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Influence of endotoxin on contractility in the rat gastric fundus

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Abstract

The influence of in vivo treatment with *E. coli* lipopolysaccharide endotoxin on the contractility of the rat gastric fundus was studied. Four h after lipopolysaccharide treatment (20 mg/kg i.p.), the contractile responses to prostaglandin $F_{2\alpha}$ in longitudinal muscle strips from the gastric fundus were not different from those in control animals, while the well-known decreased response to noradrenaline in rings of the thoracic aorta was confirmed. Incubation of the tissues with L-arginine did not depress the response to prostaglandin $F_{2\alpha}$ in fundus strips of lipopolysaccharide-treated rats. Twelve h after lipopolysaccharide treatment (6.7 mg/kg i.p.), the prostaglandin $F_{2\alpha}$-induced contractions were consistently depressed. The impairment of the prostaglandin $F_{2\alpha}$-induced responses by lipopolysaccharide treatment was not reversed by the nitric oxide synthase inhibitors N^G-nitro-L-arginine (L-NNA, 10^{-4} M), N^G-nitro-L-arginine methyl ester (L-NAME, 3×10^{-4} M), aminoguanidine (10^{-4} M) and L-N^6-1-iminoethyl-lysine (L-NIL, 10^{-4} M) nor by the cyclooxygenase inhibitor indomethacin (10^{-5} M). The impairment was prevented by pretreating the animals with dexamethasone (5 mg/kg i.p.), which had no effect per se on the contractile response to prostaglandin $F_{2\alpha}$. Lipopolysaccharide treatment did not influence the contractile responses to KCl and serotonin. The nonadrenergic noncholinergic relaxant responses to transmural electrical stimulation were not influenced 4 h after lipopolysaccharide treatment but were moderately reduced after 12 h. The results illustrate that the selective impairment of prostaglandin $F_{2\alpha}$-induced contractions in the rat gastric fundus by lipopolysaccharide treatment is not mediated via generation of nitric oxide; downregulation of the prostaglandin $F_{2\alpha}$-receptor by lipopolysaccharide treatment might be involved. © 1997 Elsevier Science B.V.

Keywords: Gastric fundus, rat; Endotoxin; Contractility; Nitric oxide (NO); Prostaglandin $F_{2\alpha}$

1. Introduction

Nitric oxide (NO) is derived from L-arginine by NO synthase. Constitutive isoforms of NO synthase are present in endothelial cells (NO synthase 3 or endothelial NO synthase) and central and peripheral neurones (NO synthase 1 or neuronal NO synthase; Förstermann and Kleinert, 1995), NO being involved in tonic vasodilatation (Moncada et al., 1991), long-term potentiation and neurotoxicity (Zhang and Snyder, 1995) and nonadrenergic noncholinergic neurotransmission (Sanders and Ward, 1992). Bacterial lipopolysaccharide endotoxin leads to the expression of an inducible isoform of NO synthase (NO synthase 2 or inducible NO synthase) in many cell types such as macrophages, endothelial cells and vascular smooth muscle cells (Marletta et al., 1988; Knowles et al., 1990; Radomski et al., 1990), NO being involved in host defence mechanisms against infectious organisms (Kröncke et al., 1995). However, inducible NO synthase is a high output enzyme (Förstermann et al., 1995) and there is good evidence that excessive NO production is involved in hypotension and vascular hyporeactivity during septic shock (Wright et al., 1992; Szabó et al., 1993). Vascular tissues obtained from animals after pretreatment with endotoxin in vivo show a reduced response to contractile agents, such as noradrenaline, that can be restored by NO synthase inhibitors (Julou-Schaeffer et al., 1990; Vallance et al., 1992).

The increased NO production during septicaemia might also damage systems other than the cardiovascular system. In the gastrointestinal tract, endotoxin-induced vascular injury in rat jejunum and colon has been described (Boughton-Smith et al., 1993). Inducible NO synthase is also expressed by rat gastric mucosal cells after exposure to endotoxin, which might contribute to gastritis (Brown et al., 1994). As for gastrointestinal motility, endotoxin de-

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lays gastric emptying of liquids and decreases fasting intestinal motility in dogs (Cullen et al., 1995). In rats, endotoxin slows gastric emptying but increases intestinal transit of nonabsorbable liquid markers, and this effect on gastric emptying is not reversed by NO synthase inhibitors (Wirthlin et al., 1996). Recently, Takakura et al. (1996) reported that incubation of mucosa-free muscle strips of rat gastric fundus with endotoxin for 6 h induced inducible NO synthase activity together with a decreased contractile response to prostaglandin $F_{2\alpha}$ and the occurrence of a relaxant response to L-arginine. However, after in vivo pretreatment with endotoxin, no inducible NO synthase activity or messenger RNA was found in the stomach of rats in contrast to the other parts of the gastrointestinal tract (Salter et al., 1991; Chen et al., 1996). The aim of this study was therefore to investigate the influence of in vivo pretreatment with endotoxin on the contractility of the rat gastric fundus. As this tissue responds to electrical field stimulation with relaxation, mediated by nonadrenergic noncholinergic nerves with NO being at least one of the neurotransmitters involved (Li and Rand, 1990; Boeckxstaens et al., 1991; D'Amato et al., 1992), we also investigated the influence of endotoxin pretreatment on the relaxant responses to electrical field stimulation under nonadrenergic noncholinergic conditions.

2. Material and methods

2.1. Animals, treatment and preparation

Male Wistar rats (260–500 g) were used and in all experiments they were fasted with free access to water for at least 12 h before they were killed by a blow on the head and bleeding.

In the first sets of experiments, the rats were injected intraperitoneally with 20 mg/kg of lipopolysaccharide from Escherichia coli (O55:B5; Difco) or with an equivalent volume of physiological salt solution (0.1 ml/100 g; controls) 4 h before killing. Two longitudinal muscle strips (approximately 15 mm long $\times$ 3 mm wide) were prepared from the gastric fundus and mounted under a load of 1 g in 7.5 ml organ baths containing Krebs solution at 37°C and bubbled with carbogen. The composition of the Krebs solution in mM was: NaCl 118.5, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.9, $NaHCO_3$ 25.0 and glucose 10.1. The tension of the tissues was recorded auxotonically with Grass FT03 force-displacement transducers coupled in series with a 1 g/cm spring and registered on a Graphtec linearcorder FWR 3701. Transmural electrical stimulation was performed via 2 parallel platinum plate electrodes (22×7 mm, distance in between 6 mm) that were connected to a Grass S88 stimulator with a constant voltage unit. When the tissues were stimulated, the Krebs solution contained 10^{-6} M atropine and 4×10^{-6} M guanethidine to block cholinergic and noradrenergic responses, respectively. In one set of rats (lipopolysaccharide-treated and controls), the thoracic aorta was also removed, cleaned of adherent connective tissue and cut into 3 mm wide rings. The rings were mounted in 7.5 ml organ baths under a load of 2 g in Krebs solution containing 10^{-6} M ascorbic acid. Contractions were recorded isometrically.

In all other experiments, the rats were injected intraperitoneally with 6.7 mg/kg of lipopolysaccharide or with an equivalent volume of physiological salt solution 12 h before killing. Gastric fundus strips were then prepared and mounted as described above. In one experiment (lipopolysaccharide-treated and controls), dexamethasone, 5 mg/kg, was injected intraperitoneally just before the injection of lipopolysaccharide or saline. In another experiment, dexamethasone was injected 12 h before killing without subsequent administration of lipopolysaccharide or saline, and the results were compared with those of rats that were not treated.

2.2. Protocols

All tissues were equilibrated for 1 h with rinsing every 15 min. In the first set of experiments with rats killed 4 h after lipopolysaccharide or saline treatment, cumulative concentration-response curves for noradrenaline (10^{-9}–10^{-5} M) were obtained in the aortic rings and for prostaglandin $F_{2\alpha}$ (10^{-9}–3×10^{-6} M) in the fundus strips. In a second set, cumulative concentration-response curves for prostaglandin $F_{2\alpha}$ were obtained in fundus strips after incubation for 30 min with either 10^{-3} M L-arginine or 10^{-4} M N^G-nitro-L-arginine (L-NNA). In a third set, fundus strips were contracted by administration of 3×10^{-7} M prostaglandin $F_{2\alpha}$. Once a stable plateau was reached, the tissues were transmurally stimulated (40 V, 1 ms, 1–16 Hz) with 10 s trains at 4 min intervals.

In the first set of experiments with rats killed 12 h after lipopolysaccharide or saline treatment, cumulative concentration-response curves for prostaglandin $F_{2\alpha}$ (10^{-9}–3×10^{-6} M) were obtained in the gastric fundus strips. The same was done with strips prepared from rats pretreated with dexamethasone. To study the influence of NO synthesis and prostaglandin synthesis inhibition on the contractile response to prostaglandin $F_{2\alpha}$, a cumulative concentration-response curve for prostaglandin $F_{2\alpha}$ was obtained before and after incubation with L-NNA (10^{-4} M; 30 min incubation), N^G-nitro-L-arginine methyl ester (L-NAME; 3×10^{-4} M; 30 min), aminoguanidine (10^{-4} M; 30 min), L-N^6-1-iminoethyl-lysine (L-NIL; 10^{-4} M; 90 min) or indomethacin (10^{-5} M; 20 min). In parallel tissues, the concentration-response curve for prostaglandin $F_{2\alpha}$ was studied before and after incubation with the solvent of the inhibitor under study; in some of these tissues, the response to cumulative administration of KCl (10–80×10^{-3} M after incubation with 3×10^{-6} M tetrodotoxin for 10 min) or to serotonin (10^{-10}–3×10^{-6} M) was studied at the end of the experiment. In a last set

of experiments, the responses to transmural electrical stimulation were studied as described above, before and after addition of L-NAME (3×10^{-4} M; 30 min incubation) or its solvent; in the strips from the saline-treated control rats, the plateau contraction was induced with 10^{-7} M prostaglandin $F_{2\alpha}$, while in those from the lipopolysaccharide-treated rats, the plateau contraction was induced with 2×10^{-6} M prostaglandin $F_{2\alpha}$. At the end of all experiments, the tissues were blotted and weighed.

2.3. Statistical analysis

Contractions are expressed as g of tension per mg (aortic rings) or g (fundus strips) of tissue, while relaxations are expressed as per cent reduction of the prostaglandin $F_{2\alpha}$-induced tone. The EC_{50} for contraction was determined by linear interpolation from each concentration-response curve as the concentration responsible for 50% of the maximal contractile response and is expressed as its negative logarithm (pD_2); the EF_{50} for relaxation was determined by linear interpolation from each frequency-response curve as the frequency responsible for 50% of the response obtained at a stimulation frequency of 16 Hz. The percentage inhibition by L-NAME of electrically induced relaxations was calculated as (R before-R after) $\times 100/R$ before, where R before and R after indicate the relaxation before and after addition of L-NAME.

Data are given as mean ± SEM values, n referring to tissues obtained from different animals unless otherwise indicated. Results between tissues from saline- and lipopolysaccharide-treated animals were compared by the unpaired t-test, and results within tissues were compared by the paired t-test. A P value of ≤ 0.05 was considered to be statistically significant.

2.4. Substances used

Aminoguanidine hemisulphate, L-arginine hydrochloride, L-ascorbic acid, atropine sulphate, guanethidine sulphate, indomethacin, N^G-nitro-L-arginine, N^G-nitro-L-arginine methyl ester hydrochloride, L-noradrenaline bitartrate and prostaglandin $F_{2\alpha}$ were from Sigma (St. Louis, MO, USA), serotonin creatinine sulphate and tetrodotoxin were from Janssen Chimica (Beerse, Belgium) and L-N^6-1-iminoethyl-lysine hydrochloride was from Alexis Corporation (San Diego, CA, USA). Commercially available ampoules of dexamethasone (Decadron®, MSD, Brussels, Belgium) were used. All other drugs were dissolved in deionized water except for indomethacin, which was dissolved in 90% ethanol. Drug solutions were prepared on the day of the experiment except for prostaglandin $F_{2\alpha}$ and tetrodotoxin, for which stock solutions were kept at −20°C. The stock solution of noradrenaline contained 5.7×10^{-6} M ascorbic acid.

3. Results

3.1. Responses obtained after 4 h in vivo pretreatment with 20 mg/kg lipopolysaccharide

Noradrenaline induced a concentration-dependent contraction in aortic rings from control animals. Lipopolysaccharide pretreatment shifted the concentration-response curve for noradrenaline to the right (Fig. 1A), with a shift of the pD_2 from 7.29 ± 0.11 ($n = 6$) to 6.91 ± 0.07 ($n = 6$; $P < 0.05$). The contractile response to 10^{-5} M noradrenaline was not influenced (1.38 ± 0.09 g/mg in controls, $n = 6$, versus 1.16 ± 0.17 g/mg in lipopolysaccharide-treated animals, $n = 6$). In contrast, the concentration-response curve for the contractile effect of prostaglandin $F_{2\alpha}$ in gastric fundus strips was not different between control and lipopolysaccharide-treated animals (Fig. 1B), with a pD_2 of 7.36 ± 0.21 ($n = 5$) in controls and 7.26 ± 0.10 ($n = 5$) in lipopolysaccharide-treated rats. In vitro pretreatment with 10^{-4} M L-arginine or 10^{-4} M L-NNA did not induce a difference in the contractile response to prostaglandin $F_{2\alpha}$ between strips from controls and lipopolysaccharide-treated rats. After incubation for 30 min with 10^{-4} M L-arginine, the E_{max} and pD_2 values were 12.4 ± 0.8 g/g and 7.58 ± 0.17 for strips from control rats ($n = 6$) and 12.6 ± 1.4 g/g and 7.37 ± 0.10 for strips from lipopolysaccharide-treated rats ($n = 6$); after incubation for 30 min with 10^{-4} M L-NNA, these values were 12.5 ± 1.2 g/g and 7.50 ± 0.13 in strips from controls ($n = 6$) and 12.6 ± 2.0 g/g and 7.41 ± 0.07 in strips from lipopolysaccharide-treated animals ($n = 6$). Transmural electrical stimulation at increasing frequencies under nonadrenergic noncholinergic conditions induced frequency-dependent relaxations that did not differ between strips from controls

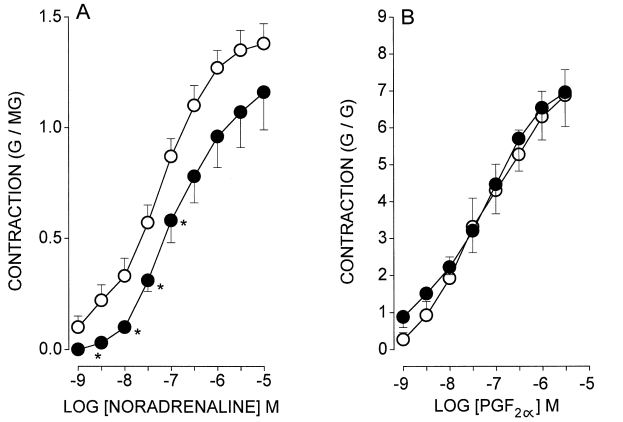

Fig. 1. Mean contractions elicited by noradrenaline in aortic rings (A) and by prostaglandin $F_{2\alpha}$ in gastric fundus strips (B) from control (○) and lipopolysaccharide-treated (●) animals. Lipopolysaccharide, 20 mg/kg, was administered i.p. 4 h before killing. Mean ± SEM of $n = 6$ in (A) and 5 in (B). * $P < 0.05$, significantly different from the response of preparations from control rats (unpaired t-test).

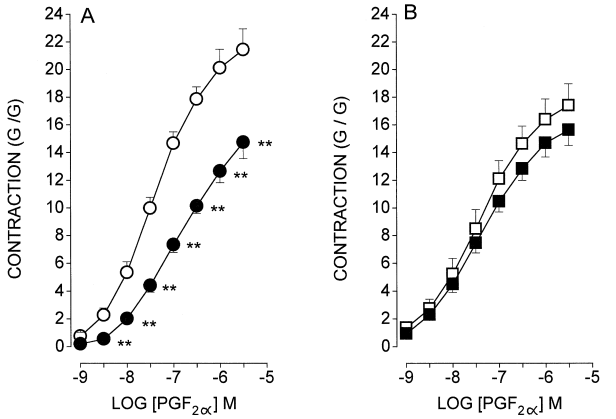

Fig. 2. (A) Mean contractions elicited by prostaglandin $F_{2\alpha}$ in gastric fundus strips from control (○) and lipopolysaccharide-treated (●) animals. Lipopolysaccharide, 6.7 mg/kg, was administered i.p. 12 h before killing. (B) Mean contractions elicited by prostaglandin $F_{2\alpha}$ in gastric fundus strips from control (□) and lipopolysaccharide-treated (■) rats, pretreated with dexamethasone, 5 mg/kg i.p., immediately before the injection of saline or lipopolysaccharide. Mean ± SEM of $n = 8$ in (A) and (B). ** $P < 0.01$, significantly different from the response of preparations from control rats (unpaired *t*-test).

and lipopolysaccharide-treated rats, the response for stimulation at 16 Hz being 67.7 ± 7.8% ($n = 6$) and 56.5 ± 6.1% ($n = 6$), respectively.

3.2. Responses obtained after 12 h in vivo pretreatment with 6.7 mg / kg lipopolysaccharide

Preliminary experiments showed that rats in general did not survive for 12 h after intraperitoneal administration of 20 mg/kg lipopolysaccharide; the dose of lipopolysaccharide was, therefore, reduced to one third.

Pretreatment with 6.7 mg/kg lipopolysaccharide i.p. shifted the concentration-response curve for prostaglandin $F_{2\alpha}$ to the right with a reduction of the E_{max} (Fig. 2A). E_{max} and pD_2 values were 21.4 ± 1.5 g/g and 7.44 ± 0.10 in strips from control rats ($n = 8$) and 14.7 ± 1.2 g/g ($P < 0.01$) and 7.01 ± 0.12 ($P < 0.05$) in strips from lipopolysaccharide-treated rats ($n = 8$). Even when the highest concentration of prostaglandin $F_{2\alpha}$ was increased from 3×10^{-6} M to 3×10^{-5} M in the fundus strips of lipopolysaccharide-treated rats, the E_{max} was still depressed (13.9 ± 1.0 g/g, $n = 9$ versus 19.1 ± 1.7 g/g in controls, $n = 9$, $P < 0.05$). When rats were pretreated with dexamethasone (5 mg/kg i.p.) immediately before lipopolysaccharide, the responses to prostaglandin $F_{2\alpha}$ were similar in tissues from control and lipopolysaccharide-treated animals (Fig. 2B). Dexamethasone per se did not suppress the response to prostaglandin $F_{2\alpha}$ as the contractile responses of strips from rats pretreated with dexamethasone 12 h before killing (E_{max}: 14.0 ± 1.3 g/g and pD_2: 7.33 ± 0.09, $n = 6$) did not differ from those of strips from untreated rats (E_{max}: 13.7 ± 0.7 g/g and pD_2: 7.55 ± 0.04, $n = 6$). In the experiments in which the influence of NO synthesis

Table 1
Influence of inhibition of NO synthesis and prostaglandin synthesis on the contractile responses to prostaglandin $F_{2\alpha}$ of fundus strips from control and lipopolysaccharide-treated rats

Inhibitor	Controls		Lipopolysaccharide-treated	
	before	after	before	
Aminoguanidine (10^{-4} M; $n = 8$)				
E_{max} (g/g)	22.8 ± 2.1	20.8 ± 2.5	10.7 ± 1.9	11.1 ± 2.2
pD_2	7.52 ± 0.16	7.62 ± 0.09	6.97 ± 0.18	7.00 ± 0.19
L-NIL (10^{-4} M; $n = 8$)				
E_{max} (g/g)	18.5 ± 1.4	16.5 ± 1.3[a]	11.5 ± 0.7	11.8 ± 0.7
pD_2	7.63 ± 0.07	7.71 ± 0.06[a]	7.02 ± 0.11	7.18 ± 0.09[a]
Indomethacin (10^{-5} M; $n = 6$)				
E_{max} (g/g)	23.7 ± 2.7	28.9 ± 3.3[a]	18.8 ± 2.3	25.2 ± 2.6[a]
pD_2	7.47 ± 0.16	7.31 ± 0.13	6.88 ± 0.10	6.71 ± 0.06

[a] $P < 0.05$, significantly different from the response before incubation with the inhibitor (paired *t*-test).

inhibitors was tested, the concentration-response curve for prostaglandin $F_{2\alpha}$ was always depressed in strips from lipopolysaccharide-treated animals as compared to that in strips from saline-treated rats. 10^{-4} M L-NNA ($n = 3$) and 3×10^{-4} M L-NAME ($n = 3$) did not influence the contractile response to prostaglandin $F_{2\alpha}$ in strips from lipopolysaccharide-treated animals. Two larger series of experiments were performed with aminoguanidine and L-NIL, which are reported to show selectivity towards inducible NO synthase (Table 1). In strips from both control and lipopolysaccharide-treated animals, the concentration-response curve for prostaglandin $F_{2\alpha}$ was reproducible when repeated after incubation with the solvent of aminoguanidine ($n = 8$) or L-NIL ($n = 8$; Fig. 3B). Aminoguanidine did not influence the responses to prostaglandin $F_{2\alpha}$ in strips from control and lipopolysaccharide-treated rats (Ta-

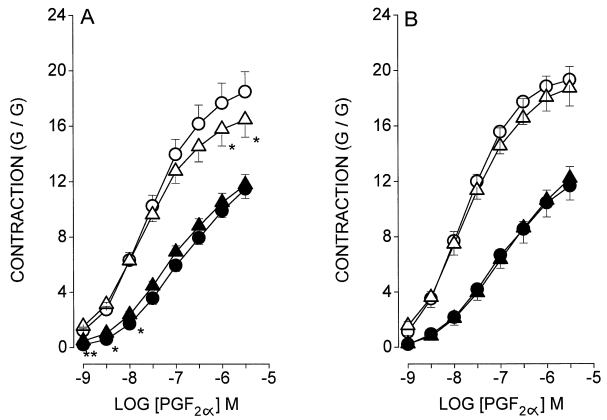

Fig. 3. Mean contractions elicited by prostaglandin $F_{2\alpha}$ in gastric fundus strips from control (open symbols) and lipopolysaccharide-treated (closed symbols) animals. Lipopolysaccharide, 6.7 mg/kg, was administered i.p. 12 h before killing. Contractions were obtained before (○, ●) and after (△, ▲) 90 min incubation with 10^{-4} M L-NIL (A) or its solvent (B). Mean ± SEM of $n = 7$–8. * $P < 0.05$, significant difference between the response before and after incubation with L-NIL (paired *t*-test).

ble 1). In strips from control rats, the contraction in response to 10^{-6} and 3×10^{-6} M prostaglandin $F_{2\alpha}$ was significantly decreased (Fig. 3A) and the pD_2 was significantly increased (Table 1) after incubation with L-NIL. In strips from lipopolysaccharide-treated rats, the contraction in response to 10^{-9}, 3×10^{-9} and 10^{-8} M prostaglandin $F_{2\alpha}$ was significantly increased after incubation with L-NIL (Fig. 3A), as was the pD_2 (Table 1). However, the responses to prostaglandin $F_{2\alpha}$ in strips from lipopolysaccharide-treated rats after incubation with L-NIL were still clearly less pronounced than those in strips from control rats (Fig. 3A), illustrating that L-NIL is not able to reverse the lipopolysaccharide-induced hyporeactivity. The NO synthesis inhibitors per se induced a small contraction that was maintained during the incubation period. Contractions in response to prostaglandin $F_{2\alpha}$ were measured with the newly developed tone as base line. The contractile response to the NO synthesis inhibitors in strips from controls and lipopolysaccharide-treated animals was: 2.35 $\pm$ 0.55 and 1.89 $\pm$ 0.56 g/g for 10^{-4} M L-NNA, $n = 3$, 2.58 $\pm$ 0.90 and 3.88 $\pm$ 0.26 g/g for 3×10^{-4} M L-NAME, $n = 3$, 2.30 $\pm$ 0.45 and 1.02 $\pm$ 0.23 g/g for 10^{-4} M aminoguanidine, $n = 8$, $P < 0.05$, and 2.92 $\pm$ 0.56 and 3.45 $\pm$ 0.86 g/g for 10^{-4} M L-NIL, $n = 8$.

During incubation with 10^{-5} M indomethacin, the tone of the tissue moderately decreased (with 3.96 $\pm$ 1.22 g/g in fundus strips from control rats, $n = 6$, and 3.43 $\pm$ 0.58 g/g in fundus strips from lipopolysaccharide-treated rats, $n = 6$), while the solvent had no influence. In strips from both control and lipopolysaccharide-treated rats, indomethacin significantly increased the contractile responses to 10^{-6} and 3×10^{-6} M prostaglandin $F_{2\alpha}$, without causing significant alterations of the pD_2 (Table 1, Fig. 4). In strips from control rats, the concentration-response curve for prostaglandin $F_{2\alpha}$ was reproducible when repeated after incubation with the solvent of indomethacin ($n = 6$), while

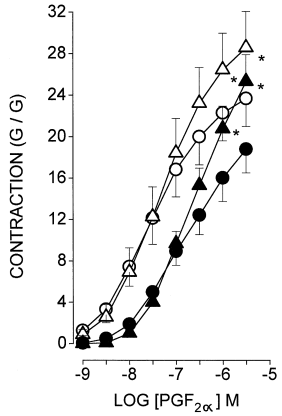

Fig. 4. Mean contractions elicited by prostaglandin $F_{2\alpha}$ in gastric fundus strips from control (open symbols) and lipopolysaccharide-treated (closed symbols) animals. Contractions were obtained before (○, ●) and after (△, ▲) 20 min incubation with 10^{-5} M indomethacin. Mean ± SEM of $n = 6$. * $P < 0.05$, significantly different from the response before incubation with indomethacin (paired t-test).

in tissues from lipopolysaccharide-treated rats, the responses to prostaglandin $F_{2\alpha}$ were significantly increased over the range of 10^{-8} to 3×10^{-6} M ($P < 0.05$ except for 10^{-7} M where $P < 0.01$) in the presence of the solvent of indomethacin (the E_{max} increased from 19.0 $\pm$ 2.5 g/g to 22.8 $\pm$ 2.5 g/g, $n = 6$).

When KCl was added cumulatively (10–80 mM) in the absence of tetrodotoxin during preliminary experiments, the contractile response to 20 mM quickly faded or a relaxation occurred immediately after 20 mM KCl was added. Similar results were observed for circular muscle strips from the rat gastric fundus; the relaxant responses were changed to contraction by treatment with tetrodotoxin and were ascribed to KCl-induced release of NO from inhibitory nonadrenergic noncholinergic nerves (Kamata et al., 1993). KCl was, therefore, investigated in the presence of tetrodotoxin in our study. Under these conditions, KCl induced well-maintained concentration-dependent contractions that did not differ between strips from control and lipopolysaccharide-treated rats (E_{max}: 19.8 $\pm$ 2.1 g/g in strips from controls, $n = 8$, and 19.3 $\pm$ 2.1 g/g in strips from lipopolysaccharide-treated animals, $n = 9$). Serotonin (10^{-10}–3×10^{-6} M) induced concentration-dependent contractions that did not differ between strips from control and lipopolysaccharide-treated animals (E_{max}: 14.6 $\pm$ 0.8 g/g in strips from controls, $n = 9$, and 13.9 $\pm$ 0.5 g/g in strips from lipopolysaccharide-treated animals, $n = 8$).

To study the relaxant responses to transmural electrical stimulation under nonadrenergic noncholinergic conditions, strips were contracted with prostaglandin $F_{2\alpha}$. To obtain a similar degree of contraction, the concentration of prostaglandin $F_{2\alpha}$ used was 10^{-7} M with control strips and 2×10^{-6} M for strips from lipopolysaccharide-treated rats in view of the decreased response to prostaglandin $F_{2\alpha}$ after lipopolysaccharide treatment. The resulting contraction (9.2 $\pm$ 1.2 g/g in strips from controls, $n = 6$, and 11.4 $\pm$ 1.0 g/g in strips from lipopolysaccharide-treated animals, $n = 6$) was not significantly different. The relaxations induced by transmural electrical stimulation were 24.6 $\pm$ 3.5, 55.8 $\pm$ 4.4, 66.5 $\pm$ 4.4, 76.9 $\pm$ 5.6 and 98.4 $\pm$ 7.2% at 1, 2, 4, 8 and 16 Hz in strips from control rats ($n = 6$) and 12.4 $\pm$ 4.2, 40.6 $\pm$ 5.4, 54.1 $\pm$ 6.2, 66.4 $\pm$ 7.2 and 93.1 $\pm$ 9.4% in strips from lipopolysaccharide-treated animals ($n = 6$). The response at 1 Hz was significantly decreased in strips from the treated animals ($P < 0.05$) while the EF_{50} was increased from 1.8 $\pm$ 0.1 to 3.0 $\pm$ 0.3 Hz ($P < 0.01$). The administration of L-NAME or its solvent did not influence the amplitude of the contraction elicited by prostaglandin $F_{2\alpha}$. Frequency-response curves obtained again in the presence of the solvent of L-NAME were comparable to those obtained before administration of the solvent, except for a significant reduction of the response at 4 Hz ($P < 0.05$) in tissues from control rats. L-NAME, 3×10^{-4} M, significantly reduced the responses in strips from both control and treated rats (Fig. 5). In strips from controls, the EF_{50} increased from 1.8 $\pm$ 0.2 to

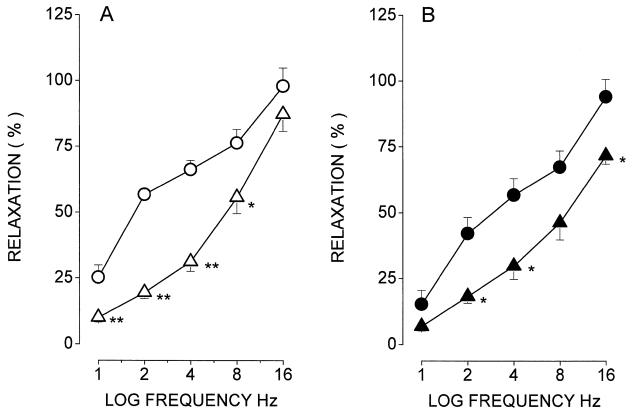

Fig. 5. Mean relaxant responses to transmural electrical stimulation (40 V, 1 ms) in gastric fundus strips from control (A) and lipopolysaccharide-treated (B) animals. Responses were obtained before (○, ●) and after (△, ▲) incubation with 3×10^{-4} M L-NAME. Mean ± SEM of $n=6$. * $P<0.05$, ** $P<0.01$, significantly different from the response before incubation with L-NAME (paired *t*-test).

6.1 ± 0.5 Hz ($n=6$, $P<0.01$), and in strips from lipopolysaccharide-treated rats, the EF_{50} was 2.8 ± 0.4 before and 5.7 ± 1.1 Hz ($n=6$) in the presence of L-NAME. The degree of inhibition by L-NAME was not different between strips from controls and lipopolysaccharide-treated animals.

4. Discussion

The aim of this study was to investigate the influence of in vivo pretreatment with lipopolysaccharide on contractility and nonadrenergic noncholinergic inhibition in the rat gastric fundus. The contractile agent used was prostaglandin $F_{2\alpha}$, a potent contractor in the rat gastric fundus as this tissue contains a prostaglandin F receptor (Dong et al., 1986). Throughout the different experiments with preparations from control rats, the maximal response to prostaglandin $F_{2\alpha}$ differed. We have no explanation for this observation. As preparations from lipopolysaccharide-treated animals were always used pairwise with preparations from controls, this should be no problem for interpretation of the results.

The dose of lipopolysaccharide (20 mg/kg i.p.) used in the first set of experiments has been shown to decrease the contractile response to noradrenaline in rat aorta 4 h after treatment (Julou-Schaeffer et al., 1990; Fleming et al., 1991). The decreased vascular reactivity after 4 h was confirmed in our study, although the maximal response was not significantly reduced. The contractile response to prostaglandin $F_{2\alpha}$ in the rat gastric fundus was, however, not influenced by lipopolysaccharide treatment and no influence of the NO synthesis inhibitor L-NNA was observed. This contrasts with the reduced contraction in response to prostaglandin $F_{2\alpha}$ in circular muscle strips from the rat gastric fundus after in vitro incubation with lipopolysaccharide for 6 h, which was ascribed to the induction of inducible NO synthase (Takakura et al., 1996) but corresponds with the inability of lipopolysaccharide (10 mg/kg i.p.) to induce inducible NO synthase mRNA in the rat stomach 4 h after treatment (Chen et al., 1996). Inducible NO synthase mRNA is induced by lipopolysaccharide in the other parts of the rat gastrointestinal tract (esophagus, duodenum, jejunum, ileum and colon; Chen et al., 1996). Decreased ileal muscle contractility to carbachol has been reported 5 h after 20 mg/kg lipopolysaccharide i.p. but L-arginine was required in the bathing medium to show the depression of the contractile response to carbachol in preparations from lipopolysaccharide-treated animals (Weisbrodt et al., 1996). Also in vascular tissue, endogenous L-arginine is not sufficient for maximal NO production by inducible NO synthase and addition of exogenous L-arginine is required (Schneider et al., 1992). An insufficient availability of L-arginine cannot explain why lipopolysaccharide treatment did not decrease the response to prostaglandin $F_{2\alpha}$ in the rat gastric fundus as addition of L-arginine did not induce a different response in strips from controls and lipopolysaccharide-treated rats.

The induction of inducible NO synthase by lipopolysaccharide is dose- but also time- and tissue-dependent (Salter et al., 1991; Cook et al., 1994). Lipopolysaccharide, 4 mg/kg i.p., induced inducible NO synthase in the epithelium of the rat small intestine, observable by immunostaining at 3 and 5 h after treatment but not at 12 h (Cook et al., 1994), whereas in the whole ileum, increased inducible NO synthase activity was still observed 18 h after treatment (Salter et al., 1991). In most studies in which the time-dependency of the response was studied, inducible NO synthase activity peaked later than 4 h after treatment (Wakabayashi et al., 1987; Salter et al., 1991; Bandaletova et al., 1993). To avoid overlooking a slowly induced effect of lipopolysaccharide treatment in the rat gastric fundus, we investigated contractility 12 h after treatment. Rats did not survive for 12 h after 20 mg/kg lipopolysaccharide i.p. but survived to termination of the experiment after the dose of lipopolysaccharide was reduced to 6.7 mg/kg i.p. The contractile reactivity to prostaglandin $F_{2\alpha}$ was clearly reduced 12 h after lipopolysaccharide treatment and this reduction was abolished by administration of dexamethasone before lipopolysaccharide. The latter result is not due to suppression by dexamethasone of the contractile response to prostaglandin $F_{2\alpha}$ in strips from the control rats, as dexamethasone per se did not influence the reactivity to prostaglandin $F_{2\alpha}$. Glucocorticoids have been shown to inhibit the induction of inducible NO synthase by lipopolysaccharide (Knowles et al., 1990; Radomski et al., 1990) and dexamethasone was used at a dose reported to prevent the lipopolysaccharide-induced decrease of the contractile response to noradrenaline in rat aorta (Paya et al., 1993). Although this finding was suggestive for the induction of inducible NO synthase, this was not confirmed by the results with the NO synthe-

sis inhibitors as none clearly influenced the hyporeactivity to prostaglandin $F_{2\alpha}$ of tissues from the lipopolysaccharide-treated rats. Although L-NNA and L-NAME show some selectivity towards endothelial NO synthase (Southan and Szabó, 1996), they have been shown to reverse lipopolysaccharide-induced suppression of vascular reactivity (Fleming et al., 1991; Palacios et al., 1996). Both L-arginine analogues were ineffective in the rat gastric fundus. Aminoguanidine and L-NIL show selectivity towards inducible NO synthase in some models (Griffiths et al., 1993; Misko et al., 1993; Stenger et al., 1995) but did not reverse the hyporeactivity to prostaglandin $F_{2\alpha}$ in the rat gastric fundus, even when the incubation time for L-NIL was increased to 90 min. As in many tissues (Rand and Li, 1995), the NO synthase inhibitors increased the basal tone of the rat gastric fundus preparations, which might correspond with the inhibition of continuously released NO. This effect has been described before and as it is not mimicked by the neuronal conductance blocker tetrodotoxin, leakage of NO out of nitrergic nerves, independently of fast sodium channels, has been suggested as explanation (Boeckxstaens et al., 1991). One would expect then that this continuously leaking NO would also counteract to some extent the contractions in response to prostaglandin $F_{2\alpha}$ in strips from control rats, but the NO synthase inhibitors did not influence the concentration-response curves for prostaglandin $F_{2\alpha}$ in preparations from the control animals. A direct action of the NO synthase inhibitors on smooth muscle tone should, therefore, be considered. The observation that the contractile effect of aminoguanidine was less pronounced in preparations from lipopolysaccharide-treated rats might suggest that treated animals are less sensitive to this direct action, but this was not confirmed with the 3 other NO synthase inhibitors. That inducible NO synthase is not involved in the hyporeactivity of the rat gastric fundus to prostaglandin $F_{2\alpha}$, 12 h after lipopolysaccharide treatment, is consistent with the inability of lipopolysaccharide (10 mg/kg i.p.) to induce inducible NO synthase mRNA in the rat stomach 8 and 24 h after treatment (Chen et al., 1996).

Lipopolysaccharide can also induce the inducible isoform of cyclooxygenase (Swierkosz et al., 1995). Prostaglandins are involved in the pyrogenic response to lipopolysaccharide (Parrott et al., 1995) and have also been implicated in the haemodynamic changes induced by lipopolysaccharide. In a pig model of endotoxin shock, the cyclooxygenase inhibitor indomethacin prevented the haemodynamic changes (Mozes et al., 1991) but in a rabbit model the haemodynamic derangements induced by lipopolysaccharide were not influenced by indomethacin (Fink et al., 1988). Indomethacin (10^{-5} M incubated for 20 min) restored the decreased response to sympathetic nerve stimulation in the perfused mesenteric bed of lipopolysaccharide-treated rats (Fatehi-Hassanabad et al., 1995) and we tested this concentration in our model. A reduction of the tone induced by indomethacin has been reported before (Lefebvre and Burnstock, 1990) and suggests that prostaglandins to some extent contribute to the maintenance of basal tone in the rat gastric fundus, as is also the case in other isolated gastrointestinal smooth muscle preparations (see Bennett and Stockley, 1977). Indomethacin increased the contractile response to prostaglandin $F_{2\alpha}$ in the fundus of lipopolysaccharide-treated rats but the same effect was observed in the fundus of controls so that no evidence for the involvement of relaxant prostaglandins in the hyporeactivity to the contractile prostaglandin $F_{2\alpha}$ after lipopolysaccharide treatment was obtained.

The influence of dexamethasone suggests that a protein, whose induction can be suppressed, is involved in the hyporeactivity to prostaglandin $F_{2\alpha}$, 12 h after lipopolysaccharide treatment. The process seems to interfere selectively with contractions elicited by prostaglandin $F_{2\alpha}$, as those elicited by KCl and serotonin were not influenced. This contrasts with the hyporeactivity related to inducible NO synthase in vascular tissue, where the response to contractile agents, acting through different mechanisms, is suppressed (Bigaud et al., 1990), which can be explained by functional antagonism of the contractile agents by relaxant NO. The KCl-induced contraction is due to entry of extracellular calcium via voltage-operated calcium channels (Godfraind and Kaba, 1972) but the contractions elicited by both prostaglandin $F_{2\alpha}$ and serotonin are related to phosphatidylinositol metabolism. Prostaglandin F receptors are coupled to phosphatidylinositol turnover via Gq proteins (Ito et al., 1994; Lake et al., 1994). The 5-HT receptor mediating contraction in the rat gastric fundus has proved difficult to characterize. It most closely resembles the 5-HT_{2B} receptor subtype and is also coupled to phosphatidylinositol turnover (Baxter et al., 1994; Cox and Cohen, 1995). If a mediator induced by lipopolysaccharide interfered with the phosphatidylinositol pathway, a depression of both prostaglandin $F_{2\alpha}$- and serotonin-induced contractions would be expected, which was not the case. A possible explanation for the selective depression of the contractions elicited by prostaglandin $F_{2\alpha}$ is downregulation of the prostaglandin F receptors by lipopolysaccharide treatment. This has been described in rat liver plasma membranes after chronic lipopolysaccharide treatment (Deaciuc and Spitzer, 1991). Ligand binding studies should be performed to investigate this possibility.

In the rat anococcygeus muscle, a tissue in which NO is the major neurotransmitter of the inhibitory nonadrenergic noncholinergic neurones (Gillespie et al., 1989; Song et al., 1993), responses to inhibitory nonadrenergic noncholinergic nerve stimulation were markedly reduced 4 h after pretreatment with lipopolysaccharide (20 mg/kg i.p.). Because the response to sodium nitroprusside was impaired, a postjunctional mechanism was suggested (Guc et al., 1991). In the rat gastric fundus, the electrically induced nonadrenergic noncholinergic relaxations were not influenced 4 h after lipopolysaccharide treatment and were only moderately reduced 12 h after lipopolysaccharide treat-

ment. This seemed not related to a decreased release of NO as the inhibitory effect of L-NAME was maintained.

In conclusion, the contractility of the rat gastric fundus to prostaglandin $F_{2\alpha}$ is not influenced by 4 h pretreatment with lipopolysaccharide but is depressed 12 h after treatment with lipopolysaccharide. This decreased response is not observed with KCl and serotonin, and is not mediated via generation of nitric oxide. Downregulation of prostaglandin F receptors by lipopolysaccharide treatment might be involved.

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