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Role of thromboxane TP and angiotensin AT_1 receptors in lipopolysaccharide-induced arterial dysfunction in the rabbit: An *in vivo* study

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ABSTRACT

Inflammation plays a major role in pathological conditions leading to cardiovascular events. Administration of lipopolysaccharide to animals decreases arterial blood flow, in contrast to the dilatations that occur in microvessels. The purpose of the present study was to determine whether or not lipopolysaccharide, *in vivo*, evokes arterial constriction and if so the underlying mechanisms. Rabbits were anaesthetized, blood pressure monitored and femoral artery diameter continuously recorded with an echotracking device. Lipopolysaccharide induced leucopenia, thrombocytopenia, acidosis and a progressive hypotension with a decrease in femoral artery diameter ($-30.7 \pm 2.4\%$ after 3 h) and an increase in arterial rigidity. Three hours after lipopolysaccharide administration, the arterial dilatations to acetylcholine, arachidonic acid and iloprost were inhibited while that to sodium nitroprusside was not altered; the constrictions to norepinephrine, angiotensin II, U46619 (thromboxane analog) and serotonin were not modified. Under control conditions endothelin-1 produced an endothelin ET_B dependent dilatation, reversed after lipopolysaccharide to an endothelin ET_A dependent constriction. The thromboxane TP receptor antagonist S 18886 partially blocked the constriction; the angiotensin AT_1 receptor antagonist candesartan prevented it. S 18886 normalized the impaired dilatations to acetylcholine, antagonists of 5-HT-receptors partially restored them while candesartan was ineffective. Antagonists of the endothelin or the histamine receptors had no effect. The present data show that lipopolysaccharide-induced inflammation causes 1) a strong constriction of the femoral artery in which activation of both thromboxane and angiotensin AT_1 receptors is involved 2) a reduction of the endothelium-dependent dilatation to acetylcholine attributed to the activation of thromboxane TP receptors.

1. Introduction

Blood vessel tone depends on a delicate balance between various vasodilator and vasoconstrictor mechanisms. The endothelium, the sympathetic system as well as the renin–angiotensin system play a preponderant role in regulating this fine equilibrium. In metabolic and cardiovascular diseases, including hypertension, atherosclerosis and diabetes, this balance shifts toward vasoconstriction and is accompanied by endothelium disruption, platelet and leucocyte–vascular wall interaction, increased oxidative stress and endothelial dysfunction (Drexler and Horning, 1999; Pompilio et al., 2001). These conditions promote cardiovascular accidents, due to the reduced dilator mechanisms, rigidity and thickening of the arterial wall, atherosclerotic lesions, spasm and thrombosis.

Inflammatory mechanisms within the arterial wall play a major role in the pathological conditions leading to cardiovascular events (Huang and Vita, 2006; Granger et al., 2004; Virdis and Schiffrin, 2003). Experimental systemic inflammation has mainly been related to two pathological models: septic shock and superficial vein pathology. Indeed, systemic inflammation can lead to multiple organ dysfunction and septic shock (Parillo, 1993) which, in various animal models, have been reproduced by administration of bacterial endotoxins such as lipopolysaccharides, or cytokines (Auclair et al., 1982; Hauser et al., 1995; Julou-Schaeffer et al., 1990; Vayssettes-Courchay et al., 2002, 2003). Injection of these substances leads to dilatation of arterioles and microvessels, to plasma extravasation and to a fatal decrease in blood pressure. However, lipopolysaccharide decreases blood flow in large arteries, such as the rabbit aorta as noted by Losser et al. (1997) and the rabbit carotid artery as well as the porcine intraventricular coronary artery as observed in preliminary experiments in our laboratory. Such a decrease in blood flow suggests a constriction elicited by systemic inflammation. These constrictions could contribute to the arterial dysfunction described in the early stages of cardiovascular diseases, which are sometimes observed without any evidence for hemodynamic or structural modifications and where systemic inflammation may occur (Arcaro et al., 1995; Harris et al., 1995; Huang and Vita, 2006; Kaplan and Frishman, 2001; Virdis et al., 2005).

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In preliminary experiments we have observed that even much lower doses of lipopolysaccharide than the one used in models of venous disease and sepsis can cause a marked decrease in arterial blood flow. Thus, using a direct ultrasonic echotracking technique, the purpose of the present study was to measure *in situ* arterial diameter and to determine, *in vivo* in anesthetized rabbits, whether or not the administration of lipopolysaccharides, at a low dose to avoid septic shock and mortality, evokes arterial constriction and if so the potential underlying mechanisms. We hypothesized that inflammatory mechanisms within the vascular wall alter the smooth muscle responsiveness to vasoactive agents and cause endothelial dysfunction.

2. Material and methods

2.1. Experimental conditions

This study was in conformity with the European Community Guidelines for the use of experimental animals, with the National Institutes of Health (NIH) guide for the care and use of laboratory animals and was approved by the ethical committee on Animal Experiments of the Servier Research Institute.

Male New-Zealand rabbits (3 to 4.5 kg) were anaesthetized with an injection of sodium pentobarbital (SANOFI 30 mg/kg) into the auricular vein. Anaesthesia was maintained throughout the experiment by i.v. infusion of sodium pentobarbital (5 mg/kg) at the rate of 15 mg/3 ml/h (Braun secure perfusor) into the jugular vein. The trachea was cannulated and ventilation was performed with a respirator (TEM) at a frequency of 38–40 cycles/min, 8 ml/kg and at a pressure of 10–15 cm H_2O. Body temperature was maintained at 39–40 °C with a homeothermic blanket (Harvard) connected to a rectal probe. In some experiments, the femoral nerve was exposed, ligated upstream and activated with a stimulation at 7–10 V, 6–20 Hz, 2–4 ms. Additionally, in some experiments, blood samples (0.2 ml) were taken in order to determine leukocyte and platelet count, hematocrit as well as pO_2, pCO_2, and pH (Beckman Coulter).

Compounds were administered i.v. through the auricular vein or locally into the femoral artery via a catheter inserted into a collateral branch situated 1 cm upstream of the ultra-sound probe.

2.2. Recording

In rabbits, arterial blood pressure was recorded via a catheter inserted into the carotid artery, connected to a Statham P23 *L Gould transducer. Direct and mean arterial blood pressure were measured by a Gould pressure transducer and a Gould DC amplifier, visualized on a Gould RS 3600 recorder and analyzed with an Acknowledge acquisition and analysis system (Biopac).

A non-invasive 10 MHz ultra-sound probe was placed above the femoral artery to measure the internal diameter (Vayssettes-Courchay et al., 2000) by an ultrasonic echotracking device (Nius02, Asulab Research Laboratory). The probe was first positioned with the Doppler mode and then adjusted with the pulse-echo mode to have the focal of the probe at the center of the artery. The back-scattered echoes from both the anterior and posterior wall were tagged electronically, recorded and analyzed by the Nius2 device to measure precisely the diameter of the artery (Fig. 1). Blood velocity was also measured with a Doppler probe and blood flow was then computed with the Nius02 coupled to a computer PC Pentium-233 MHz. The pulsatile compliance of the artery was calculated as pulsatile diameter/pulsatile pressure and the arterial rigidity as 1/pulsatile compliance.

2.3. Experimental protocols

In rabbits, arterial blood pressure was continuously recorded throughout the experiments. Blood pressure and femoral artery diameter were allowed to stabilize for at least 30 min. Then,

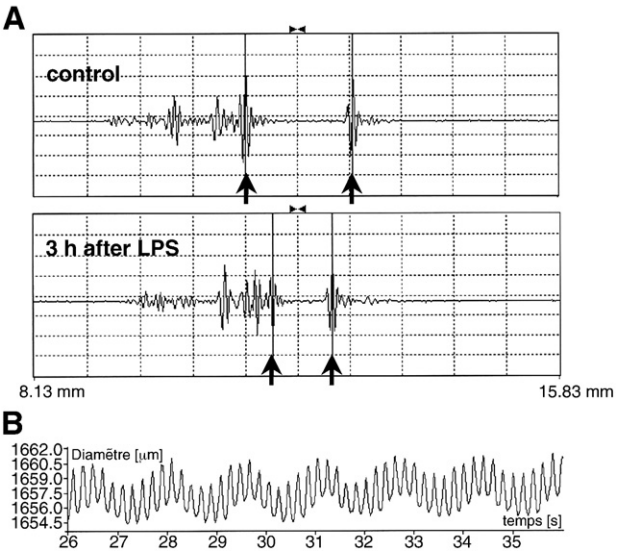

Fig. 1. Echotracking measurement of arterial diameter. A: Example of echotracking signals obtained in the rabbit femoral artery. The arrows indicate the tagged arterial wall position in control conditions and 3 h after lipopolysaccharide administration. B: Example of raw data recording of the femoral artery diameter.

vasoactive agents were administered into the collateral of the femoral artery (three compounds in one animal with 30 min interval) and these injections were repeated 3 h after either lipopolysaccharides or saline administration (4 ml/15 min). The femoral artery diameter was determined before each injection and then at 0.5, 1, 2, 3, 5 and 10 min after injection, and at 5 min interval until the diameter returned to basal values.

The effects of lipopolysaccharides or saline on other parameters were measured before infusion, every 5 min during the infusion and then every 30 min up to 3 h after the cessation of the infusion.

Antagonists were administered by continuous i.v. infusion beginning 15 min before the lipopolysaccharide administration. The parameters were determined before treatment, before lipopolysaccharide and then, as described above, during the next 3 h.

In some control and lipopolysaccharide-treated rabbits, the femoral artery was collected and cut in 5 µm-thick sections. Then, a haematoxylin–eosin staining and a RAM11 immuno-histochemical detection of inflammatory cells were performed.

2.4. Analysis

Results are expressed as mean ± S.E.M. Arterial blood pressure is expressed in mm Hg, arterial diameter in µm and arterial blood flow in ml/min. Statistical significance was analyzed by paired Student's *t* test and by one way or two way analysis of variance (ANOVA) followed by complementary Dunnett or Bonferroni test respectively. A value of $P<0.05$ was considered statistically significant. As the lipopolysaccharide treatment markedly altered the basal arterial diameter, a comparison of the effect of the compounds injected locally after lipopolysaccharide versus before could not be performed with a two way ANOVA. The data were then analyzed independently with one way ANOVA.

2.5. Drugs

Lipopolysaccharide: (*E. coli* 0127:B8), acetylcholine chloride, norepinephrine bitartrate (Arterenol), serotonin creatinine sulfate, arachidonic acid (porcine liver), angiotensin II, adrenergic α_1 adrenoceptor antagonist prazosin hydrochloride, histamine H_1

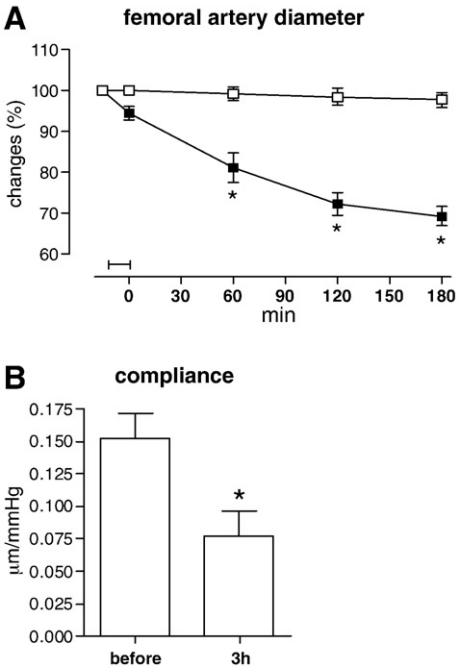

Fig. 2. Effects of lipopolysaccharide. A: Time-dependent effect of lipopolysaccharide (1 mg/kg, $n=9$, black squares), and saline ($n=8$, open squares) on rabbit femoral artery diameter. B: Effect of lipopolysaccharide on femoral artery compliance ($n=8$). The asterisk indicates that the parameters are significantly modified by lipopolysaccharide administration, $P<0.05$.

receptor antagonist mepyramine maleate (Sigma, St Quentin Fallavier, France). NO donor sodium nitroprusside (Prolabo, Fontenay-sous-Bois, France); prostacyclin analog iloprost in methyl acetate, endothelin ET_B receptor antagonist N-cis-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-l-methoxycarboyl-D-norleucine (BQ788) and ET_A receptor antagonist cyclo-[$_D$Trp-$_D$Asp-Pro-$_D$Val-Leu] (BQ123), thromboxane A_2 analog U 46619 ethyl acetate, (Cayman, Ann Arbor, MI, U.S.A.). Endothelin-1 human (Neosystem, Strasbourg, France). Serotonin 5-HT_2 receptor antagonist: ritanserin, (RBI). Thromboxane TP receptor antagonist: S 18886 (terutroban, Servier Research Institute); angiotensin AT_1 receptor antagonist: candesartan, endothelin $ET_{A/B}$ receptor antagonist: 4-ter-butyl-N-[6-(2 hydroxy-ethoxy)-5-(3-methoxy-phenoxy)-pyrimidin-4-yl]-benzenesulfonamide (RO 462005), serotonin 5-$HT_{1B/D}$ antagonist: N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide HCl (GR127935) (Servier Research Institute). All the drugs were dissolved daily in saline solution, at the exception of prazosin which required 5% DMSO.

3. Results

3.1. Effects of lipopolysaccharide administration

Three hours after lipopolysaccharide administration (1 mg/kg) the femoral artery blood flow was decreased by 56 ± 8% (from 9.3 ± 1.8 to 5.4 ± 2.0 ml/min $n=10$) and mean arterial blood pressure from 80 ± 4 to 36 ± 3 mm Hg without any lethality being observed. The heart rate was not altered (307 ± 9 from 308 ± 10 bpm). The number of circulating platelets and leukocytes was reduced from 247 ± 31 to 138 ± 18 · 10^3/µl and from 1625 ± 138 to 800 ± 58/µl, respectively and the arterial pH decreased from to 7.46 ± 0.02 to 7.24 ± 0.03 ($n=4$–5). The femoral artery diameter was significantly reduced by approximately 30%, ($n=9$, Figs. 1 and 2, Table 1), and the arterial pulsatile compliance was decreased by 47% (Fig. 2), leading to an increased arterial rigidity index from 7.5 ± 1.0 to 16.3 ± 2.1 mm Hg/µm ($n=8$; $P<0.05$).

Under similar conditions, a saline infusion ($n=8$) did not significantly affect the various measured parameters: mean blood pressure was 79 ± 7 from 82 ± 6 mm Hg, blood flow was 11.7 ± 2 from 12.5 ± 2.9 ml/min and arterial diameter was 1379 ± 54 from 1413 ± 57 µm.

There was no major histological alteration (haematoxylin–eosin staining) and no evidence for inflammatory cell infiltration (RAM11 immuno-histological staining) in the femoral artery of lipopolysaccharide-treated rabbits when compared to that of control animals (data not shown).

3.2. Effects of lipopolysaccharide (3 h) on arterial vasoreactivity

A 3 h saline infusion did not significantly modify the effects of any of the studied vasoactive mediators The different vasoactive agents injected locally into the femoral artery did not modify the mean arterial blood pressure (data not shown).

Acetylcholine (0.1 µg)-induced dilatation was significantly reduced 3 h after lipopolysaccharide administration ($n=8$, Fig. 3). Furthermore, the dilatations in response to arachidonic acid (50 µg, $n=6$) and to the prostacyclin analog iloprost (1 µg, $n=5$) were also decreased (Fig. 3); iloprost becoming a weak vasoconstrictor (−2.2 ± 0.4%). In contrast, the dilatation to sodium nitroprusside (5 µg) was not altered (Fig. 3).

In order to assess the influence of vasoconstriction on the ability of acetylcholine to produce a vasodilatation, additional control experiments were performed by reducing the arterial diameter via femoral

Table 1
Effects of various pretreatments and lipopolysaccharide on basal values of arterial pressure and femoral artery diameter.

Treatments	N	Mean arterial pressure (mm Hg)			Femoral artery diameter (µm)			Changes (%)
		Before treatment	Before LPS	3 h after LPS	Before treatment	Before LPS	3 h after LPS	Femoral diameter
Saline	8		82 ± 6	79 ± 7		1413 ± 57	1379 ± 54	−2.3 ± 1.7
LPS	10	–	80 ± 4	36 ± 3	–	1500 ± 93	1067 ± 83	−29.2 ± 2.5
S 18886	5	87 ± 3	86 ± 2	44 ± 3	1676 ± 72	1675 ± 70	1293 ± 122	−23.0 ± 6.1[a]
Candesartan	5	90 ± 8	70 ± 7	23 ± 2[a]	1528 ± 62	1490 ± 64	1443 ± 63	−3.0 ± 3.3[a]
Nerve section	5	78 ± 2	70 ± 2	37 ± 5	1613 ± 31	1618 ± 91	1113 ± 168	−32.4 ± 6.8
GR127935	4	84 ± 6	85 ± 5	37 ± 4	1416 ± 58	1414 ± 36	952 ± 77	−32.7 ± 5.0
Ritanserin	5	87 ± 6	86 ± 6	43 ± 4	1544 ± 39	1535 ± 36	1105 ± 81	−29.0 ± 4.5
RO462005	6	80 ± 4	75 ± 5	32 ± 2	1445 ± 48	1417 ± 54	925 ± 29	−33.9 ± 4.8
Mepyramine	4	86 ± 8	83 ± 8	59 ± 6[a]	1439 ± 60	1426 ± 63	989 ± 83	−30.0 ± 6.0
Prazosin	3	91 ± 5	73 ± 1	34 ± 4	1581 ± 170	1701 ± 184	1302 ± 207	−25.0 ± 4.0

The table shows the basal values, the values measured 15 min after the beginning of the infusion with the antagonists (before lipopolysaccharide administration) and 3 h after lipopolysaccharide (LPS) for arterial blood pressure and femoral artery diameter. The right column shows the % of femoral artery diameter 3 h after lipopolysaccharide versus before lipopolysaccharide.
Data are shown as means ± S.E.M.
[a] Indicates that the effect on mean arterial blood pressure and the decrease in diameter is significantly less than that measured with lipopolysaccharide without treatment, $P<0.05$.

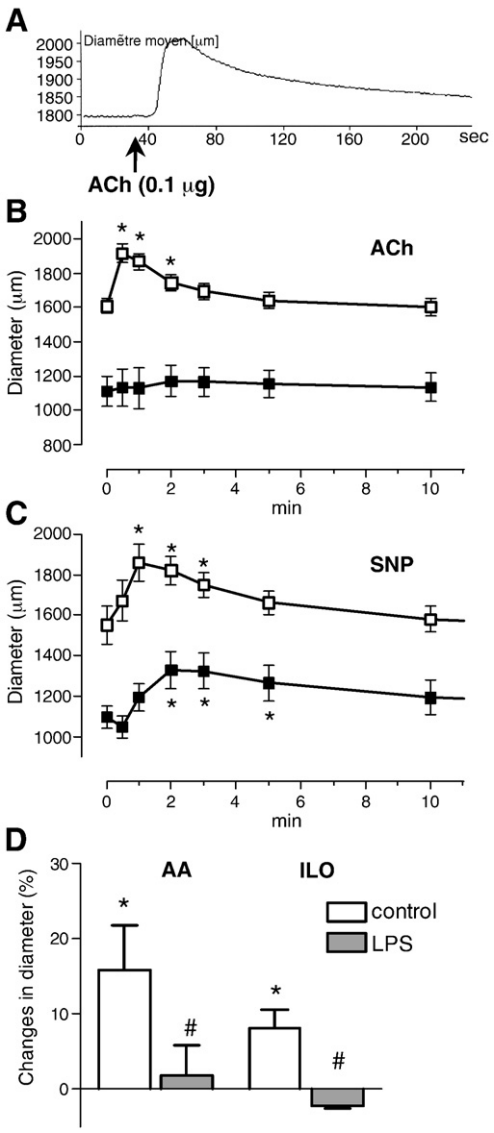

Fig. 3. Effects of vasodilators on rabbit femoral artery diameter. A: Original trace showing the changes in rabbit femoral artery diameter obtained after a local injection of 0.1 µg acetylcholine. B, C: Time-dependent effect of local intra-arterial injections of acetylcholine (ACh, 0.1 µg, $n=6$, A) and sodium nitroprusside (SNP, 5 µg, $n=5$, B) on the femoral artery diameter before (open squares) and 3 h after lipopolysaccharide administration (filled squares). The asterisk indicates a significant effect of a compound, $P<0.05$. D: Maximal effect of arachidonic acid (AA) and iloprost (ILO) on the femoral artery diameter in control (open bars) and 3 h after lipopolysaccharide administration (grey bars). The asterisk indicates a significant effect of the compound and the sharp sign indicates a significant change in the response versus before lipopolysaccharide $P<0.05$.

nerve stimulation. Acetylcholine increased the femoral artery diameter by 20.1 ± 3.2% in control conditions. After nerve stimulation which produced a significant reduction in artery diameter from 1528 ± 86 to 1303 ± 67 µm, acetylcholine still evoked a similar dilatation (20.9 ± 2.8%, $n=5$).

Lipopolysaccharide administration did not significantly influence the constricting effects of norepinephrine (0.1 µg, $n=5$), angiotensin II (0.1 µg, $n=7$), the thromboxane A_2 analog U46619 (1 µg, $n=8$) and serotonin (3 µg, $n=5$), Fig. 4.

Under control conditions, endothelin (0.5 µg) induced a dilatation (Fig. 5), which was inhibited by the endothelin EtB receptor antagonist BQ788 at 10 nmol/kg (Fig. 5B, $n=4$). In a different subgroup of rabbits, it was observed that the dilatation to endothelin-1 was abolished by lipopolysaccharide administration. Under

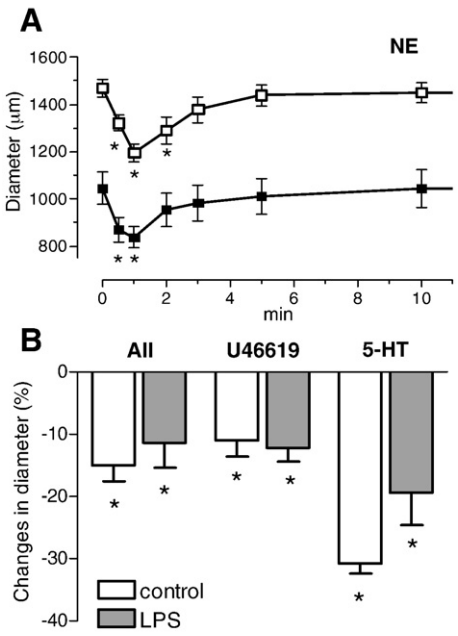

Fig. 4. Effects of vasoconstrictors on rabbit femoral artery diameter. A: Time-dependent effect of local intra-arterial injections of norepinephrine (NE, 0.1 µg, $n=5$) before (open squares) and 3 h after lipopolysaccharide administration (filled squares). The asterisk indicates a significant effect of a compound, $P<0.05$. B: Maximal effect of angiotensin II (AII 0.1 µg, $n=7$), U 46619 (1 µg, $n=8$) and 5-HT (3 µg, $n=5$) on the femoral artery diameter in control (open bars) and 3 h after lipopolysaccharide administration (grey bars). The asterisk indicates a significant effect of a compound, $P<0.05$.

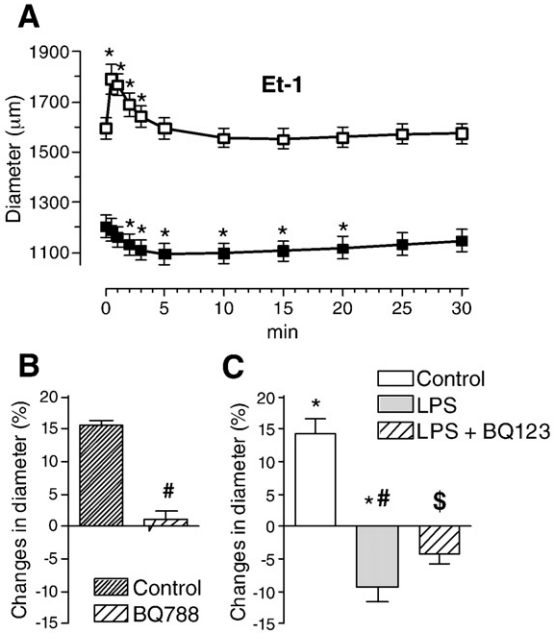

Fig. 5. Effects of ET-1 on rabbit femoral artery diameter. A: Time-dependent effect of local intra-arterial injections of endothelin-1 (Et-1, 0.5 µg, $n=5$) on the femoral artery diameter before (open squares) and 3 h after lipopolysaccharide administration (filled squares). B: Maximal effects of endothelin-1 (Et-1, 0.5 µg) on femoral artery diameter before (grey hatched bar) and after BQ 788 (10 nmol/kg, black hatched bar) in a specific subgroup of 4 rabbits. C: Maximal effects of endothelin-1 (Et-1, 0.5 µg, $n=5$) on femoral artery diameter recorded in a different subgroup of rabbits, before lipopolysaccharide (open bar), 3 h after lipopolysaccharide (grey bar) and after lipopolysaccharide plus BQ123 (5 nmol/kg, $n=5$) (black hatched bar). The asterisk indicates that the effect of the compound is significantly modified by the presence of lipopolysaccharide, $P<0.05$. The sharp sign indicates that the presence of an endothelin receptor antagonist significantly affect the effect of endothelin-1, $P<0.05$.

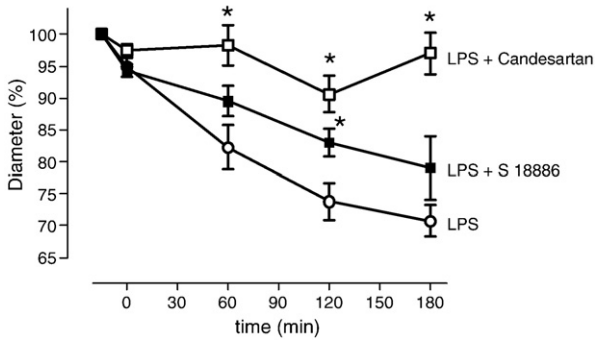

Fig. 6. Effects of an antagonist of the AT-1 receptor and an antagonist of the TP receptor on lipopolysaccharide-induced constriction of the rabbit femoral artery. Time-dependent effect of lipopolysaccharide (1 mg/kg, $n=9$) in the absence (open circle) or in the presence of an infusion of either candesartan (open squares, 0.1 mg/kg/h, $n=5$) or S 18886 (filled squares, 0.1 mg/kg/h, $n=5$). The asterisk indicates a significant effect of a compound, $P<0.05$.

these conditions, endothelin-1 caused a slowly developing constriction which was inhibited by the endothelin ET_A receptor antagonist BQ123 (5 nmol/kg) (Fig. 5C, $n=5$).

3.3. Mechanisms of lipopolysaccharide-induced femoral alterations

The antagonists were administered by continuous infusion at 0.1 mg/kg/h for the thromboxane receptor antagonist S 18886, the angiotensin AT_1 receptor antagonist candesartan, the serotonin 5-HT_2-receptor antagonist ritanserin, the serotonin 5-$HT_{1B/D}$ receptor antagonist GR 127935 and the adrenergic α_1-adrenoceptor antagonist prazosin while the endothelin $ET_{A/B}$ receptor antagonist RO462005 was infused at the rate of 1 mg/kg/h and the histamine H_1 receptor antagonist mepyramine was infused at 3 mg/kg/h. The infusion of the various antagonists started 15 min before lipopolysaccharide administration and did not significantly affect the arterial diameter or mean blood pressure, at the exception of candesartan and prazosin which produced a decrease in blood pressure (Table 1). Both femoral artery diameter and mean arterial blood pressure were stable when lipopolysaccharide was administered. The decrease in blood pressure was reduced by the treatment with mepyramine (59 ± 6 mm Hg 3 h after lipopolysaccharide administration); the other treatments did not modify the effect of lipopolysaccharide on blood pressure (Table 1).

The decrease in femoral artery diameter caused by lipopolysaccharide was significantly reduced by the presence of candesartan (Fig. 6, Table 1, $n=5$) and to a lesser extent by that of S 18886 ($n=5$) (Fig. 6). Ritanserin ($n=5$), GR 127935 ($n=4$), RO 462005 ($n=6$), mepyramine ($n=4$), prazosin ($n=3$) and femoral nerve section ($n=5$) did not alter lipopolysaccharide-induced constriction at any time over the 3 h period. The doses used have been previously shown to be effective in the rabbit *in vivo* and are confirmed by the absence of effect of angiotensin II, serotonin, U 46619, L-phenylephrine and endothelin injected locally at the end of the experiments using candesartan, 5-HT antagonists, S 18886, prazosin and RO462005 respectively (data not shown).

The response to acetylcholine before LPS was statistically comparable in each group, averaging $19\pm1.2\%$. The inhibitory effect of lipopolysaccharide on acetylcholine-induced dilation response was prevented by the presence of S 18886 ($13.7\pm3.3\%$, versus $5.4\pm1.6\%$ in untreated rabbits, Fig. 7) and to a lesser extent by that of ritanserin or GR 127935 (8.1 ± 2.3 and $9.6\pm2.8\%$ respectively). However, the inhibitory effects of lipopolysaccharide on acetylcholine-induced vasodilatation were not altered by the presence of candesartan, RO 462005, mepyramine, prazosin or nerve section (4.6 ± 2.1, 2.8 ± 1.3, 5.3 ± 3.7 and $5.2\pm1.4\%$ respectively) (Table 2).

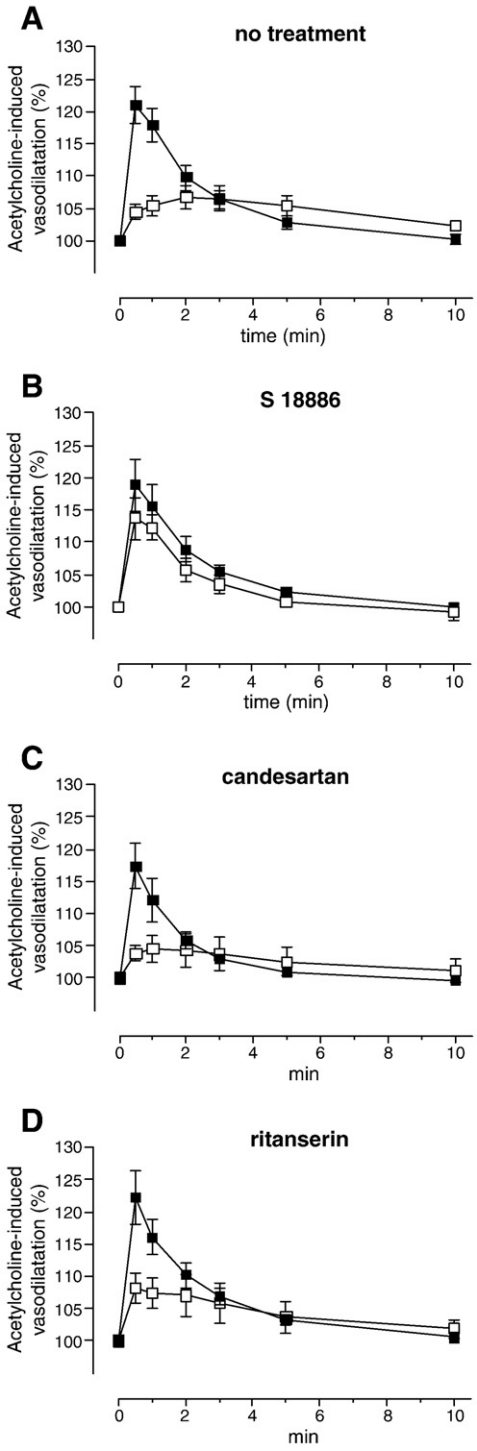

Fig. 7. Effect of acetylcholine (ACh) on rabbit femoral artery diameter. Time-dependent effect of ACh (0.1 µg) before (black circle) and after lipopolysaccharide (open squares) under control conditions (A) or after treatment with S 18886 (0.1 mg/kg) (B), candesartan (0.1 mg/kg) (C) or ritanserin (0.1 mg/kg) (D). Data are expressed in % of basal values.

4. Discussion

Using direct recording via echotracking of arterial diameter *in vivo* in the rabbit, we could investigate the effects of systemic inflammation on femoral artery diameter and reactivity and give evidence for the first time that systemic inflammation profoundly alters the functional arterial integrity. The present study demonstrates that the

Table 2
Effects of various pretreatments and lipopolysaccharide on acetylcholine-induced femoral artery dilatations.

Treatments	*N*	Acetylcholine (0.1 μg) A: before treatment dilatation (μm)	Acetylcholine (0.1 μg) B: 3 h after LPS dilatation (μm)	Inhibition by LPS B versus A (%)
No treatment	9	322 ± 38[a]	60 ± 17	78 ± 5
S 18886	5	318 ± 61[a]	184 ± 38[a]	21 ± 17[b]
Candesartan	5	275 ± 58[a]	54 ± 14	70 ± 12
Nerve section	5	254 ± 48[a]	80 ± 47	67 ± 12
GR127935	4	346 ± 49[a]	96 ± 33[a]	64 ± 16
Ritanserin	5	348 ± 61[a]	95 ± 30[a]	71 ± 8
RO462005	5	209 ± 35[a]	41 ± 11	85 ± 3
Mepyramine	4	389 ± 62[a]	61 ± 44	86 ± 14
Prazosin	3	294 ± 96	105 ± 52	73 ± 11

The table shows the effects of acetylcholine administered locally at 0.1 μg on the femoral artery diameter before treatment (A) and 3 h after lipopolysaccharide (LPS) injection (B). The inhibitory effect of lipopolysaccharide is also indicated (C).
Data are shown as means ± S.E.M.
[a] Indicates a significant effect of acetylcholine on arterial diameter.
[b] Indicates that the inhibitory effect of lipopolysaccharide on acetylcholine-induced dilatation has been significantly affected by a treatment, $P<0.05$.

decreased in blood flow observed after lipopolysaccharide administration is due to a strong and progressive arterial constriction caused by the concomitant activation of the AT-1 and TP receptors, a phenomenon associated with a major endothelial dysfunction.

Arterial blood flow depends not only on the changes in blood vessel diameter but also on changes in cardiac output and/or on modifications in the downstream microcirculation and arteriovenous shunts. In the present study, the arterial constriction induced by lipopolysaccharide is directly demonstrated by arterial diameter recording. This is in contrast with the simultaneous dilatation of resistant vessels, as previously described in arterioles and microvessels (Bouskela and Rubanyi, 1995; Mayhan, 2005) and as evidenced by the decrease in arterial blood pressure. Moreover the present data show that the arterial constriction is accompanied by a reduction of the arterial compliance, which represents a major risk factor of cardiovascular accidents (Laurent and Boutouyrie, 2007; Schiffrin, 2004). Reduced distensibility is due either to increased blood pressure or to arterial wall properties; it occurs here despite a decrease in blood pressure and is likely due to inflammatory mechanisms within the vascular wall, in agreement with previous observations in human (Mahmud and Feely, 2005).

The dose of lipopolysaccharide, used in the present study was only 1/10th of the dose commonly used for this given serotype to generate sepsis in rabbits. Therefore, in the time course of the experimental protocol, this dose did not produce any mortality and did not significantly modify venous flow and diameter (data not shown) and heart rate. However, the thrombocytopenia, leucopenia, acidosis and the decrease in blood pressure confirm the inflammatory process generated by lipopolysaccharide administration (Feletou et al., 1996). However, there was no direct evidence for an inflammation in the femoral artery wall itself since there was no structural change or immune cell infiltration observed after 3 h exposure to lipopolysaccharides

Since the femoral artery was markedly constricted during this systemic inflammation, it was hypothesized that its reactivity could be altered. The preponderant mechanisms that endogenously play a vasomotor role in the femoral artery have been tested using appropriate agonist/activators. Vasoactive compounds were administered locally into the femoral artery, thus avoiding hemodynamic effects and reflexes, which would have complicated the analysis. The arterial constrictor effect of norepinephrine, serotonin and of the stable analog of thomboxane A_2, U 46619, were not altered by the administration of lipopolysaccharides. However, the endothelium-dependent vasodilatations induced by acetylcholine and the partially endothelium-dependent vasodilatations to arachidonic acid (Campbell and Falck, 2007), were virtually abolished. The inhibition of the dilatation to acetylcholine could not be attributed to the reduced arterial diameter *per se* provoked by lipopolysaccharide, since the response produced by acetylcholine was maintained when a similar level of constriction was elicited by nerve stimulation. The vasodilatations induced by the nitrovasodilator sodium nitroprusside were unaltered whereas those to the prostacyclin analog iloprost were inhibited by systemic inflammation. These data show that, in the femoral artery, systemic inflammation produces an endothelial dysfunction and, at the smooth muscle cell, the prostacyclin IP-cAMP-pathway is dysfunctional while the cGMP pathway appears to be preserved. Iloprost tended to induce a slight constriction after lipopolysaccharide, which could possibly be attributed to the activation of thromboxane TP receptors, as it has already been shown in other pathologic blood vessels (Cheng et al., 2002; Gluais et al., 2005). Under control conditions endothelin-1 produced a vasodilatation that was inhibited by a selective endothelin ET_B receptor antagonist. Lipopolysaccharide abolished the dilatation and revealed an endothelin ET_A receptor-dependent constrictor effect. This is consistent with the impairment of the dilatations observed in response to acetylcholine, arachidonic acid and iloprost as the relaxations following endothelin Et_B receptor activation are associated with the release of nitric oxide and prostacyclin (Clozel et al., 1992; D'Orleans-Juste et al., 2002). Endothelial dysfunction is a major component of cardiovascular diseases and has been observed in humans and in animal models of atherosclerosis, hypertension, chronic heart failure and diabetes (Drexler and Horning, 1999; Schiffrin, 2001; Stähli et al., 2004; Taddei et al., 1993; Wort and Evans, 1999). It also occurs in arterioles and microcirculation during inflammation in animals and humans (Vila and Salaices, 2005). This study shows that this phenomenon is also present in a large artery during acute systemic inflammation. Endothelial dysfunction is characterized by an impairment of endothelial dilator mechanisms (nitric oxide, prostacyclin and/or endothelium-derived-hyperpolarizing-factor) but can also involve the generation of endothelial constrictors [endothelin, cyclo-oxygenase dependent arachidonic acid constrictor metabolites (endoperoxide, thromboxane A_2) and oxygen derivatives]. The present observations are thus in agreement with a redox mechanism leading to increased endothelial constrictor and decreased endothelial dilator responses via superoxide, nitric oxide inactivation and peroxinitrite formation. Indeed, in lipopolysaccharide-treated mice, the overexpression of superoxide dismutase prevented the endothelial dysfunction by accelerating superoxide elimination (Didion et al., 2004).

The arterial constriction induced by this systemic inflammation could be explained either by the deletion of a vasodilator mechanism or by the production of constricting factors. It appears unlikely that the powerful constriction induced by lipopolysaccharide would be due exclusively to a reduced dilator mechanism, since under similar experimental conditions acute inhibition of NO-synthase did not cause significant reduction of the rabbit femoral artery diameter. Therefore the generation of a constrictor mechanism is the most likely explanation for arterial lipopolysaccharide-induced constriction.

An increase in sympathetic nerve activity, which has been described during sepsis and after lipopolysaccharide-induced inflammation (Palsson et al., 1988; Vayssettes-Courchay et al., 2002, 2005), in agreement with the observed increased level of catecholamines in human and animals (Groves et al., 1973; Jones et al., 1994), could have explained the femoral artery constriction. Furthermore, an increase in sympathetic nerve activity is also produced after such a decrease in blood pressure, via the baroreflex pathway. However, since local denervation with the section of the femoral nerve and adrenergic α_1 adrenoceptor blockade with prazosin did not affect lipopolysaccharide-induced constriction, sympathetic activation is unlikely to be involved.

Vasoactive substances are released during the inflammatory process, angiotensin II, serotonin, endothelin and arachidonic acid-derived metabolites which activate thromboxane TP receptors (Ruiz-Ortega et al., 2006; Wort and Evans, 1999). These mechanisms could play a role in the arterial constrictions and/or the endothelial dysfunction. However, antagonists of serotonin-5-HT_2 or 5-$HT_{1B/D}$, histamine H_1 and endothelin $ET_{A/B}$ receptors did not affect the lipopolysaccharide-dependent femoral artery constriction, ruling out a predominant role for these mediators. In contrast, angiotensin AT_1 receptors and to a lesser extent the thromboxane/endoperoxide TP receptors appear to be involved since candesartan prevented the vasoconstriction and S 18886 partially inhibited it. The role of angiotensin receptors shown here is in agreement with *in vitro* experiments in mice aorta (Lund et al., 2007). An increase in the renin–angiotensin system activity is a physiological response to compensate the decrease in blood pressure. Angiotensin II constricts arteries by direct activation of angiotensin AT_1 receptors on smooth muscle cells and via an increased production of superoxide and other endothelium-dependent constrictors. It has been shown that thromboxane A_2 can be partly involved in the vasoconstrictor effect of angiotensin (François et al., 2004). The inhibitory effect of S 18886 on lipopolysaccharide-induced constrictions could be attributed to the release of thromboxane A_2 and/or the endoperoxide PGH_2 by angiotensin II or to an increase production of isoprostanes, also ligands of the thromboxane TP receptors. The origin of the thromboxane TP receptor activation during systemic inflammation needs to be further explored both *in vivo* and in isolated blood vessels. In the rabbit femoral artery, the possible contribution of TP-receptor activation in the mechanisms underlying the vasoconstriction to angiotensin II, *in vivo*, has never been shown and experiments are currently underway in order to assess this hypothesis.

Acetylcholine dilatations are not affected by the antagonists of the endothelin $ET_{A/B}$ and the angiotensin AT_1 receptors, but are partially restored by antagonists of the serotonin 5-HT_2 or 5-$HT_{1B/D}$ receptors and virtually normalized by S 18886, the potent and specific antagonist of the thromboxane TP receptors. Therefore serotonin, possibly derived from platelets, is likely to be involved in the endothelial dysfunction while thromboxane TP receptors are strongly implicated in this phenomenon. Thromboxane may also originate from activated platelets, but additionally, acetylcholine can induce the endothelial release of constrictor prostanoids, as it has been shown in experimental models of hypertension (Luscher and Vanhoutte, 1986; Yang et al., 2003). Thromboxane TP receptors are activated by various prostaglandins including endoperoxide and isoprostanes whose production is increased in cardiovascular pathologies (Cayatte et al., 2000; Janssen and Tazzeo, 2002). The activation of thromboxane TP receptors, is involve not only in vasoconstriction but also in platelet aggregation and cellular adhesion, and this activation plays a role in inflammatory mechanisms, hypertension, heart failure and atherogenesis (Cohen et al., 2001; Katz et al., 1993; Luscher and Vanhoutte, 1986; Schiffrin, 2001; Verbeuren, 2006). Blockade of thromboxane TP receptors with S 18886 inhibits the endothelium-dependent contraction to acetylcholine in hypertensive rats (Yang et al., 2003); reduces atherosclerosis in mouse and rabbit models (Cayatte et al., 2000; Viles-Gonzalez et al., 2005) and prevents the reduced response of the brachial artery to acetylcholine in coronary patients (Belhassen et al., 2003).

In conclusion, the systemic inflammation induced by lipopolysaccharide administration leads to a strong constriction and an increased rigidity of the artery. The loss of most of the vasodilator mechanisms is associated with a preserved sensitivity to all the vasoconstrictors tested. The artery under these conditions presents features encountered in human pathologies which predispose to vasospasm and thrombosis and therefore to cardiovascular accidents. Different mechanisms are involved in the vascular dysfunction. Thromboxane TP-receptor activation seems to be the major cause of the endothelial dysfunction whereas activation of angiotensin AT_1 receptors appears to be the major cause for the increase of arterial tone.

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