British Journal of Pharmacology (1995) 116, 3042-3048

Enhancement of the haemodynamic effects of N^G-monomethyl-L-arginine by transforming growth factor-β_1 in conscious, normal, but not endotoxaemic, rats

[1]S.M. Gardiner, P.A. Kemp, J.E. March & T. Bennett

Department of Physiology & Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 Male, Long Evans rats (350-450 g) were chronically instrumented for the measurement of regional haemodynamics, and the effects of TGF-β_1 (25 $\mu g\ kg^{-1}$ i.v. bolus) were assessed during infusion of saline ($n=9$) or lipopolysaccharide (LPS, 150 $\mu g\ kg^{-1}\ h^{-1}$; $n=12$). In the same animals, responses to N^G-monomethyl-L-arginine (L-NMMA 10 mg kg^{-1} bolus; 10 mg $kg^{-1}\ h^{-1}$ infusion) were determined 18 h after administration of TGF-β_1. In a separate experiment, the effects of the endothelin antagonist, SB 209670 (10 $\mu g\ kg^{-1}\ min^{-1}$) on responses to TGF-β_1 and to L-NMMA subsequently, were determined.

2 In the absence of LPS, TGF-β_1 had slow-onset bradycardic and pressor effects accompanied by mesenteric and hindquarters, but not renal, vasoconstriction. Eighteen hours after TGF-β_1, these effects had gone, but the bradycardic, pressor, and mesenteric vasoconstrictor responses to L-NMMA were enhanced. The haemodynamic changes following TGF-β_1, and the augmentation of the subsequent responses to L-NMMA, were inhibited by SB 209670. These results are consistent with TGF-β_1 stimulating the synthesis and release of endothelin, and an involvement of the latter in responses to L-NMMA.

3 The pressor effects of TGF-β_1 were similar in LPS-infused and saline-infused animals, but in the former group the mesenteric vasoconstriction was enhanced and the hindquarters vasoconstriction diminished. Since, in the absence of TGF-β_1, LPS-infused animals showed a developing hindquarters vasodilatation and mesenteric vasoconstriction, it is feasible that, in the presence of TGF-β_1 and LPS together, the haemodynamic profile represented an amalgam of the individual effects of the two interventions, rather than a specific effect of TGF-β_1 on the haemodynamic sequelae of endotoxaemia.

4 In the presence of LPS, haemodynamic responses to L-NMMA were suppressed, and TGF-β_1 generally did not affect this suppression. A possible explanation of this observation is that LPS increased circulating endothelin levels, and thus resulted in desensitization to the effects of endothelin released following administration of L-NMMA.

Keywords: Transforming growth factor-β_1; endothelin; lipopolysaccharide; SB 209670

Introduction

Transforming growth factor-β_1 (TGF-β_1) is the most common member of a ubiquitous family of growth and differentiation factors which have many biological effects, although the majority of these have been studied *in vitro*. For example, TGF-β_1 increases biosynthesis and release of the vasoconstrictor, endothelin (ET), *in vitro* (Kurihara *et al.*, 1989; Ohta *et al.*, 1990; Kanse *et al.*, 1991; Endo *et al.*, 1992). On the basis of this observation, Kurihara *et al.* (1989) suggested that TGF-β_1 could be involved in the local regulation of vascular tone by stimulating ET-1 release, but Lefer *et al.* (1993) reported that TGF-β_1 had no haemodynamic effects *in vivo*. However, the *in vitro* experiments of Kurihara *et al.* (1989) showed that the increase in ET mRNA was maximal 2h after TGF-β_1 stimulation, whereas in the experiments of Lefer *et al.* (1993) haemodynamic effects of TGF-β_1 were not looked for over several hours in the absence of other interventions. Hence, one objective of the present work was to assess any possible *in vivo* haemodynamic actions of TGF-β_1 over such a time course.

Recent observations in conscious (Gardiner *et al.*, 1995a) and anaesthetized (Richard *et al.*, 1995) rats have indicated that components of the pressor and regional vasoconstrictor (Gardiner *et al.*, 1995a) responses to the nitric oxide synthase (NOS) inhibitors, N^G-monomethyl-L-arginine (L-NMMA), and N^G-nitro-L-arginine methyl ester (L-NAME) are due to ET, inasmuch as the haemodynamic effects of the NOS inhibitors are attenuated by the non-selective ET antagonist, bosentan. Thus, it is feasible that a positive interaction between TGF-β_1 and ET biosynthesis might be reflected in an increase in the haemodynamic response to NOS inhibition. Therefore, another objective of our experiments was to determine if TGF-β_1 influenced the subsequent haemodynamic responses to L-NMMA.

In addition to its influence on ET synthesis and release, TGF-β_1 can have substantial modulatory influences on immune and inflammatory responses (see Attisano *et al.*, 1994 for review). In this context, it is notable that TGF-β_1 has been shown to protect against myocardial and endothelial injury caused by ischaemia and reperfusion (Lefer *et al.*, 1990; 1993), possibly through influencing processes mediated by tumour necrosis factor, or nitric oxide (NO; Lefer *et al.*, 1990; 1993). Indeed, it has been found that TGF-β_1 inhibits cytokine-induced NO production by macrophages (Ding *et al.*, 1990), human and rat aortic smooth muscle cells (Junquero *et al.*, 1992; Schini *et al.*, 1992), and rat ventricular myocytes (Pinsky *et al.*, 1995). Furthermore, TGF-β_1 also down-regulates inducible NOS (iNOS) mRNA in rat aortic smooth muscle cells (Perrella *et al.*, 1994). However, it is not known if TGF-β_1 influences *in vivo* responses to activators of the cytokine cascade, such as lipopolysaccharide (LPS). Hence a further objective of the present study was to assess the haemodynamic effects of TGF-β_1, in LPS-infused animals.

To achieve our objectives, we investigated the regional haemodynamic effects of TGF-β_1 in conscious control rats, and in rats receiving a constant infusion of LPS (Waller *et al.*, 1994). In the same animals we then determined if TGF-β_1 influenced responses to L-NMMA. Since TGF-β_1 had clear-cut haemodynamic effects and enhanced the cardiovascular responses to L-NMMA in control animals (see Results) we car-

[1] Author for correspondence.

ried out a separate experiment to determine if the non-selective ET antagonist, SB 209670 (Ohlstein *et al.*, 1994; Douglas *et al.*, 1995) affected haemodynamic responses to TGF-β_1, or its ability to enhance responses to L-NMMA.

Methods

All experiments were carried out on male, Long Evans rats (350–450 g) bred in the Medical School in Nottingham. Surgery for the implantation of pulsed Doppler probes and intravascular catheters was carried out under anaesthesia (sodium methohexitone, Brietal, Lilly, 40–60 mg kg^{-1} i.p.) as described in detail previously (Gardiner *et al.*, 1990). Animals were fully conscious and unrestrained, and had free access to food and water throughout the experiment.

Five groups of animals were investigated in separate protocols as follows:-

Effects of saline on regional haemodynamics, and on responses to L-NMMA, in saline-infused animals

Animals ($n=9$) were given a continuous infusion of sterile isotonic saline (154 mmol l^{-1} NaCl) for 6 h (to control for LPS infusion, see below) and then received a bolus injection of saline (0.1 ml flushed in with 0.1 ml saline; to control for TGF-β_1, see below). Recordings were made for the 3 h subsequently, and were begun again 18 h later (i.e. 24 h after the onset of saline infusion). At this juncture, in a sub-group of 8 animals, a primed infusion of L-NMMA (10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion) was begun and continued for 3 h.

Effects of TGF-β_1 on regional haemodynamics, and on responses to L-NMMA, in saline-infused animals

Animals ($n=9$) were given a continuous infusion of saline for 6 h and then received TGF-β_1 (25 μg kg^{-1}; 0.1 ml flushed in with 0.1 ml saline). Eighteen hours later, L-NMMA was administered as above and continued for 3 h, in a sub-group of 8 animals. The dose of TGF-β_1 was chosen on the basis of the study of Lefer *et al.* (1990) showing cardioprotection in rats.

Effects of saline on regional haemodynamics, and on responses to L-NMMA, in LPS-infused animals

Animals ($n=9$) were given a continuous infusion of LPS (150 μg kg^{-1} h^{-1}) and 6 h later received a bolus injection of saline (to control for TGF-β_1). After a further 18 h, an L-NMMA infusion (as above) was begun and continued for 3 h, together with the LPS, in a sub-group of 8 animals.

Effects of TGF-β_1 on regional haemodynamics, and on responses to L-NMMA, in LPS-infused animals

Animals ($n=12$) had a continuous infusion of LPS (as above) for 6 h before being given a bolus injection of TGF-β_1 (as above). Eighteen hours later an infusion of L-NMMA (as above) was begun, in a subgroup of 7 animals, and continued together with the LPS for a further 3 h.

Effects of SB 209670 on responses to TGF-β_1 and on subsequent responses to L-NMMA in saline-infused animals

Animals ($n=8$) were given a continuous infusion of saline for 5 h before the onset of a continuous infusion of SB 209670 (10 μg kg^{-1} min^{-1}) (Douglas *et al.*, 1995); the latter was given for 1 h before and for 3 h after a bolus injection of TGF-β_1 (as above). Seventeen hours later the infusion of SB 209670 was resumed, 1 h before an infusion of L-NMMA (as above) was started; SB 209670 and L-NMMA were co-infused for 3 h. In pilot experiments ($n=3$) we found this dose of SB 209670 completely reversed the effects of an infusion of ET-1 (120 pmol h^{-1}) which increased mean arterial pressure by 30–40 mmHg.

Data analysis

Within-group analysis was by Friedman's test, and between-group analysis by the Kruskal-Wallis test, applied to resting values or integrated responses (areas under or over curves (AUC, AOC, respectively)). A P value <0.05 was taken as significant.

Materials

Human recombinant TGF-β_1 was a gift from Genentech (California, U.S.A.). LPS (*E. coli* serotype 0127:B8) was purchased from Sigma (U.K.), and L-NMMA hydrochloride was a gift from Wellcome Research Labs. (Dr Daryl Rees). (±)-SB 209670 ([(±)-1S, 2R, 3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxy-phenyl)-5-(prop-1-yloxy) indane 2-carboxylic acid]) was a gift from Dr E. Ohlstein (SmithKline Beecham Pharmaceuticals, U.S.A.).

Results

At the start of the experiments, there were no significant differences between resting haemodynamics in any of the groups of animals studied (Table 1).

Effects of saline or TGF-β_1 in saline-infused animals

Just prior to injection of saline or TGF-β_1 (i.e., 6 h after the onset of saline infusion) resting haemodynamics were similar in the 2 groups (Table 2). During the 3 h following saline injection there were no haemodynamic changes (Figure 1), whereas after injection of TGF-β_1, there was a significant increase in

Table 1 Resting cardiovascular variables in the 5 groups of animals studied

	Saline/ saline ($n=9$)	*Saline/ TGF-β_1* ($n=9$)	*LPS/ saline* ($n=9$)	*LPS/ TGF-β_1* ($n=12$)	*SB209670/ TGF-β_1* ($n=8$)
Heart rate (beats min^{-1})	342±7	349±7	340±6	347±5	343±7
Mean blood pressure (mmHg)	104±3	102±2	102±2	104±1	103±1
Renal Doppler shift (kHz)	6.8±0.7	7.4±0.6	6.8±0.4	6.1±0.4	5.9±0.4
Mesenteric Doppler shift (kHz)	7.0±0.6	6.4±0.4	7.6±0.5	6.8±0.6	5.8±0.5
Hindquarters Doppler shift (kHz)	4.2±0.3	4.2±0.4	4.9±0.4	4.5±0.2	4.2±0.5
Renal vascular conductance ([kHz mmHg^{-1}]10^3)	66±5	73±6	66±3	59±4	58±4
Mesenteric vascular conductance ([kHz mmHg^{-1}]10^3)	68±6	63±5	75±5	66±7	57±5
Hindquarters vascular conductance ([kHz mmHg^{-1}]10^3)	40±2	41±4	48±5	44±2	44±5

The treatments subsequently given are indicated by the headings. Values are mean±s.e. mean; n=number of animals.

mean arterial pressure (MAP), associated with bradycardia, and reductions in mesenteric and hindquarters flows and vascular conductances (Figure 1).

Twenty four hours after the onset of saline infusion (i.e. 18 h after injection of saline or TGF-β_1), there was no difference between the haemodynamic status of the 2 groups (Table 3).

Effects of saline or TGF-β_1 in LPS-infused animals

Six hours after the onset of LPS infusion, there was tachycardia and renal and mesenteric vasodilatation, but no hypotension (Table 2).

During the 3 h following saline injection in LPS-infused animals, there was further tachycardia, and increases in renal

Table 2 Resting cardiovascular variables after 6 h infusion of saline or LPS, and immediately before injection of saline, or TGF-β_1

	(a) *Saline/ saline* ($n=9$)	(b) *Saline/ TGF-β_1* ($n=9$)	(c) *LPS/ saline* ($n=9$)	(d) *LPS/ TGF-β_1* ($n=12$)
Heart rate (beats min^{-1})	329±4	334±7	387±7^{ab}	390±7^{ab}
Mean blood pressure (mmHg)	99±2	103±2	101±2	101±1
Renal Doppler shift (kHz)	6.4±0.8	7.1±0.6	9.4±0.5^{ab}	8.7±0.8^{a}
Mesenteric Doppler shift (kHz)	5.5±0.5	6.4±0.5	8.9±0.6^{ab}	7.4±0.6^{a}
Hindquarters Doppler shift (kHz)	3.6±0.2	3.7±0.3	4.2±0.5	4.1±0.2
Renal vascular conductance ([kHz mmHg^{-1}]10^3)	64±7	69±5	94±5^{ab}	86±7^{a}
Mesenteric vascular conductance ([kHz mmHg^{-1}]10^3)	56±5	62±4	88±6^{ab}	73±6^{ac}
Hindquarters vascular conductance ([kHz mmHg^{-1}]10^3)	37±2	36±4	42±5	40±2

Values are mean±s.e.mean; n=number of animals.
Superscripts = $P<0.05$ versus column indicated (Kruskal-Wallis test).

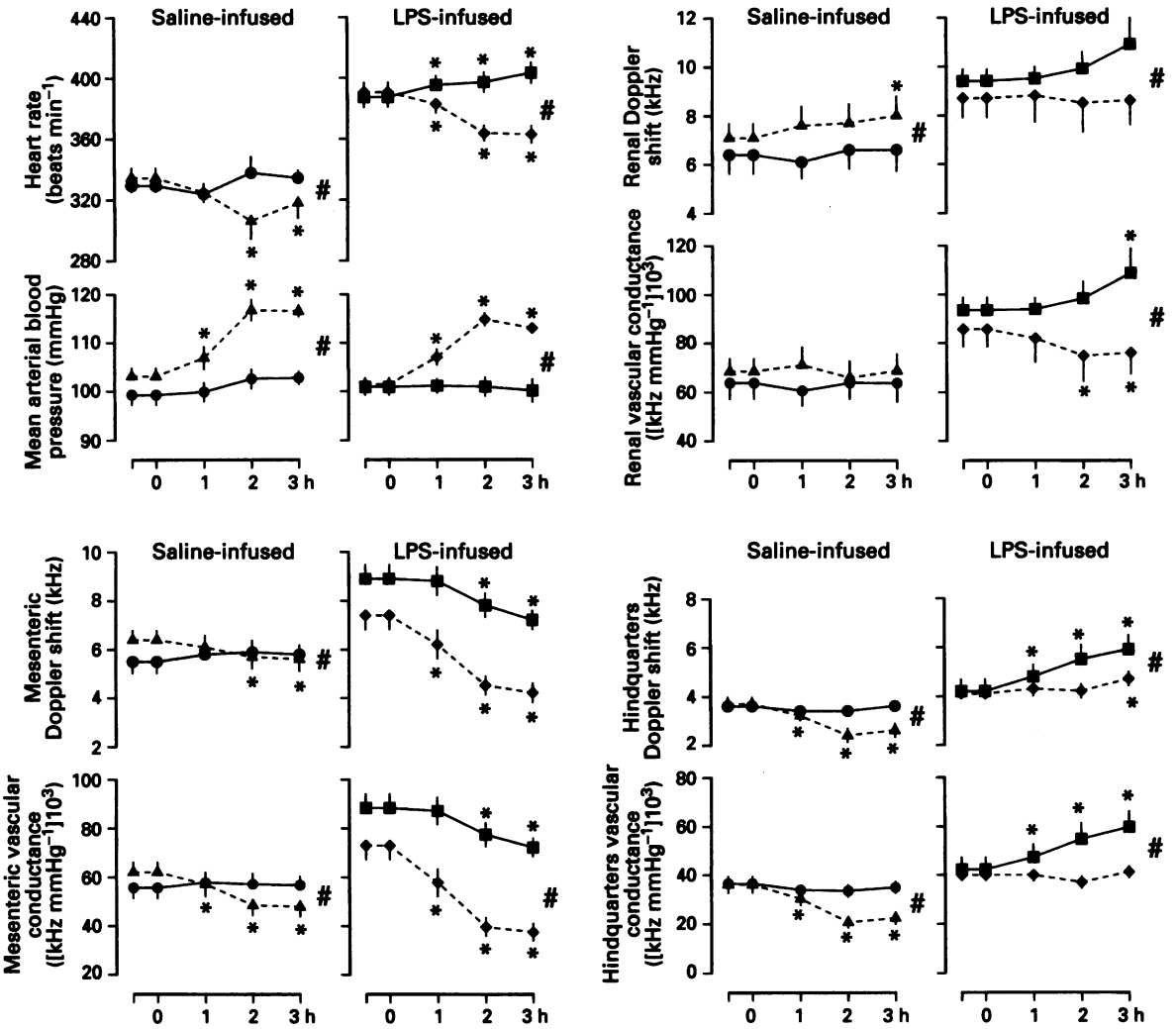

Figure 1 Cardiovascular changes in saline-infused rats or lipopolysaccharide (LPS)-infused rats over 3 h following injection of saline or TGF-β_1 (saline-infused: ●—● saline-injected ($n=9$), ▲—▲ TGF-β_1-injected ($n=9$); LPS-infused: ■—■ saline-injected ($n=9$), ◆—◆ TGF-β_1-injected ($n=12$). Values are mean, and vertical bars show s.e. mean. *$P<0.05$ versus baseline (Friedman's test); #$P<0.05$ for corresponding integrated responses.

and hindquarters vascular conductances, but a reduction in mesenteric flow and vascular conductance (Figure 1). In contrast, in the LPS-infused animals given TGF-β_1, there was a rise in MAP, bradycardia, a small renal vasoconstriction, and marked mesenteric vasoconstriction (Figure 1); the latter was significantly greater than that seen in the LPS-infused animals injected with saline, and in the saline-infused animals injected with TGF-β_1 (Table 4). The LPS-infused animals given TGF-β_1 showed no change in hindquarters vascular conductance (Figure 1), in contrast to the vasoconstriction seen in the saline-infused animals given TGF-β_1 (Figure 1, Table 4), and the vasodilatation seen in the LPS-infused animals injected with saline (Figure 1, Table 4).

Twenty four hours after the onset of LPS infusion (i.e., 18 h after injection of saline or TGF-β_1), there was no difference between the haemodynamic status of the 2 groups (Table 3). Compared to saline-infused animals, the LPS-infused groups showed tachycardia, hypotension, and hyperaemic vasodilatation in the renal and hindquarters vascular beds (Table 3).

Responses to L-NMMA in saline-infused animals 18 h after injection of saline or TGF-β_1

In both groups of animals being infused with saline, L-NMMA caused an increase in MAP, bradycardia, marked and persistent vasoconstriction in the mesenteric and hindquarters vascular beds, and a variable, less persistent, renal vasoconstriction (Figure 2). Over the 3 h period during L-renal and NMMA infusion, the bradycardia, rise in MAP and renal and mesenteric vasoconstrictions were significantly greater in the animals that had received TGF-β_1 18 h previously (Figure 2,Table 5).

Responses to L-NMMA in LPS-infused animals 18 h after injection of saline or TGF-β_1

In the 2 groups of LPS-infused animals, L-NMMA caused similar increases in MAP and regional vasoconstrictions, although the bradycardia and mesenteric vasoconstriction were significantly greater in the animals that had received TGF-β_1 (Figure 2, Table 5). Compared to the corresponding saline-infused groups, the rise in MAP and falls in mesenteric and hindquarters vascular conductances caused by L-NMMA were significantly smaller in both groups of animals infused with LPS (Table 5). In the saline-injected group, the renal vasoconstrictor response to L-NMMA was enhanced in the presence of LPS (Table 5).

Effects of SB 209670 on responses to TGF-β_1 in saline-infused animals

Pretreatment with SB 209670 for 1 h had no significant haemodynamic effects (data not shown). Over the 3 h period following TGF-β_1 injection in the presence of SB 209670, there was a rise in MAP (AUC, 7 ± 2 mmHg h), bradycardia (AOC 222 ± 66 beats 10^{-1}) and mesenteric and hindquarters vasoconstriction (AOC 11 ± 5; 11 ± 3 [kHz mmHg^{-1}] 10^3 h) (Table 4). With the exception of the bradycardia, the cardiovascular responses to TGF-β_1 were significantly smaller in the presence of SB 209670 (see above) than in its absence (Table 4).

Effects of SB 209670 on responses to L-NMMA in saline infused animals, 18 h after injection of TGF-β_1

Pretreatment with SB 209670 for 1 h had no effect on resting haemodynamics (heart rate, before $=343\pm6$, after $=345\pm8$ beats min^{-1}; MAP, before $=105\pm2$, after $=102\pm1$ mmHg; renal vascular conductance, before $=54\pm2$, after $=58\pm2$

Table 3 Resting cardiovascular variables 24 h after the onset of LPS infusion, and 18h after injection of saline or TGF-β_1

	(a) *Saline/ saline* ($n=9$)	(b) *Saline/ TGF-β_1* ($n=9$)	(c) *LPS/ saline* ($n=9$)	(d) *LPS/ TGF-β_1* ($n=12$)
Heart rate (beats min^{-1})	332 ± 5	342 ± 6	434 ± 7^{ab}	432 ± 8^{ab}
Mean blood pressure (mmHg)	102 ± 2	104 ± 2	96 ± 2^{ab}	96 ± 2^{ab}
Renal Doppler shift (kHz)	6.4 ± 0.9	7.5 ± 0.8	11.7 ± 1.1^{ab}	11.3 ± 0.9^{ab}
Mesenteric Doppler shift (kHz)	7.1 ± 0.5	7.7 ± 0.7	7.2 ± 0.4	6.0 ± 0.5
Hindquarters Doppler shift (kHz)	3.9 ± 0.3	4.1 ± 0.3	7.2 ± 0.7^{ab}	6.4 ± 0.3^{ab}
Renal vascular conductance ([kHz mmHg^{-1}]10^3)	62 ± 8	73 ± 8	122 ± 12^{ab}	119 ± 10^{ab}
Mesenteric vascular conductance ([kHz mmHg^{-1}]10^3)	70 ± 6	75 ± 8	75 ± 4	63 ± 5
Hindquarters vascular conductance ([kHz mmHg^{-1}]10^3)	38 ± 3	39 ± 3	75 ± 8^{ab}	67 ± 3^{ab}

Values are mean ± s.e.mean; n = number of animals.

Table 4 Integrated (areas under or over curves) cardiovascular responses over the 3 h following injection of saline or TGF-β_1 in animals being infused with saline or LPS

	(a) *Saline/ saline* ($n=9$)	(b) *Saline/ TGF-β_1* ($n=9$)	(c) *LPS/ saline* ($n=9$)	(d) *LPS/ TGF-β_1* ($n=12$)
Heart rate (beats 10^{-1})	126 ± 60	-264 ± 102^{a}	156 ± 48^{b}	-300 ± 60^{ac}
Mean blood pressure (mmHg h)	6 ± 1	24 ± 2^{a}	-2 ± 1^{b}	25 ± 2^{ac}
Renal Doppler shift (kHz h)	-0.6 ± 0.2	2.1 ± 0.9^{a}	1.5 ± 0.8^{a}	-1.5 ± 0.4^{bc}
Mesenteric Doppler shift (kHz h)	1.2 ± 0.4	-1.5 ± 0.3^{a}	-2.2 ± 0.4^{a}	-5.7 ± 0.6^{abc}
Hindquarters Doppler shift (kHz h)	-0.7 ± 0.2	-2.5 ± 0.3^{a}	2.6 ± 0.3^{ab}	0.9 ± 0.2^{abc}
Renal vascular conductance ([kHz mmHg^{-1}]10^3 h)	-6 ± 1	-9 ± 2	17 ± 7^{ab}	-26 ± 5^{abc}
Mesenteric vascular conductance ([kHz mmHg^{-1}]10^3 h)	10 ± 4	-26 ± 3^{a}	-21 ± 4^{a}	-67 ± 7^{abc}
Hindquarters vascular conductance ([kHz mmHg^{-1}]10^3 h)	-8 ± 2	-29 ± 4^{a}	27 ± 3^{ab}	-5 ± 2^{bc}

Values are mean ± s.e.mean; n = number of animals.
Superscripts = $P<0.05$ versus corresponding columns (Kruskal-Wallis test).

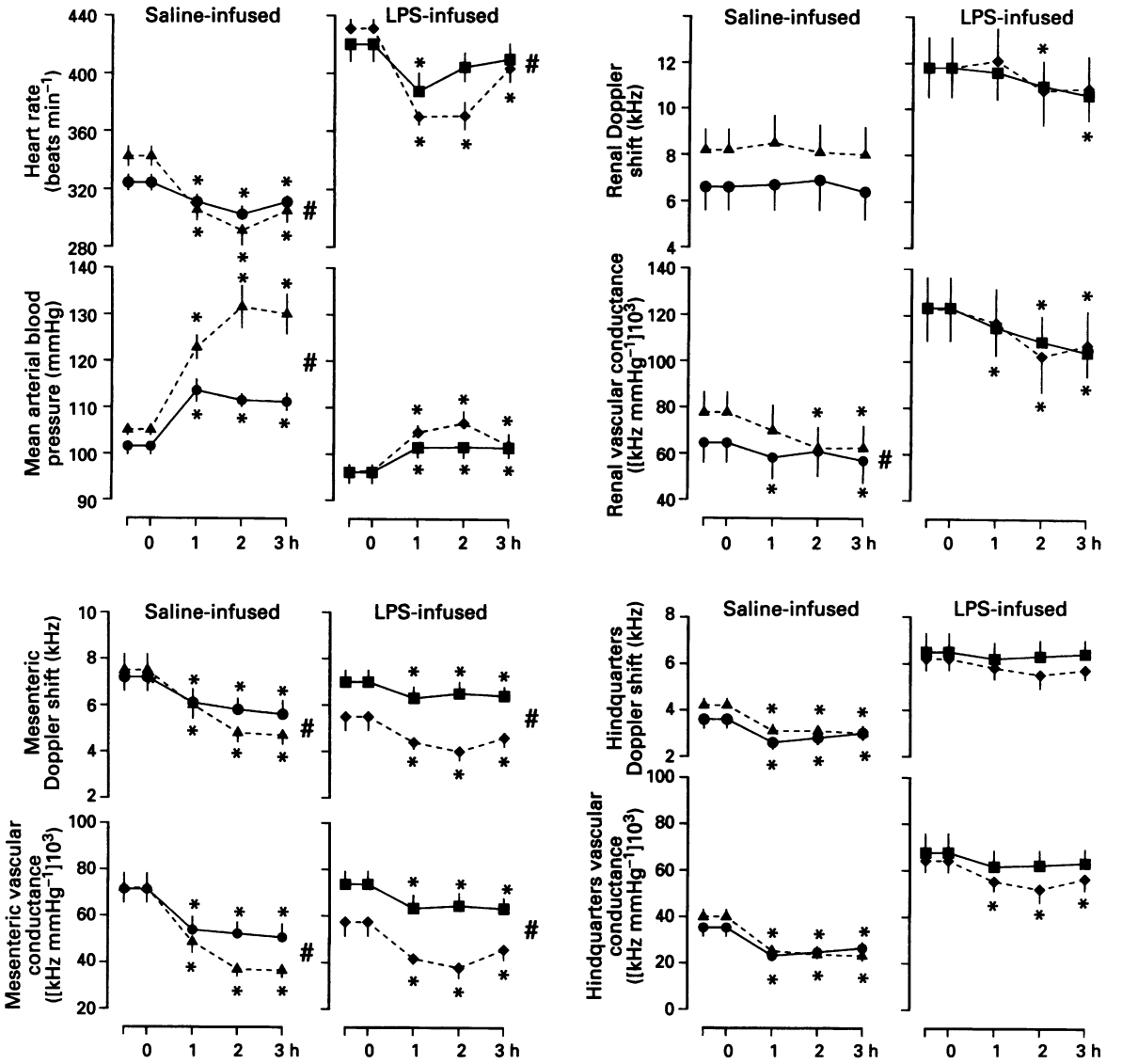

Figure 2 Cardiovascular changes during 3 h infusion of N^G-monomethyl-L-arginine (L-NMMA) in saline-infused or lipoplysaccharide (LPS)-infused rats which, 18 h previously, had received an injection of saline or TGF-β_1 (saline-infused: ●—● saline-injected ($n=8$), ▲—▲ TGF-β_1-injected ($n=8$); LPS-infused: ■—■ saline-injected ($n=8$), ◆—◆ TGF-β_1-injected ($n=7$). Values are mean, and vertical bars show s.e. mean. $^*P<0.05$ versus baseline (Friedman's test); $^\#P<0.05$ for corresponding integrated responses.

[kHz mmHg^{-1}]10^3; mesenteric vascular conductance, before = 59 ± 5, after = 54 ± 5 [kHz mmHg^{-1}]10^3; hindquarters vascular conductance, before = 42 ± 4, after = 41 ± 4 [kHz mmHg^{-1}]10^3).

Following administration of L-NMMA, the maximum changes in all the haemodynamic variables occurred at 2 h. At this juncture, the increase in MAP (+12 ± 3 mmHg) and falls in heart rate (−24 ± 6 beats min^{-1}) and renal, mesenteric and

Table 5 Integrated (areas under or over curves) cardiovascular responses over the 3 h during infusion of L-NMMA in animals being infused with saline or LPS and injected 18 h previously with saline or TGF-β_1

	(a) *Saline/ saline* ($n=8$)	(b) *Saline/ TGF-β_1* ($n=8$)	(c) *LPS/ saline* ($n=8$)	(d) *LPS/ TGF-β_1* ($n=7$)
Heart rate (beats 10^{-1})	−260 ± 87	−641 ± 49^a	−328 ± 87^b	−806 ± 130^{ac}
Mean blood pressure (mmHg h)	27 ± 2	57 ± 8^a	14 ± 2^{ab}	22 ± 6^b
Renal Doppler shift (kHz h)	−0.6 ± 0.3	−1.0 ± 0.4	−1.7 ± 0.6^a	−2.7 ± 1.2
Mesenteric Doppler shift (kHz h)	−3.4 ± 0.5	−5.7 ± 0.8^a	−1.6 ± 0.3^{ab}	−3.1 ± 0.8^{bc}
Hindquarters Doppler shift (kHz h)	−2.2 ± 0.5	−2.8 ± 0.3	−0.9 ± 0.3^{ab}	−1.4 ± 0.2^b
Renal vascular conductance ([kHz mmHg^{-1}]10^3 h)	−16 ± 4	−32 ± 6^a	−32 ± 8^a	−38 ± 14^a
Mesenteric vascular conductance ([kHz mmHg^{-1}]10^3 h)	−47 ± 5	−76 ± 11^a	−25 ± 3^{ab}	−41 ± 10^{bc}
Hindquarters vascular conductance ([kHz mmHg^{-1}]10^3 h)	−30 ± 4	−40 ± 5	−17 ± 5^{ab}	−25 ± 5^b

Values are mean ± s.e.mean; n = number of animals.
Superscripts = $P<0.05$ versus corresponding columns (Kruskal-Wallis test).

hindquarters vascular conductances (-6 ± 3, -17 ± 5, and -7 ± 2[kHz mmHg^{-1}]10^3, respectively) were all significantly smaller than the corresponding changes in the saline-infused animals injected with TGF-β_1, but not receiving SB 209670 ($+27\pm5$ mmHg; -51 ± 5 beats min^{-1}; -15 ± 2, -35 ± 5, and -16 ± 3[kHz mmHg^{-1}]10^3, respectively).

Discussion

The results of this study show that bolus injection of TGF-β_1 in normal rats causes haemodynamic changes and influences the cardiovascular responses to NOS inhibition (with L-NMMA) 18 h later, possibly via an effect on ET synthesis and release. Furthermore, we have shown that TGF-β_1 also exerts haemodynamic effects in animals infused with LPS, but does not appear to influence markedly the diminished cardiovascular responses to NOS inhibition in those animals.

As mentioned in the Introduction, there is evidence from *in vitro* studies that TGF-β_1 increases synthesis and release of ET. Hence, it is feasible that this action was responsible for the TGF-β_1-induced rise in MAP, and the accompanying mesenteric and hindquarters vasoconstrictions. However, generalized up-regulation of ET synthesis and release would not explain the absence of any effect of TGF-β_1 on renal haemodynamics, since exogenous ET-1 is a potent renal vasoconstrictor (Gardiner *et al.*, 1990). It may be that TGF-β_1-induced activation of vasodilator mediators (Jackson *et al.*, 1993) opposed the renal vasoconstrictor effect of endogenous ET, or TGF-β_1 differentially influences local synthesis and release of ET. Whatever the explanation, the finding that SB 209670 significantly attenuated the pressor, and mesenteric and hindquarters vasoconstrictor responses to TGF-β_1 is consistent with an involvement of endogenous ET in these effects. The time course of the cardiovascular action of TGF-β_1 i.e., developing slowly over 2 h, is in line with the *in vitro* data of Kurihara *et al.* (1989), which indicated that the increase in ET mRNA was maximal 2 h after stimulation with TGF-β_1. The slow onset of the *in vivo* effects of the latter indicate they were not a simple consequence of the interaction of TGF-β_1 with its receptor(s).

The finding that TGF-β_1, injected 18 h prior to L-NMMA, enhanced the haemodynamic actions of the latter, and the observation that this effect was abolished by SB 209670, are consistent with up-regulation of ET synthesis by TGF-β_1, and an involvement of ET in the responses to L-NMMA. Other recent evidence indicates that a component of the haemodynamic effect of NOS inhibition is due to ET release, inasmuch as the ET antagonist, bosentan, suppressed the haemodynamic effects of L-NMMA in conscious rats (Gardiner *et al.*, 1995a), and of L-NAME in anaesthetized rats (Richard *et al.*, 1995), and in the latter, L-NAME caused a modest elevation in plasma ET-1 (Richard *et al.*, 1995). Since TGF-β_1 did not enhance the maximal haemodynamic effects of L-NMMA over the first 3 min (data not shown), but, rather, enhanced the more prolonged effects of L-NMMA, it is not likely that the ability of TGF-β_1 to enhance the effects of NOS inhibition was due to an increased pool of releasable ET. This proposal is consistent with the observation that, 18 h after injection of TGF-β_1 (i.e., just before L-NMMA administration), cardiovascular variables in saline-infused animals were not different from the original baseline, and were not influenced by SB 209670. Therefore, it is possible that TGF-β_1 primes the ET system such that, following suppression of NO production, there is increased synthesis and subsequent release of ET.

The haemodynamic effects of LPS clearly involve many vasodilator and vasoconstrictor factors. One of the original reasons for this study was to investigate the possibility that TGF-β_1 might inhibit iNOS effects (Perella *et al.*, 1994), and thereby suppress vasodilator responses to LPS. In our model of chronic endotoxaemia, iNOS activity is significantly elevated in many tissues 6 h after the onset of LPS infusion, but is not increased 24 h after the onset of LPS infusion (Bennett *et al.*, 1995). Hence, administration of TGF-β_1, 6 h after the onset of LPS infusion, should have optimised our chances of detecting any functional effect of down-regulating iNOS. In this circumstance TGF-β_1 did appear to prevent the renal and hindquarters vasodilatation seen between 6–9 h after the onset of LPS infusion, and substantially enhanced the mesenteric vasoconstriction usually seen. But this apparent effect must be viewed against the background of the haemodynamic changes seen in saline-infused animals given TGF-β_1. Thus, the changes following TGF-β_1 in LPS-infused animals may, to a large extent, have represented a summation of the ongoing effects of LPS (developing renal and hindquarters vasodilatation and mesenteric vasoconstriction), and the superimposed 'normal' effects of TGF-β_1 (i.e., a rise in MAP with mesenteric and hindquarters vasoconstriction). This may not have been the case in the renal vascular bed, where TGF-β_1 appeared to cause vasoconstriction only in LPS-infused animals. It is feasible that this was due to inhibition of iNOS (Perrella *et al.*, 1994), and unmasking of underlying vasoconstrictor influences (Gardiner *et al.*, 1995b).

The diminution in the pressor and mesenteric and hindquarters vasoconstrictor responses to L-NMMA, 24 h after the onset of LPS infusion, was unexpected and has not been commented on previously. However, this finding is consistent with a component of the response to L-NMMA being due to ET (see above) to which there was desensitization consequent upon LPS-induced ET release (see Rubanyi & Polokoff (1994) for review). However, this leaves unexplained the enhanced renal vasoconstrictor effect of L-NMMA at this stage, although the marked resting renal vasodilatation makes difficult any direct comparison with saline-treated animals. Whatever the full explanation of the changes in response to L-NMMA after 24 h infusion of LPS, these changes were generally uninfluenced by TGF-β_1, and, in addition, treatment with the latter at 6 h did not influence haemodynamic status 24 h after the onset of infusion of LPS. It is clear that iNOS activity cannot be directly responsible for the renal and hindquarters vasodilatation seen at this time (Bennett *et al.*, 1995), thus it appears that the other factors involved are not suppressed by pretreatment with TGF-β_1. Equally, the ability of TGF-β_1 to stimulate prostanoid synthesis (Jackson *et al.*, 1993) does not seem to exacerbate the effects of LPS.

References

ATTISANO, L., WRANA, J.L., LÓPEZ-CASILLAS, F. & MASSAGUÉ, J. (1994). TGF-β receptors and actions. *Biochim. Biophys. Acta*, **1222**, 71–80.

BENNETT, T., GARDINER, S.M., KEMP, P.A., MARCH, J.È., REES, A. & MONCADA, S. (1995). Transient changes in inducible nitric oxide synthase activity during continuous infusion of lipopolysaccharide (LPS) in conscious rats. *Br. J. Pharmacol.*, **115**, Suppl. 67P.

DING, A., NATHAN, C.F., GRAYCAR, J., DERYNCK, R., STUEHR, D.J. & SRIMAL, S. (1990). Macrophage deactivating factor and transforming growth factor-β1, -β2, and -β3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-g. *J. Immunol.*, **145**, 940–944.

DOUGLAS, S.A., EDWARDS, R.M., ELLIOTT, J.D. & OHLSTEIN, E.H. (1995). *In vivo* pharmacological characterization of the non-peptide endothelin receptor antagonist SB 209670. *Br. J. Pharmacol.*, **114**, 405–413.

ENDO, T., UCHIDA, Y., MATSUMOTO, H., SUZUKI, N., NOMURA, A., HIRATA, F. & HASEGAWA, S. (1992). Regulation of endothelin-1 synthesis in cultured guinea pig airway epithelial cells by various cytokines. *Biochem. Biophys. Res. Commun.*, **186**, 1594–1599.

GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1990). Regional haemodynamic effects of endothelin-1 and endothelin-3 in conscious Long Evans and Brattleboro rats. *Br. J. Pharmacol.*, **99**, 107–112.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1995a). Effects of the non-peptide, non-selective endothelin antagonist, bosentan, on regional haemodynamic responses to N^G-monomethyl-L-arginine (L-NMMA) in conscious rats. *Br. J. Pharmacol.*, **114**, Suppl. 74P.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1995b). Involvement of angiotensin II and vasopressin in the haemodynamic responses to lipopolysaccharide (LPS) in conscious rats. *Br. J. Pharmacol.*, **114**, Suppl. 243 P.

JACKSON, B.A., GOLDSTEIN, R.H., ROY, R., COZZANI, M., TAYLOR, L. & POLGAR, P. (1993). Effects of transforming growth factor β and interleukin-1β on expression of cyclooxygenase 1 and 2 and phospholipase A_2 mRNA in lung fibroblasts and endothelial cells in culture. *Biochem. Biophys. Res. Commun.*, **197**, 1465–1474.

JUNQUERO, D.C., SCOTT-BURDEN, T., SCHINI, V.B. & VANHOUTTE, P.M. (1992). Inhibition of cytokine-induced nitric oxide production by transforming growth factor-β1 in human smooth muscle cells. *J. Physiol.*, **454**, 451–465.

KANSE, S.M., TAKAHASHI, K., LAM, H-C., REES, A., WARREN, J.B., PORTA, M., MOLINATTI, P., GHATEI, M. & BLOOM, S.R. (1991). Cytokine stimulated endothelin release from endothelial cells. *Life Sci.*, **48**, 1379–1384.

KURIHARA, H., YOSHIZUMI, M., SUGIYAMA, T., TAKAKU, F., YANAGISAWA, M., MASAKI, T., HAMAOKI, M., KATO, H. & YAZAKI, Y. (1989). Transforming growth factor-β stimulates the expression of endothelin mRNA by vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **159**, 1435–1440.

LEFER, A.M., MA, X-L., WEYRICH, A.S. & SCALIA, R. (1993). Mechanism of the cardioprotective effect of transforming growth factor β_1 in feline myocardial ischemia and reperfusion. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 1018–1022.

LEFER, A.M., TSAO, P., AOKI, N. & PALLADINO, JR, M.A. (1990). Mediation of cardioprotection by transforming growth factor-β. *Science*, **249**, 61–64.

OHLSTEIN, E.H., NAMBI, P., DOUGLAS, S.A., EDWARDS, R.M., GELLAI, M., LAGO, A., LEBER, J.D., COUSINS, R.D., GAO, A., FRAZEE, J.S., PEISHOFF, C.E., BEAN, J.W., EGGLESTON, D.S., ELSHOURBAGY, N.A., KUMAR, C., LEE, J.A., YUE, T-L., LOUDEN, C., BROOKS, D.P., WEINSTOCK, J., FEUERSTEIN, G., POSTE, G., RUFFOLO, R.R., GLEASON, J.G. & ELLIOTT, J.D. (1994). SB 209670, a rationally designed potent nonpeptide endothelin receptor antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 8052–8056.

OHTA, K., HIRATA, Y., IMAI, T., KANNO, K., EMORI, T., SHICHIRI, M. & MARUMO, F. (1990). Cytokine-induced release of endothelin-1 from porcine renal epithelial cell line. *Biochem. Biophys. Res. Commun.*, **169**, 578–584.

PERELLA, M.A., YOSHIZUMI, M., FEN, Z., TSAI, J-C., HSIEH, C-M., KOUREMBANAS, S. & LEE, M-E. (1994). Transforming growth factor-β1, but not dexamethasone, down-regulates nitric-oxide synthase mRNA after its induction by interleukin-1β in rat smooth muscle cells. *J. Biol. Chem.*, **269**, 14595–14600.

PINSKY, D.J., CAI, B., YANG, X., RODRIGUEZ, C., SCIACCA, R.R. & CANNON, P.J. (1995). The lethal effects of cytokine-induced nitric oxide on cardiac myocytes are blocked by nitric oxide synthase antagonism or transforming growth factor β. *J. Clin. Invest.*, **95**, 677–685.

RICHARD, V., HOGIE, M., CLOZEL, M., LÖFFLER, B-M. & THUILLEZ, C. (1995). *In vivo* evidence of an endothelin-induced vasopressor tone after inhibition of nitric oxide synthesis in rats. *Circulation*, **91**, 771–775.

RUBANYI, G.M. & POLOKOFF, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol. Rev.*, **46**, 325–415.

SCHINI, V.B., DURANTE, W., ELIZONDO, E., SCOTT-BURDEN, T., JUNQUERO, D.C., SCHAFER, A.I. & VANHOUTTE, P.M. (1992). The induction of nitric oxide synthase activity is inhibited by TGF-b_1, $PDGF_{AB}$ and $PDGF_{BB}$ in vascular smooth muscle cells. *Eur. J. Pharmacol.*, **216**, 379–383.

WALLER, J., GARDINER, S.M. & BENNETT, T. (1994). Regional haemodynamic responses to acetylcholine, methoxamine, salbutamol and bradykinin during lipopolysaccharide infusion in conscious rats. *Br. J. Pharmacol.*, **112**, 1057–1064.

(Received May 2, 1995
Revised July 14, 1995
Accepted August 14, 1995)