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The suppression by lipopolysaccharide of cytochrome P450-dependent renal vasodilation in the rat is mediated by nitric oxide

Adebayo O. Oyekan *

Faculty of Medical Sciences, The University of the West Indies, St Augustine, Trinidad and Tobago

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Abstract

The isolated perfused kidney of the rat was used to examine the hypothesis that lipopolysaccharide-induced nitric oxide (NO) production inhibits cytochrome P450-dependent vasodilation. The vasodilator responses to arachidonic acid and bradykinin were examined as the response to arachidonic acid is wholly dependent, and that to bradykinin partly dependent on cytochrome P450 metabolism. In endotoxin-treated rats, the vasodilator response to arachidonic acid was inhibited, and those to bradykinin and acetylcholine were enhanced. Following treatment with phenobarbitone, the inducer of certain isoforms of cytochrome P450 enzymes, the vasodilator effects of all three agonists, especially that of arachidonic acid, were amplified. Lipopolysaccharide inhibited the effect of phenobarbitone on the vasodilator effect of arachidonic acid and bradykinin but enhanced that of acetylcholine. The effect of lipopolyysaccharide was antagonized by haemoglobin, a NO antagonist, and N^{ω}-nitro-L-arginine, an inhibitor of NO synthase, suggesting that the inhibitory effect of lipopolysaccharide on arachidonic acid- and bradykinin-induced vasodilation was mediated by NO/NO synthase. N^{ω}-Nitro-L-arginine enhanced vasodilation induced by arachidonic acid while that induced by bradykinin or acetylcholine was reduced, implying that endogenous NO inhibits vasodilator cytochrome P450 metabolites in the rat kidney. Pretreatment with dexamethasone, an inhibitor of inducible NO synthase, resulted in inhibition of the lipopolysaccharide modulation of arachidonic acid-induced vasodilation, suggesting that the inducible NO synthase is the target of the inhibitory effect of lipopolysaccharide. The inhibitory effect of lipopolysaccharide was mimicked by nitroprusside, the L-arginine-independent NO donor, and by L-arginine, the biosynthetic precursor of NO. The effect of L-arginine, but not of nitroprusside, was antagonized by N^{ω}-nitro-L-arginine, suggesting a specific role for NO synthase in the inhibitory effect of lipopolysaccharide in the inhibition of cytochrome P450-dependent vasodilation in the rat kidney.

Keywords: Lipopolysaccharide; Cytochrome *P*-450; Arachidonic acid; Nitric oxide (NO)

1. Introduction

Lipopolysaccharide, the endotoxin from the cell walls of Gram-negative bacteria, is a potent immunoactivator which stimulates certain cell types (macrophages and endothelial cells) to release cytokines and reactive oxygen species (Hofmann et al., 1989; Hauschildt et al., 1990). Only recently has nitric oxide (NO) been identified as a mediator of the L-arginine-dependent cytostasis in tumour target cells (Stuehr and Nathan, 1989). NO is a short-lived, lipophilic free radical that is the primary nitrogen oxide formed by macrophages and some tumour cells in response to lymphokines and immunostimulants (Gross et al., 1990; Delaporte et al., 1993). NO has therefore been identified to play not only a physiological role in blood pressure regulation, but also a pathological role in the cardiovascular collapse associated with bacterial endotoxin (Kilbourn et al., 1990; Hauschildt et al., 1990).

In recent studies, the effects of exposure to lipopolysaccharide in vitro and in vivo were attributed to the induction of an NO synthase in the endothelium and smooth muscles (Knowles et al., 1990; Rees et al., 1990). The NO synthase responsible for the pathological release of NO is distinct from the constitutive NO synthase in the vascular endothelium responsible for the physiological regulation of vascular tone and blood pressure (Smith et al., 1991).

Immunological stimuli have long been known to inhibit cytochrome P450-mediated hepatic metabolism of various xenobiotics and endogenous substances. In-

* Corresponding author. Fax (1-809) 645 7428.

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deed, reduced cytochrome P450 activity was reported in animals following bacterial and viral infection or after treatment with cytokines and immunostimulants (Mannering and Deloria, 1986). The mechanism involved in this phenomenon was unknown for a long time. However, recent studies by Wink et al. (1993) and Khatsenko et al. (1993) showed that exposure of rat hepatic microsomes to NO inhibited cytochrome P450 activity. Evidence was also provided that NO produced by immunoactivated cells mediates the suppression of cytochrome P450 enzymes in vitro and in vivo. Therefore, the hypothesis to be tested in this study is that NO inhibits the responses of agents that depend on the cytochrome P450 pathway for expression of biological activity. We have therefore examined the effects of endotoxin, nitroprusside and L-arginine, generators of NO on the vasodilator effects of two agonists, namely, arachidonic acid which depends wholly (Oyekan et al., 1991a), and bradykinin which depends partially (Fulton et al., 1992) on the cytochrome P450 enzyme for expression of vasodilator activity in the rat kidney.

2. Materials and methods

These studies were performed using adult male Sprague-Dawley rats (weight, 283 ± 9 g), bred at the Laboratory Animal facility of the Faculty of Medical Sciences, the University of the West Indies, St Augustine, Trinidad.

2.1. Isolated rat perfused kidney

The rats were anaesthetized with pentobarbital sodium (60 mg/kg i.p.), after which the right kidney was exposed by midline laparotomy and the mesenteric and right renal arteries were cleared of surrounding tissue. Ties were loosely placed around these vessels and the vena cava just above and below the junction with the right renal vein. The right renal artery was then cannulated with a 19-gauge needle via the mesenteric artery to avoid interruption of blood flow and the vena cava was occluded and cut to provide an exit for the perfusate. The right ureter was also cut, and the animal was killed by an intracardiac injection of 10 mg pentobarbital. The kidney was then removed and suspended in a water-jacketed bath at 37°C.

Kidneys were perfused at a constant flow rate by means of a Gilson peristaltic pump (Model MP2 Minipuls) with Krebs' solution at 37°C and gassed with 95% O_2, 5% CO_2. The Krebs' solution used had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.5. Flow was adjusted to obtain a basal perfusion pressure of 75–90 mm Hg. Mean perfusate flow rate at this pressure was 9.4 ± 0.3 ml/min. To amplify vasodilator responses, vascular tone was elevated with phenylephrine ($5–7.5 \times 10^{-7}$ M), which increased perfusion pressure by 83.3 ± 2.8 mm Hg. Vascular perfusion pressure was measured with a Statham transducer and recorded on a Gilson Duograph recorder (Model ICT-2H).

Since the flow was maintained at a constant rate, the change in perfusion pressure was used as an index of change in the resistance of the artery: a fall in perfusion pressure indicated vasodilation; a rise, vasoconstriction. The perfusion pump produced a pulsatile pressure, which was not electrically meaned.

All responses were measured from the minimum of the pulse pressure and are the peak responses.

2.2. Experimental protocol

Renal vascular responses to arachidonic acid and bradykinin

Arachidonic acid (5–20 μg) and bradykinin (0.5, 1 and 2 μg) were administered randomly as a bolus into the perfusate line proximal to the kidney. Responses to acetylcholine (0.1, 0.25 and 0.5 μg) were also determined. Dose-response curves were established to these agonists in kidneys that were treated with certain agents or their respective vehicles. The responses obtained in the treated kidneys were compared with those in the untreated (control) kidneys. The vasodilator response to arachidonic is observed only after the inhibition of cyclooxygenase (Oyekan et al., 1991a); hence, indomethacin (2.8 μM) was routinely included in the perfusate. The final concentration of ethanol was 0.01%. This concentration was without effect on the vascular responses to phenylephrine and the vasodilators tested.

In the first series of experiments, the effect of endotoxin (lipopolysaccharide) on the vasodilator activity of arachidonic acid, bradykinin and acetylcholine was tested. A bolus dose of lipopolysaccharide was administered (2 mg/kg i.p.) 4 days before the kidneys were isolated. Dose-response curves were constructed for the agonists in kidneys preconstricted with phenylephrine (10^{-6} M). Control kidneys were from rats that received normal saline (0.9% NaCl; 1 ml/kg i.p.) and were preconstricted with $5–7.5 \times 10^{-7}$ M phenylephrine. Dose-response curves were established for the agonists in lipopolysaccharide- and normal saline-treated kidneys. In order to evaluate the inhibition by lipopolysaccharide of cytochrome P450-dependent vasodilation in response to arachidonic acid and bradykinin, phenobarbitone, an inducer of cytochrome P450 enzymes, was administered (80 mg/kg i.p. for 3 days) to a group of the rats that received lipopolysaccharide or an equivalent volume of normal saline diluent. Responses in kidneys taken from both groups of rats were compared. To assess whether antagonism of NO will alter the lipopolysaccharide-modulated

changes in response to cytochrome P450-dependent vasodilation induced by arachidonic acid and bradykinin, dose-response curves were constructed for these agonists in the absence (control) and presence of haemoglobin (5 μg/ml), an antagonist of NO. As a positive control, responses to acetylcholine were also determined. Responses obtained in haemoglobin-treated kidneys were compared to those in controls. Evidence was also sought for the involvement of NO synthase in the effect of lipopolysaccharide. To this end, the effect of N^{ω}-nitro-L-arginine, a specific and competitive inhibitor of the constitutive and inducible NOS, was evaluated. In these experiments, N^{ω}-nitro-L-arginine was administered (2 mg/kg i.p.) to rats treated with lipopolysaccharide and kidneys were isolated from the rats 4 days later. Control rats received normal saline diluent (instead of N^{ω}-nitro-L-arginine). Dose-response relationships were established for arachidonic acid, bradykinin and acetylcholine in both groups of rats. In order to distinguish between the involvement of the constitutive and the inducible NO synthase in lipolysaccharide-mediated effects, dexamethasone, an inhibitor of the inducible NO synthase (Rees et al., 1990), was administered (80 mg/kg i.m. for 3 days) to rats that concurrently received lipopolysaccharide. The effect of dexamethasone was assessed by comparing the dose-response curves for arachidonic acid and bradykinin in kidneys isolated from rats that received lipopolysaccharide alone and those that received lipopolysaccharide and dexamethasone.

In other experiments, the effects of endogenous levels of NO on the vasodilator responses to arachidonic acid and bradykinin were evaluated. N^{ω}-nitro-L-arginine (10^{-5} M) was included in the perfusate and, after a 10-min infusion, various concentrations of phenylephrine were added. (The concentrations of phenylephrine added were adjusted to give a comparable elevation of tone as in other experiments since N^{ω}-nitro-L-arginine elevated tone on its own.) After a sustained elevation of tone was obtained (following the addition of phenylephrine), responses to arachidonic acid and bradykinin were obtained. The responses obtained were compared with those of the kidneys that did not receive N^{ω}-nitro-L-arginine. Further evidence for the role of NO was obtained by testing the vasodilator responses to these agonists in the presence of nitroprusside (5 μg/ml), an L-arginine-independent NO donor. The role of NO was determined by comparing the vasodilator responses in the presence of nitroprusside with those of the saline-treated controls. To ascertain the selectivity of the effect of N^{ω}-nitro-L-arginine, L-arginine (3×10^{-4}–10^{-3} M), the biosynthetic precursor of endothelium-derived NO and a substrate for NOS, was added to the perfusate before phenylephrine (5×10^{-7} M) was added. Following a stable elevation of tone in the kidneys, the responses to arachidonic acid and bradykinin were redetermined. The responses to acetylcholine, the effect of which is mediated by NO synthesis/release, were also determined. A reversal of the inhibitory effects of N^{ω}-nitro-L-arginine on the vasodilator actions of these agonists by L-arginine is evidence for the involvement of NO/NO synthase in the vasodilator responses to arachidonic acid, bradykinin, and acetylcholine.

Phenylephrine, bradykinin, haemoglobin (from sheep), acetylcholine, sodium nitroprusside, phenobarbitone and L-arginine, N^{ω}-nitro-L-arginine (all from Sigma, St. Louis, MO, USA) were dissolved in Krebs buffer. Lipopolysaccharide from *Escherichia coli* (serotype 026:B6) was also from Sigma and was dissolved in 0.9% NaCl. Dexamethasone was from Sonofi, Quebec, Canada. Indomethacin, a gift from Merck Sharp and Dohme, was dissolved in 95% ethanol.

2.3. Statistical analysis

All data are expressed as means ± S.E. Analysis of variance (ANOVA) was used to compare dose-response curves between control and treated groups, Duncan's test (Antonipillai et al., 1989) being used to determine the significance of differences in mean values within each group. Individual points were compared using a Newman-Keuls test and in all cases, $P \leq 0.05$ was regarded as significant. All statistical analyses were done using the application 'Statworks' on a Macintosh Plus computer.

3. Results

3.1. The effect of lipopolysaccharide treatment on vasodilator responses to arachidonic acid and bradykinin

The basal perfusion pressure in kidney vessels perfused with Krebs' solution was 83.5 ± 2.3 mm Hg (n = 45). In control kidneys, infusion of phenylephrine (5–7.5×10^{-7} M) caused a sustained elevation of arterial perfusion pressure (83.3 ± 2.8 mm Hg, n = 41). In kidneys harvested from rats treated with lipopolysaccharide, the concentration of phenylephrine needed to elevate tone to levels comparable to those in control kidneys was 10^{-6} M. Control vasodilator responses to arachidonic acid 5, 10 and 20 μg were −19.7 ± 2.7, −26.7 ± 2.9, and −42.8 ± 3.0 mm Hg, respectively. For bradykinin, bolus doses of 0.5, 1, and 2 μg elicited reductions in perfusion pressure of −13.2 + 1.9, −22.2 ± 2.6, and −30.2 ± 3.1 mm Hg, respectively. The control vasodilator responses to acetylcholine 0.1, 0.25 and 0.5 μg were −16.8 ± 4.4, −23.9 ± 2.3, and −41.6 ± 2.5 mm Hg, respectively.

Following treatment with lipopolysaccharide (Fig. 1), the vasodilator responses to arachidonic acid were

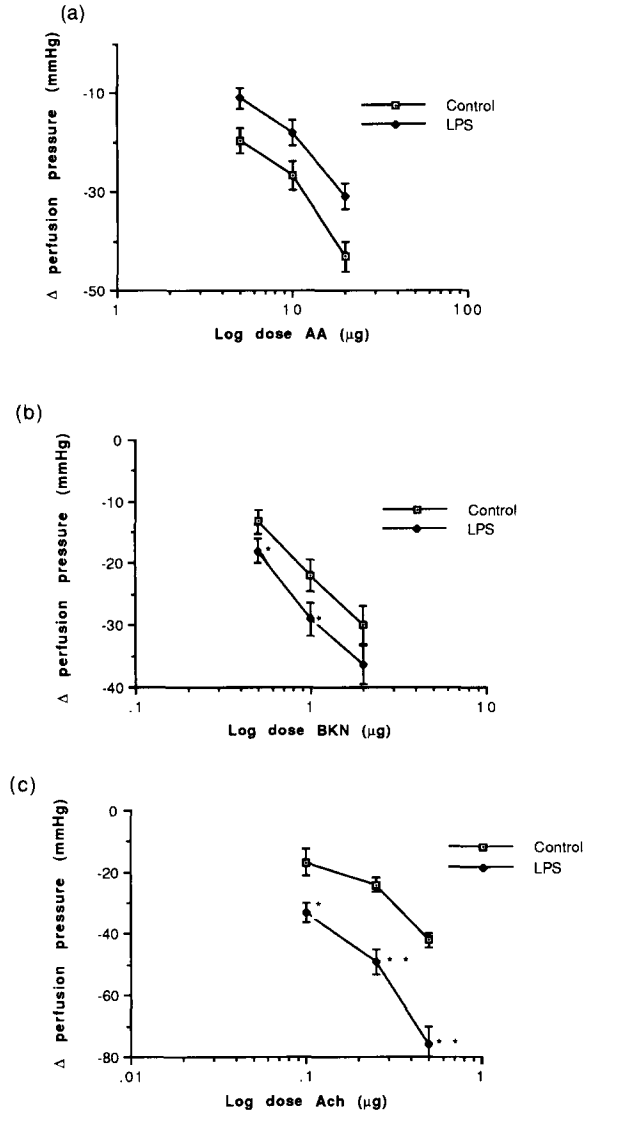

Fig. 1. The effects of endotoxin (lipopolysaccharide; LPS) on the vasodilator effects of arachidonic acid (AA) (a), bradykinin (BKN) (b) and acetylcholine (Ach) in indomethacin-treated kidneys preconstricted with phenylephrine (10^{-6} M). * $P < 0.05$, ** $P < 0.01$. Data were compared between control ($n = 7$) and LPS-treated ($n = 6$) kidneys.

reduced ($P < 0.05$) while bradykinin- and acetylcholine-induced vasodilations were enhanced ($P < 0.05$–0.01). The enhancing effect of lipopolysaccharide was more pronounced for acetylcholine, with a percentage increase (from control) of $-89.7 \pm 6.4\%$ ($P < 0.01$) as against $-30.4 \pm 4.5\%$ ($P < 0.05$) for bradykinin.

3.2. Effect of lipopolysaccharide on phenobarbitone-evoked enhancement of arachidonic acid-induced vasodilatation

Phenobarbitone, an inducer of cytochrome P450 enzymes, amplified the vasodilator response to arachidonic acid by $42.3 \pm 5.6\%$ ($P < 0.01$). The vasodilator responses to bradykinin and acetylcholine were also amplified by phenobarbitone (Fig. 2). The amplification was highest for arachidonic acid ($P < 0.01$). In kidneys taken from rats treated with lipopolysaccharide and phenobarbitone, the phenobarbitone enhancement of arachidonic acid-induced vasodilation was inhibited ($P < 0.05$), that to bradykinin was unchanged while that to acetylcholine was further enhanced ($P < 0.05$).

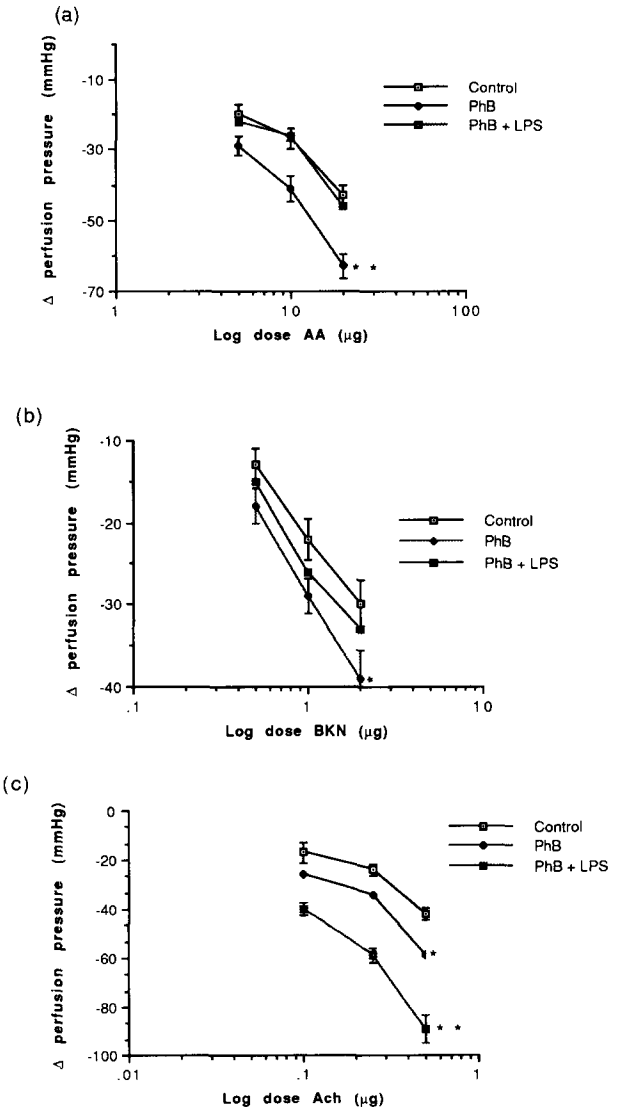

Fig. 2. Effect of phenobarbital (PhB) pretreatment alone or in the presence of lipopolysaccharide (PhB+LPS) on the vasodilator responses to arachidonic acid (AA) (a), bradykinin (BKN) (b) and acetylcholine (Ach) (c). The responses in the control kidneys are shown for comparison. PhB pretreatment enhanced the vasodilator effect of arachidonic acid, bradykinin and acetylcholine. Lipopolysaccharide inhibited the effect of PhB on arachidonic acid-induced vasodilation but enhanced that of bradykinin (b) and acetylcholine (c). * $P < 0.05$, ** $P < 0.01$. Data were compared between control ($n = 7$) and PhB-treated ($n = 5$) kidneys.

3.3. Effect of haemoglobin on lipopolysaccharide-modulated vasodilator responses

Haemoglobin, an antagonist of NO, was used as a probe to assess the role of NO and to evaluate whether responses to arachidonic acid are enhanced in kidneys treated with haemoglobin as a result of antagonism of NO. Arachidonic acid-induced vasodilation was increased ($P < 0.05$) while bradykinin- and acetylcholine-induced vasodilations were reduced ($P < 0.05$) in phenylephrine-preconstricted kidneys treated with haemoglobin (5 μg/ml; $n = 5$) (Fig. 3).

3.4. N^{ω}-Nitro-L-arginine inhibition of lipolysaccharide-modulated vasodilator responses to arachidonic acid and bradykinin

In the presence of N^{ω}-nitro-L-arginine, the dose-response curve for arachidonic acid was shifted to the left, the inhibition by lipopolysaccharide of arachidonic acid-induced vasodilation being completely inhibited ($P < 0.05$; Fig. 4). Under the same conditions, the lipopolysaccharide enhancement of bradykinin- and acetylcholine-induced vasodilation was also completely inhibited ($P < 0.01$), the response curves being shifted to the right. For bradykinin, the inhibition of vasodilator responses in the presence of N^{ω}-nitro-L-arginine was such that the responses were reduced to levels lower than those for lipopolysaccharide.

3.5. Dexamethasone inhibition of lipopolysaccharide-evoked modulation of vasodilator responses to arachidonic acid and bradykinin

Dexamethasone was used to probe the possible participation of the inducible NO synthase in the modulation by lipopolysaccharide of the vasodilator responses to arachidonic acid and bradykinin. Compared to responses in kidneys from rats that received lipopolysaccharide alone, the vasodilator responses to arachidonic acid were increased ($P < 0.05$), those to bradykinin were unchanged while those to acetylcholine were reduced ($P < 0.01$) in kidneys harvested from rats pretreated with dexamethasone and lipopolysaccharide (Fig. 5).

3.6. Effect of nitroprusside and L-arginine with or without N^{ω}-nitro-L-arginine on the vasodilator responses to arachidonic acid and bradykinin

The data obtained above demonstrated a role for the involvement of NO/NO synthase in the inhibitory effect of lipopolysaccharide on vasodilation induced by arachidonic acid and bradykinin. To address the role of NO more directly, the responses to arachidonic acid and bradykinin were assessed in kidneys treated with

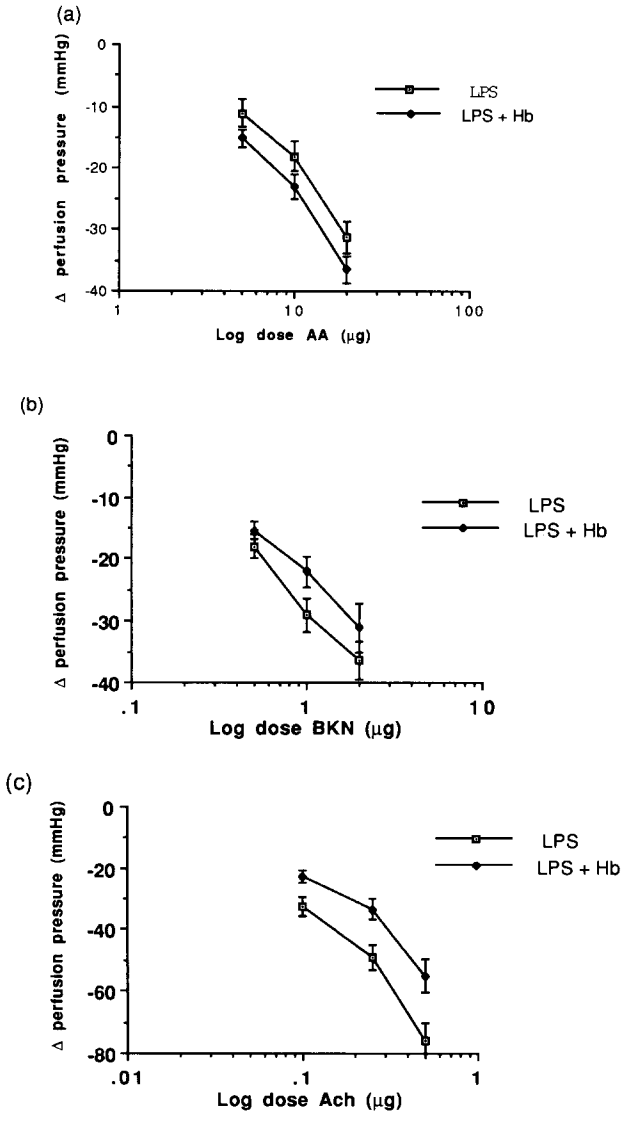

Fig. 3. Effect of haemoglobin (Hb) on vasodilator responses to arachidonic acid (AA) (a), bradykinin (BKN) (b) and acetylcholine (Ach) (c) in indomethacin-treated kidneys prepared from rats treated with lipopolysaccharide (LPS). Arterial tone was elevated with phenylephrine (10^{-6} M). Data were compared between kidneys treated with lipopolysaccharide alone ($n = 6$) and those treated additionally with haemoglobin (LPS + Hb; $n = 5$). In every case, the difference observed was significant at $P < 0.05$.

N^{ω}-nitro-L-arginine, a specific inhibitor of the synthesis/release of NO, nitroprusside, an NO donor, and L-arginine, a substrate for NO synthase, in the presence or absence of N^{ω}-nitro-L-arginine. Nitroprusside (5 μg/ml) reduced the vasodilator response to arachidonic acid ($P < 0.05$) but was without effect on the vasodilator responses to bradykinin and acetylcholine (Fig. 6). Blockade of the endogenous synthesis/release of NO with N^{ω}-nitro-L-arginine (10^{-5} M) was accompanied by an increased vasodilator response to arachidonic acid ($P < 0.05$). However, the vasodilator responses to bradykinin and acetylcholine were reduced

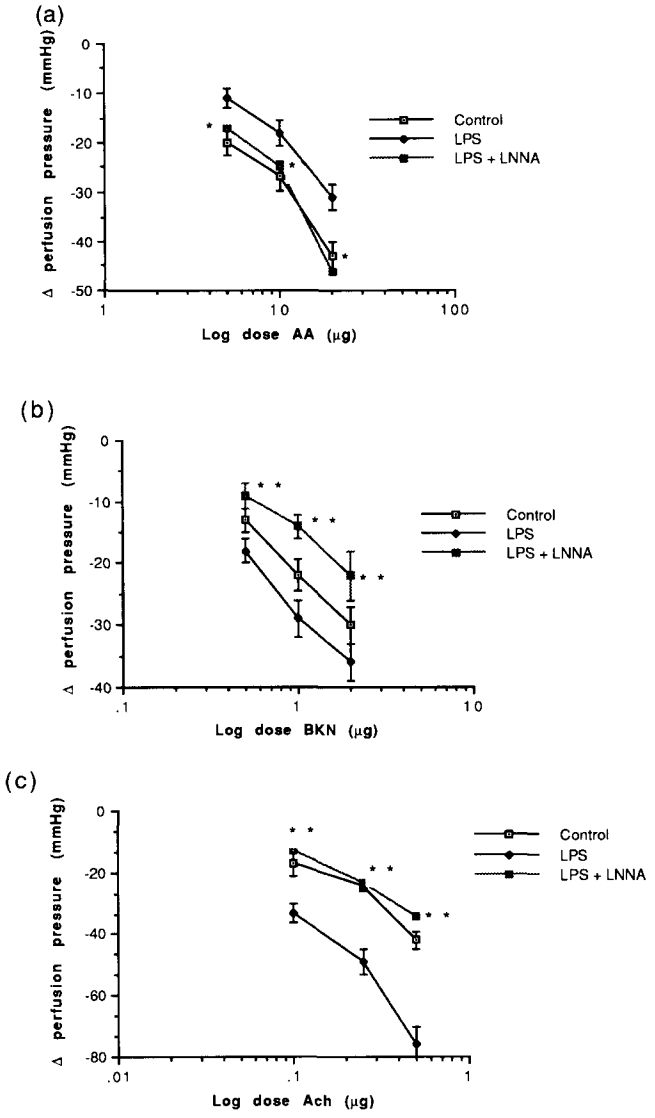

Fig. 4. Effect of N^{ω}-nitro-L-arginine (LNNA) pretreatment on the lipopolysaccharide (LPS)-modulated changes in the vasodilator effects of arachidonic acid (AA) (a), bradykinin (BKN) (b) and acetylcholine (Ach) (c) in indomethacin-treated kidneys preconstricted with phenylephrine (10^{-6} M). * $P < 0.05$, ** $P < 0.01$. Data comparisons were between lipopolysaccharide (LPS; $n = 6$) and lipopolysaccharide + N^{ω}-nitro-L-arginine (LPS + LNNA; $n = 5$) groups.

($P < 0.05$–0.01) (Fig. 7). The nitroprusside effect on the arachidonic acid response was not affected by N^{ω}-nitro-L-arginine (data not shown). L-Arginine (3×10^{-4} and 10^{-3} M) markedly inhibited arachidonic acid-induced vasodilation ($P < 0.01$). At the same concentrations, the vasodilation elicited by bradykinin was moderately reduced ($P < 0.05$) while that induced by acetylcholine was increased ($P < 0.05$) (Fig. 8). The addition of N^{ω}-nitro-L-arginine (10^{-5} M) to the perfusate containing 3×10^{-4} M L-arginine annulled the effect of L-arginine on the vasodilation elicited by arachidonic acid, bradykinin, or acetylcholine (Fig. 8).

4. Discussion

The results of this study demonstrate that lipopolysaccharide, the bacterial endotoxin, attenuated arachidonic acid-induced vasodilation in the rat kidney. The vasodilator activity of bradykinin was slightly enhanced while that of acetylcholine was markedly enhanced. The enhancement of the vasodilator activity of bradykinin and especially of acetylcholine is consistent with the concept that overproduction of NO in endotoxic shock is a result of stimulation of NO synthase (Khatsenko et al., 1993). The inhibition of arachidonic

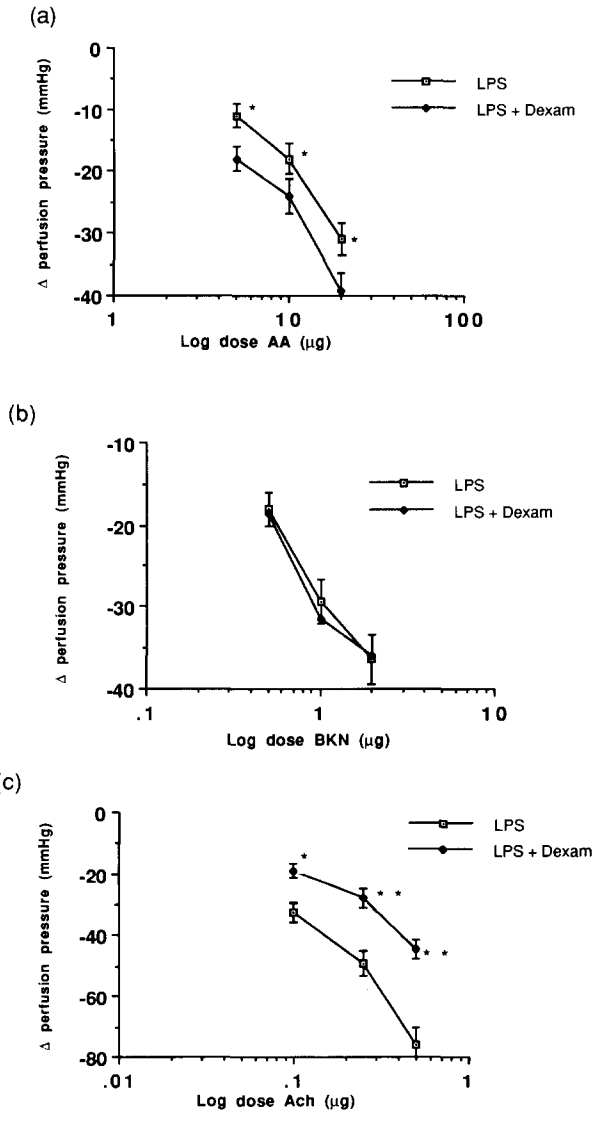

Fig. 5. Effect of dexamethasone pretreatment (Dexam) on the lipopolysaccharide-modulated changes in the vasodilator responses to arachidonic acid (AA) (a), bradykinin (BKN) (b) and acetylcholine (Ach) (c) in indomethacin-treated kidneys preconstricted with phenylephrine (10^{-6} M). * $P < 0.05$, ** $P < 0.01$. Data were compared between kidneys from rats that received lipopolysaccharide alone (LPS; $n = 6$) and those that received lipopolysaccharide and dexamethasone (LPS + Dexam; $n = 7$).

acid-induced vasodilation by NO in this study is novel and constitutes the first evidence that NO directly interferes with the activity of another endothelium-dependent vasodilator i.e. the cytochrome P450-dependent vasodilator metabolite of arachidonic acid. This observation is in agreement with studies that showed that immunostimulants inhibit cytochrome P450 activity (Mannering and Deloria, 1986; Delaporte et al., 1993).

The kidney is replete with cytochrome P450 enzymes and in previous studies (Oyekan et al., 1991a,b) conclusive evidence was presented to show that arachidonic acid is metabolized to vasodilator metabolites by the endothelial cytochrome P450-dependent enzymes in the rat kidney. The inhibition, therefore, of arachidonic acid-induced vasodilation by endotoxin is interpreted as being the result of the inhibition of the conversion of arachidonic acid to cytochrome P450 vasodilator metabolites by the NO generated following the administration of endotoxin. In the study by Khatsenko et al. (1993), the novel observation was made that the administration of lipopolysaccharide to rats was associated with the induction of NO synthase which showed a strong positive correlation with the severity of inhibition of cytochrome P450 enzymes.

In another study by our group (Fulton et al., 1992),

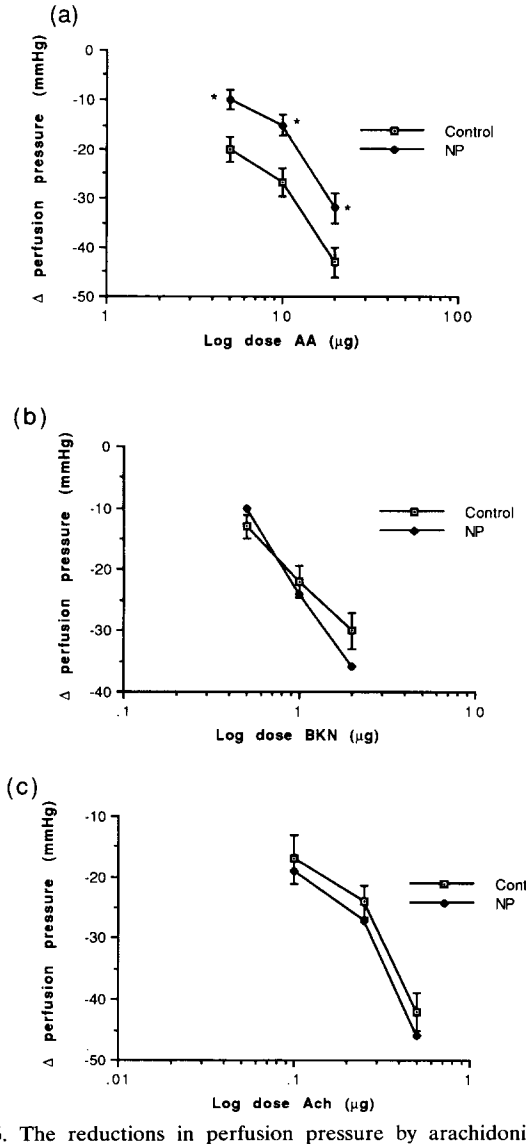

Fig. 6. The reductions in perfusion pressure by arachidonic acid (AA) (a), bradykinin (BKN) (b) and acetylcholine (Ach) (c) in indomethacin-treated phenylephrine (5×10^{-7} M)-preconstricted kidneys perfused with nitroprusside (NP; 5 μg/ml). Data from control kidneys are included for comparison. * $P < 0.05$. Data were compared between control ($n = 7$) and nitroprusside-treated ($n = 6$) kidneys.

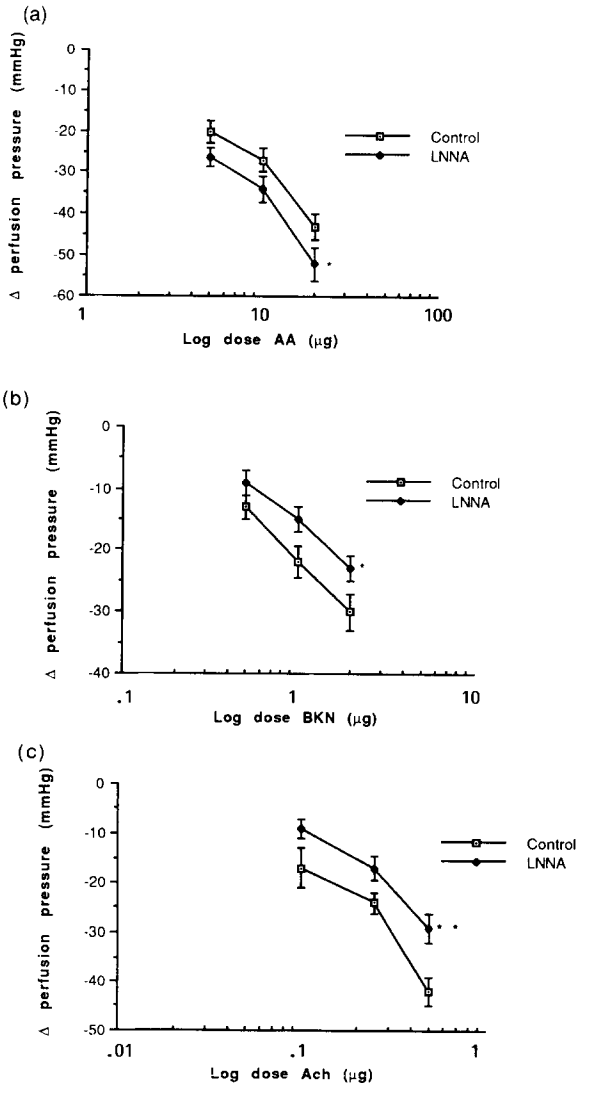

Fig. 7. The effect of N^{ω}-nitro-L-arginine (LNNA; 10^{-5} M) on the vasodilator responses to arachidonic acid (AA) (a), bradykinin (BKN) (b) and acetylcholine (Ach) (c) in kidneys treated with indomethacin. Arterial tone was elevated with phenylephrine (5×10^{-7} M). LNNA amplified the vasodilation elicited by AA but reduced that elicited by BKN and Ach. Responses in control kidneys ($n = 7$) were compared to those of kidneys treated with LNNA ($n = 5$). * $P < 0.05$.

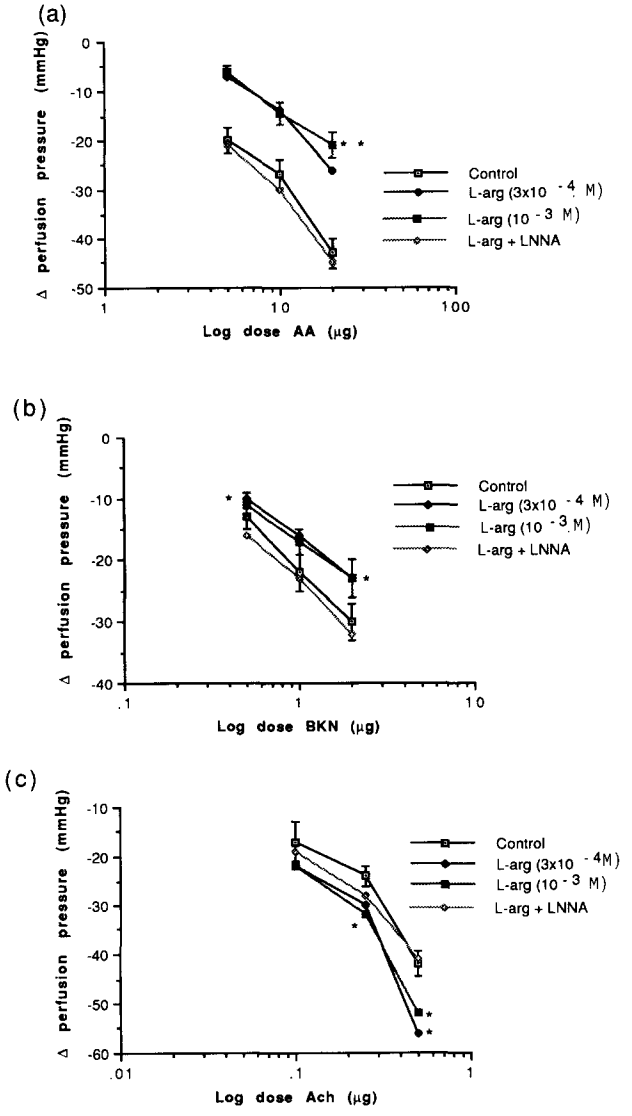

Fig. 8. The effect of L-arginine (L-arg) (3×10^{-4} and 10^{-3} M) on the vasodilator responses to arachidonic acid (AA) (a), bradykinin (BKN) (b) and acetylcholine (Ach) (c) in indomethacin-treated phenylephrine (5×10^{-7} M)-preconstricted kidneys. The effect of the addition of N^{ω}-nitro-L-arginine (LNNA; 10^{-5} M) to the perfusate containing L-arginine (L-arg+LNNA) is also shown. L-Arginine and N^{ω}-nitro-L-arginine were added to the perfusate from the beginning of the experiment. * $P < 0.05$, * * $P < 0.01$. Data comparisons were between L-arginine-treated ($n = 5$–6) kidneys and controls ($n = 7$). The asterisks refer to all data points on the respective graphs.

the use of inhibitors of cytochrome P450 enzymes led to the conclusion that 70% of the renal vasodilator response to bradykinin in the rat was subserved by cytochrome P450 metabolites. This being so, we evaluated if NO inhibits the cytochrome P450 component of the vasodilator activity of bradykinin as was observed for arachidonic acid. The effects of NO generated from lipopolysaccharide, nitroprusside and L-arginine were therefore examined. Lipopolysaccharide rather enhanced bradykinin-induced vasodilation. This observation is incongruent with the study that reported that bradykinin-induced vasodilation is largely mediated by cytochrome P450 metabolites. This discrepancy is a reflection of the multicomponental nature of the vasodilator response to bradykinin, unlike the response to arachidonic acid. Depending on species and tissue, the vasodilator response to bradykinin has been reported to be mediated by prostaglandins (McGiff et al., 1972), oxygen-derived free radicals (Kontos et al., 1984), endothelium-derived relaxing factor/NO (Cachofeiro and Nasjletti, 1991) and cytochrome P450 metabolites (Fulton et al., 1992). Considering this multiplicity of mediators, it would appear that the response to bradykinin is the net effect of the contribution by these mediators. Hence in a situation as in this study, where there is overproduction of a component, i.e. NO, this component will be enhanced and will therefore diminish the cytochrome P450 component. This is typified by the responses to arachidonic acid, bradykinin, and acetylcholine (Fig. 1).

The lipopolysaccharide/NO inhibition of cytochrome P450 enzymes was further seen in experiments where phenobarbitone, an inducer of microsomal enzymes, was administered. Phenobarbitone amplified the vasodilator responses to bradykinin and acetylcholine, and especially the responses to arachidonic acid. The amplification of arachidonic acid-induced vasodilation is consistent with stimulation of cytochrome P450 enzymes, as phenobarbitone preferentially induces the cytochrome P2B1 isoform (Coon et al., 1992). The amplified response to acetylcholine, and by extrapolation, to bradykinin is in agreement with the study by Randall and Hiley (1988). Pretreatment with lipopolysaccharide inhibited the effect of phenobarbitone on the arachidonic acid response and enhanced that on the acetylcholine response. For bradykinin, endotoxin administration did not affect the amplification elicited by phenobarbitone of the vasodilation, probably because of the opposing signals from NO overproduction due to lipopolysaccharide treatment and increased P450 enzymes from phenobarbitone treatment.

Further investigation revealed that haemoglobin antagonized the effect produced by lipopolysaccharide, further implicating NO generated following lipopolysaccharide administration in the inhibition of arachidonic acid-induced vasodilation. This observation is consistent with the fact that haemoglobin is an antagonist of NO. Higher concentrations of haemoglobin did not completely abolish the effect, suggesting an additional axis of interaction. That the lipopolysaccharide effect was mediated by stimulation of NO synthase was revealed by the observation that N^{ω}-nitro-L-arginine, a competitive inhibitor of the constitutive and inducible NOS (Rees et al., 1990; Smith et al., 1991) and dexa-

methasone, the inhibitor of the inducible NO synthase (Rees et al., 1990; Knowles et al., 1990), antagonized the effects of lipopolysaccharide on arachidonic acid, bradykinin and acetylcholine responses. This observation is consistent with the fact that lipopolysaccharide-induced inhibition of hepatic metabolism in vivo is prevented by dexamethasone (Bertini et al., 1989). Our data further implicate the inducible NO synthase as the target of the lipopolysaccharide effect as dexamethasone, which on its own was without effect on acetylcholine responses, antagonized the lipopolysaccharide-induced enhancement of the vasodilator response to acetylcholine. The lack of effect of dexamethasone on the acetylcholine-induced vasodilation is in agreement with the fact that dexamethasone is without an effect on the constitutive NO synthase (Rees et al., 1990).

Evidence has been provided that NO is normally produced at a significant basal rate (Kilbourn et al., 1990) and both NO and cytochrome P450 metabolites have been reported to play a role in vascular haemodynamics. In this study, the inhibition of endogenous NO by N^{ω}-nitro-L-arginine resulted in an enhancement of arachidonic acid-induced vasodilation, suggesting that endogenous NO inhibits cytochrome P450-dependent arachidonic acid-induced vasodilation. Further proof of the inhibition of cytochrome P450 enzymes by NO was demonstrated by showing that nitroprusside, the arginine-independent NO donor, and L-arginine, the biosynthetic precursor of NO, exerted inhibitory effects on arachidonic acid- and bradykinin-induced vasodilation. That the effect of L-arginine, but not of nitroprusside, was reversed by N^{ω}-nitro-L-arginine suggests a specific role for NO synthase in the inhibition of cytochrome P450-dependent vasodilation.

In conclusion, the results of this study revealed that endogenous and exogenous NO antagonized cytochrome P450-dependent arachidonic acid-induced vasodilation. The inconsistent effect on bradykinin is a reflection of the multicomponental nature of its response.

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