).

In SHR aortic rings with endothelium a significant depolarization was observed by addition of IBTX. In these preparations the hyperpolarization induced by LPS was partially, but significantly, inhibited by IBTX or L-NNA or glibenclamide, but was totally inhibited by glibenclamide plus IBTX (Figure 6a).

In de-endothelized SHR rings IBTX, but not L-NNA, also caused a significant depolarization. In these preparations, the hyperpolarizing effect of LPS was similar to that observed in the rings with endothelium (Figure 6b). This response was not affected by L-NNA, but was totally inhibited by IBTX.

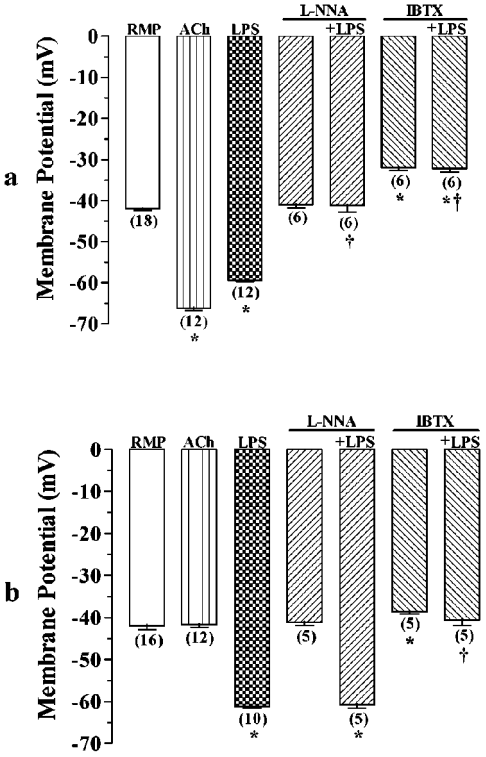

Figure 2 Membrane potentials measured in NWR mesenteric artery rings with (a) or without (b) endothelium. The presence of endothelium is indicated by the response to 10 μM acetylcholine (ACh). The resting membrane potential (RMP) and the effects of 10 μg ml^{-1} LPS in the absence and in the presence of 50 μM L-NNA or 10 nM IBTX are shown. For each mesenteric ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5 to 12 cells were impaled, and the averages of the respective measurements were used to obtain the means ± s.e.means. *$P<0.05$ versus respective RMP. †$P<0.05$ versus the response to LPS (Newman-Keuls test).

Table 1 Effect of LPS (10 μg ml^{-1}) on the membrane potential (in mV) of mesenteric arteries and aortic rings with and without endothelium

	Mesenteric arteries				*Aortic rings*			
	With endothelium		*Without endothelium*		*With endothelium*		*Without endothelium*	
	RMP	*LPS*	*RMP*	*LPS*	*RMP*	*LPS*	*RMP*	*LPS*
NWR	−42.0±0.4	−59.3±0.4*	−42.0±0.9	−61.3±0.4*	−50.6±0.5	−49.3±0.5	−52.1±0.5	−51.5±0.3
SHR	−32.7±0.7	−50.3±0.7*	−28.5±2.2	−32.2±1.0	−69.9±0.3	−89.7±0.3	−68.1±0.9	−87.9±0.6

RMP, resting membrane potential; LPS, lipopolysaccharide. For each artery ring from individual rats ($n=8$), 5 to 12 cells were impaled, and the averages of the respective measurements were used to obtain the mean ± s.e.mean. *$P<0.05$ versus RMP (Newman–Keuls test).

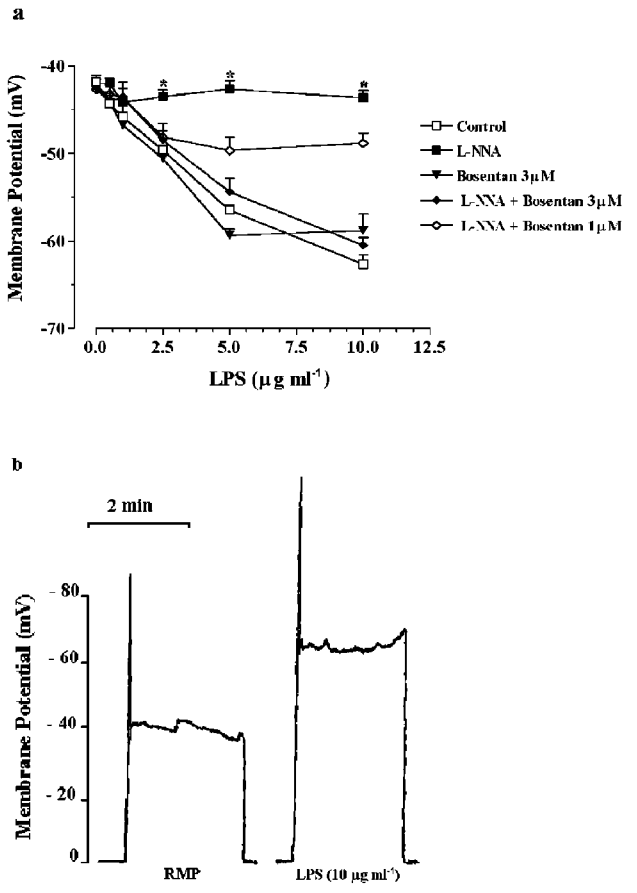

Figure 3 (a) Concentration-response curves for the hiperpolarization induced by LPS on intact mesenteric arteries from NWR in the absence (control) and in the presence of 50 μM L-NNA or 3 μM Bosentan or 50 μM L-NNA plus 1 μM Bosentan or 50 μM L-NNA plus 3 μM Bosentan. *$P<0.05$ versus controls (Newman-Keuls test); (b) Representative experimental trace of the record of smooth muscle membrane potential from NWR mesenteric artery rings in the absence (RMP) and in the presence of 10 μg ml^{-1} LPS.

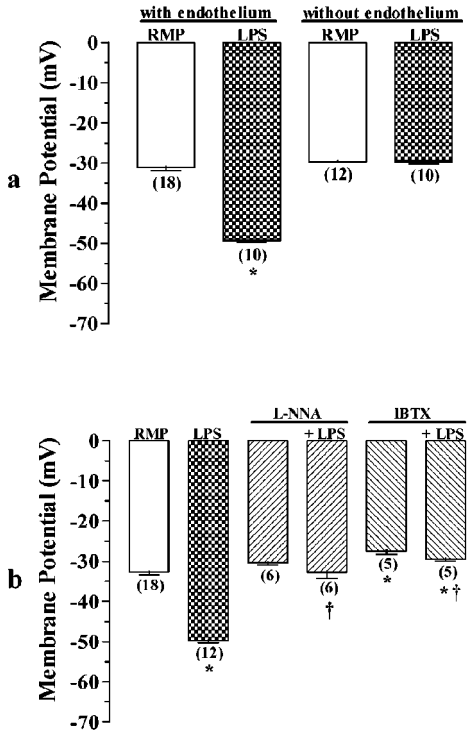

Figure 4 (a) Membrane potential measured in SHR mesenteric artery rings, with or without endothelium, in the absence (RMP) and in the presence of 10 μg ml^{-1} LPS. (b) Effect of 10 μg ml^{-1} LPS on the membrane potential of intact SHR mesenteric artery rings in the absence and in the presence of 50 μM L-NNA or 10 nM IBTX. For each ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5 to 12 cells were impaled, and the averages of the respective measurements were used to obtain the mean and s.e.mean. *$P<0.05$ versus RMP. †$P<0.05$ versus LPS alone (Newman-Keuls test).

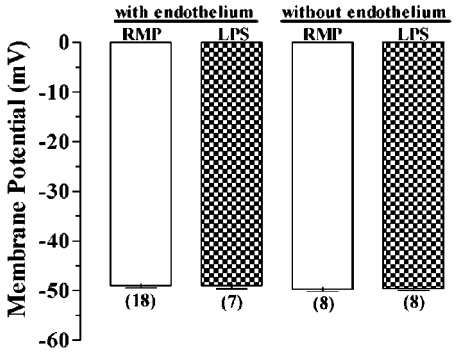

Figure 5 Membrane potential measured in aortic rings from NWR, with and without endothelium, in the absence (RMP) and in the presence of 10 μg ml^{-1} LPS. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5 to 12 cells were impaled, and the averages of the respective measurements were used to obtain the mean and s.e.mean.

Discussion

Most of the previous studies of the mechanisms responsible for the hypotension induced by LPS were performed in isolated arteries or organs from animals pre-treated with this drug. In the present work we studied the direct effect of LPS in isolated arteries from normotensive and from hypertensive rats, avoiding the interference of plasma factors. Since EDRF (NO) has been shown to play an important role in the hyporeactivity induced by LPS (Li *et al*., 1997; Wu *et al*., 1994; Thiemermann & Vane, 1990; Rees *et al*., 1990), the direct effect of LPS was also tested in the presence of the NO synthase inhibitor L-NNA.

The mechanisms underlying the vascular relaxation induced by LPS differed in normotensive and SHR animals, depending on the type of vessel studied.

In the NWR mesenteric vascular bed, preincubation with L-NNA had no effect on the relaxant response to LPS (Figure 1b), indicating that this response is not mediated by NO. In this case, a direct effect on the smooth muscle or the release of EDHF by the endothelium could be involved, possibly through the activation of high-conductance Ca^{2+}-dependent K^+ channels, since it was blocked by IBTX.

The behaviour of the SHR mesenteric vascular bed towards LPS differed from that of the NWR, in which preincubation with L-NNA had no effect. In SHR preparations, L-NNA efficiently reduced the responses to LPS,

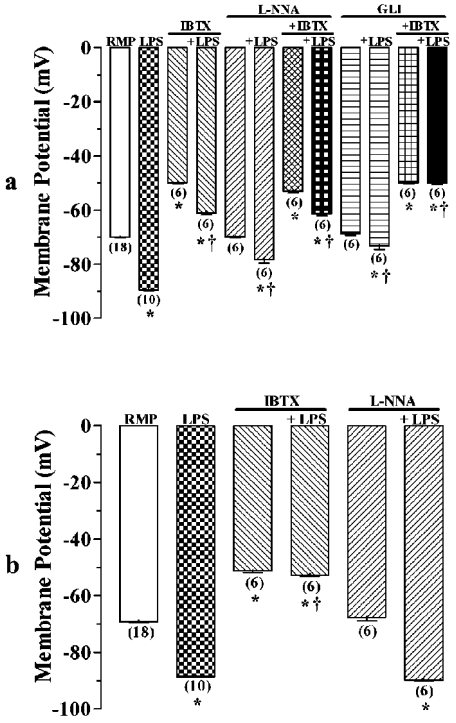

Figure 6 (a) Effect of 10 μg ml^{-1} LPS on the membrane potential of SHR aortic rings with endothelium in the absence (RMP) or in the presence of 10 nM IBTX, 50 μM L-NNA, or 1 nM glibenclamide (Gli). (b) Effect of 10 μg ml^{-1} LPS on the membrane potential of SHR aortic rings without endothelium in the absence (RMP) or in the presence of 10 nM IBTX or 50 μM L-NNA. For each aortic ring obtained from individual rats (the number of which is shown in parentheses below the bars), 5 to 12 cells were impaled, and the averages of the respective measurements were used to obtain the mean and s.e.mean. *$P<0.05$ versus RMP. †$P<0.05$ versus LPS alone (Newman-Keuls test).

indicating that these responses are largely mediated by NO, which also activates the Ca^{2+}-dependent K^+ channels sensitive to IBTX. This was probably a direct effect of NO, since it was not affected by the guanylate cyclase inhibitor.

To visualize the relaxant response to LPS in mesenteric vascular beds, the preparations must be pre-contracted with vasoconstrictor agonists which inhibit K^+ channels (Quayle *et al*., 1997), blunting the relaxant response due to the activation of these channels. To avoid this problem, intracellular microelectrodes were used to obtain direct measurements of the smooth muscle cell membrane potential in mesenteric and aortic rings.

In the NWR mesenteric rings, with or without endothelium, no differences were observed in the resting membrane potential or in the hyperpolarizing responses to LPS (Figure 2). L-NNA blocked the responses of endothelized but not those of de-endothelized NWR rings, indicating the participation of NO release, in contrast with the mesenteric vascular bed results. This may be explained by the hyperpolarization and vasodilatation being mostly due to NO (EDRF) in larger arteries and to EDHF in small resistance arteries (Hwa *et al*., 1994; Shimokawa *et al*., 1996).

The hyperpolarizing response to LPS in de-endothelized NWR mesenteric rings suggest that the toxin acts directly on the smooth muscle, without the mediation of NO release by the endothelium. If this is the case, it should not be expected that L-NNA would block the hyperpolarization of NWR endothelized rings in response to LPS, as was observed (Figure 2a). Therefore we investigated the possibility that endothelin released by LPS might have a depolarizing effect that would mask the direct effect on the smooth muscle. Endothelin (ET-1) is a potent vasoconstrictor released by the endothelium which is markedly increased in endotoxic shock, but its role in sepsis remains obscure (Ishimaru *et al*., 2001). Although no data in isolated vascular preparations have been reported, studies involving LPS administration to toxaemic rats showed complex responses that involved release of endothelin-1 and different effects in the expression of ETA and ETB receptors (e.g. Rossi *et al*., 2001). Our results (Figure 3) show that bosentan, an antagonist of these receptors, blocked the inhibition by L-NNA of the hyperpolarizing response to LPS in endothelized NWR mesenteric rings. This suggests that LPS induces the release of both NO and endothelin, which have opposite effects on the membrane potential. A better understanding of this finding awaits further study.

The responses induced by LPS in either endothelized or de-endothelized NWR mesenteric rings were both blocked by IBTX, in agreement with previous reports that the NO produced by vascular expression of inducible NO synthase activates Ca^{2+}-dependent K^+ channels in vascular smooth muscle (Murphy & Brayden, 1995; Taguchi *et al*., 1996). In fact, the inhibitory effect of IBTX, observed in either endothelized or de-endothelized arteries, indicates that these Ca^{2+}-dependent K^+ channels are located in the smooth muscle.

In SHR mesenteric rings, LPS caused hyperpolarization only in endothelized preparations (Figure 4) suggesting that it did not directly stimulate potassium channels in the smooth muscle. Indeed, these channels were shown to be impaired in the SHR (Borges *et al*., 1999).

The above assumptions are in agreement with the report that the ability of acetylcholine to relax the SHR mesenteric vascular bed through EDHF is impaired while there is a parallel increase in NO release (Mantelli *et al*., 1995). Furthermore, Feres *et al*. (1998) also verified that the impaired hyperpolarizing response to stimulation of α_2-adrenoceptors in SHR is compensated by the increased endothelial production of NO in this strain (Wu & Yen, 1999).

The results in the NWR mesenteric vascular bed lead us to conclude that LPS induces relaxation by directly opening Ca^{2+}-dependent K^+ channels located in the smooth muscle cell membrane, whereas in SHR, these effects are mediated by NO.

Our results indicate that LPS induces hyperpolarization in NWR mesenteric rings by opening smooth muscle Ca^{2+}-dependent K^+ channels both directly and indirectly through release of NO from the endothelium. In SHR rings, the indirect effect through NO release from the endothelium predominates.

Regarding the NWR aorta, LPS did not cause hyperpolarization in either endothelized or de-endothelized preparations, indicating that the toxin does not act on the ATP-sensitive K^+ channels that were shown to predominate in aortic smooth muscle (Fauaz *et al*., 2000).

In contrast, the SHR aorta is a good model to study the direct effect of LPS, since it presents an increased expression

of Ca^{2+}-sensitive K^+ channels (Liu *et al*., 1997). These channels in SHR are constitutively open, since a significant depolarization was observed by the addition of IBTX, which would stimulate the sodium/potassium pump, shown to be hyperactive in this strain (Silva *et al*., 1994). These conditions would contribute to the hyperpolarized state of SHR aortic rings. The resting membrane potential did not differ between endothelized and de-endothelized SHR aortic rings, and in both conditions similar hyperpolarizing responses to LPS were observed. The endothelized ring responses were partially affected by the NO inhibitor L-NNA, by IBTX or by glibenclamide, but total inhibition was only observed by the combination of IBTX plus glibenclamide. This indicates that LPS was also able to open the ATP-sensitive K^+ channels present in SHR aorta (Landry & Oliver, 1992). Since in the de-endothelized SHR aortic rings IBTX totally inhibited the hyperpolarization induced by LPS, the activation of ATP-dependent potassium channels observed in intact rings was probably due to NO release from the endothelium.

In conclusion, our results in the resistance vessels (mesenteric vascular bed) suggest that relaxation induced by LPS in NWR is due to direct activation of large conductance Ca^{2+}-sensitive K^+ channels located in the smooth muscle cell membrane. In the SHR, LPS causes relaxation indirectly, mainly through NO release from the endothelium, which is also able to open Ca^{2+}-sensitive K^+ channels.

In medium size vessels (mesenteric arteries), our results indicate that the hyperpolarization induced by LPS in NWR is due to both direct and indirect (through NO release from the endothelium) activation of the Ca^{2+}-sensitive K^+ channels, while in SHR the response is only due to NO release from the endothelium.

In aortic rings, LPS induces hyperpolarization only in the SHR, by direct activation of Ca^{2+}-sensitive and ATP-dependent K^+ channels, or indirectly, through NO release from endothelium.

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