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Effects of the non-peptide, non-selective endothelin antagonist, bosentan, on regional haemodynamic responses to N^G-monomethyl-L-arginine in conscious 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 to allow monitoring of mean arterial blood pressure, heart rate and changes in renal, mesenteric and hindquarters haemodynamics. In the first experiment, animals ($n=8$) were given a bolus i.v. injection of the nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine hydrochloride (L-NMMA; 30 mg kg^{-1}) on four consecutive days. Fifteen min prior to L-NMMA administration on the fourth day, the endothelin, ET_A-, ET_B-receptor antagonist, bosentan, was injected (30 mg kg^{-1}, i.v.). Relative to the response on the third day, bosentan caused $33\pm4\%$, $24\pm3\%$, $14\pm3\%$, and $18\pm5\%$ inhibition of the pressor, and renal, mesenteric and hindquarters vasoconstrictor effects of L-NMMA, respectively.

2 In the second experiment, bosentan was given 15 min before L-NMMA in a group of rats ($n=6$) which had not received L-NMMA previously. Relative to the responses to L-NMMA on the first day in the previous experiment, bosentan caused a 30%, 24%, 18% and 27% inhibition of the pressor, and renal, mesenteric and hindquarters vasoconstrictor effects of L-NMMA, respectively.

3 The results indicate a significant contribution from endothelin to the haemodynamic effects of L-NMMA in conscious rats. However, since our previous studies have shown the renin-angiotensin and sympathoadrenal systems are not involved, it is likely that the major component of the cardiovascular response to L-NMMA in conscious rats is due to loss of vasodilator action of endothelial nitric oxide.

Keywords: Nitric oxide; endothelin; N^G-monomethyl-L-arginine; haemodynamics

Introduction

Inhibition of nitric oxide synthase (NOS) with arginine analogues such as N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl ester (L-NAME) causes marked hypertension accompanied by widespread vasoconstriction in conscious rats (e.g., Gardiner *et al.*, 1990a, b). Under some experimental conditions there is evidence that sympathoadrenal and renin-angiotensin systems may contribute to the hypertensive action of NOS inhibitors (Vargas *et al.*, 1990; Lacolley *et al.*, 1991; Sigmon *et al.*, 1992). However, in conscious unrestrained rats, in the presence of combined ganglion blockade and angiotensin-converting enzyme inhibition, L-NAME caused substantial pressor and vasoconstrictor effects (Gardiner *et al.*, 1990c), and in other experiments, antagonism of AT_1-receptors had no effect on the haemodynamic responses to L-NMMA (Gardiner *et al.*, 1994a), consistent with loss of vasodilator action of endothelial NO being responsible for the haemodynamic effects of NOS inhibition. But, since there is evidence from *in vitro* studies that NO inhibits release of the vasoconstrictor, endothelin (ET), from endothelial cells (Boulanger & Luscher, 1990), it is feasible the *in vivo* haemodynamic effects of NOS inhibitors involve disinhibition of ET release. In the present work we investigated this possibility by assessing the effects of the ET_A-, ET_B-receptor antagonist, bosentan (Clozel *et al.*, 1994; Gardiner *et al.*, 1994b) on the haemodynamic responses to L-NMMA. Some of these results have been presented to the British Pharmacological Society (Gardiner *et al.*, 1995).

Methods

All experiments were carried out on male, Long Evans rats (350-450 g) bred in the Biomedical Services Unit in Nottingham. All surgery was carried out under sodium methohexitone anaesthesia (Brietal, Lilly; 40-60 mg kg^{-1}, i.p., supplemented as required). The procedures for implantation of miniaturized pulsed Doppler probes, to monitor renal, mesenteric hindquarters blood flow, and intravascular catheters to allow recording of systemic arterial blood pressure and heart rate, and to permit i.v. administration of drugs, have been described in detail previously (Gardiner *et al.*, 1990a, b). Experiments were carried out on conscious, unrestrained rats, at least 24 h after surgery for implantation of catheters.

In one group of animals ($n=8$) a bolus injection of L-NMMA (30 mg kg^{-1}) was given on 4 consecutive days, but, on the fourth day, bosentan (30 mg kg^{-1}; Gardiner *et al.*, 1994b) was administered 15 min prior to L-NMMA. From those experiments we found that repeated injections of L-NMMA tended to evoke increasing pressor and vasoconstrictor responses (see Results). Therefore, in a second experiment, a group of rats ($n=6$) was given bosentan (30 mg kg^{-1}) 15 min prior to injection of L-NMMA (30 mg kg^{-1}) for the first time. Measurements were made at 1 min intervals over the 5 min following injection of L-NMMA, since this covered the period of maximum change.

Data analysis

Within-group analysis was by Friedman's test, and between group analysis was by the Mann-Whitney U test; a P value <0.05 was taken as significant.

Drugs

Bosentan was a gift from Dr M Clozel (Hoffman LaRoche, Basel, Switzerland), and L-NMMA hydrochloride was a gift

[1]Author for correspondence.

from Dr Daryl Rees (Wellcome Research Laboratories, Beckenham, Kent).

Results

Resting cardiovascular variables for the animals studied on 4 consecutive days are shown in Table 1. Injection of L-NMMA on the first 3 days had an increasing pressor effect but a relatively constant bradycardic action (Figure 1). Although the reductions in renal, in mesenteric, and in hindquarters flows were not significantly different on the 3 experimental days, there was a progressive increase in the vasoconstrictor response to L-NMMA in all vascular beds (Figure 1).

On the fourth experimental day, 15 min after bosentan, injection of L-NMMA caused pressor and renal, mesenteric and hindquarters vasoconstrictor effects, all of which were less than those seen on the previous two days in the absence of bosentan (Figure 1); the pressor and mesenteric vasocontrictor actions of L-NMMA in the presence of bosentan were also significantly less than the responses on the first experimental day, in the absence of bosentan (Figure 1). However, the bradycardic response to L-NMMA was not affected by bosentan.

In the second group of rats, given bosentan prior to L-NMMA without having been exposed to L-NMMA previously, the rise in mean arterial blood pressure and the reductions in renal, mesenteric and hindquarters vascular conductances were all significantly less than the responses seen in the first group of rats on their first exposure to L-NMMA. However, bosentan did not affect the bradycardic response to L-NMMA (Figure 1).

Discussion

The hypothesis tested in this work was that the regional haemodynamic responses to administration of L-NMMA in conscious rats involve ET, due to its release being disinhibited by suppression of NO production (Boulanger & Luscher, 1990). The finding that the non-selective, ET-receptor antagonist, bosentan, significantly inhibited the pressor and regional vasoconstrictor responses to L-NMMA is consistent with this hypothesis, and corroborates and extends the recent findings of Richard *et al.* (1995). The latter workers reported that bosentan inhibited the pressor effects of L-NAME in anaesthetized rats, but they did not assess regional haemodynamics. Here we show that bosentan antagonized the renal, mesenteric and hindquarters vasoconstrictor effects of L-NMMA, indicating that disinhibition of ET release was involved in all vascular beds. Thus it seems that the relative resistance of the hindquarters vasoconstrictor effect of L-NMMA or L-NAME to reversal by L-arginine (Gardiner *et al.*, 1990a, b) is not due to a greater involvement of ET in that vascular bed.

Our results, showing a modest effect of bosentan on responses to L-NMMA, differ from a very recent study (Thompson *et al.*, 1995) in which it was reported that the selective ET_A-receptor antagonist, BQ-610 caused a much greater inhibition of the pressor (76%) and renal vasoconstrictor (40%) effects of L-NAME in pentobarbitone anaesthetized rats. One possible explanation of this difference is that blockade of ET_A-receptors unmasks depressor and dilator

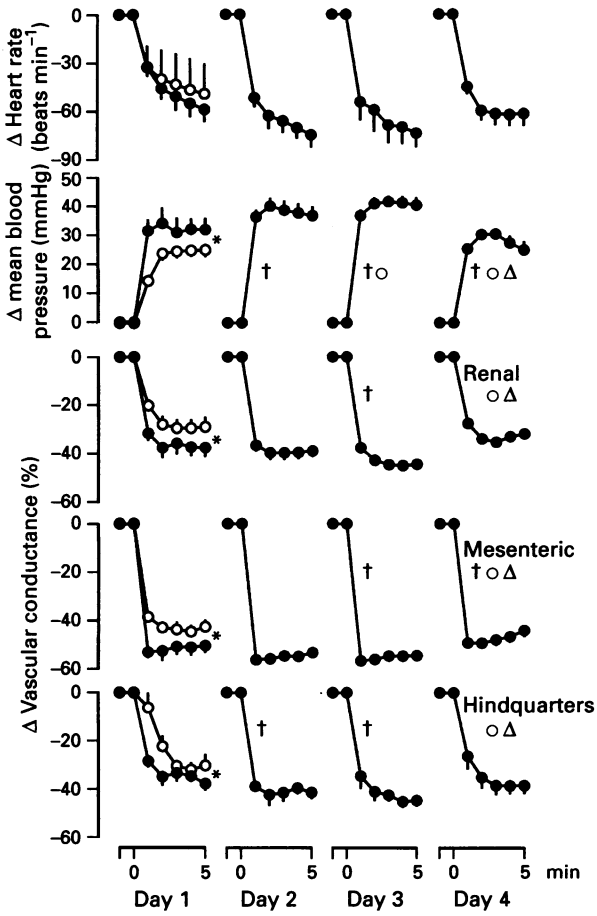

Figure 1 Cardiovascular changes following i.v. injection of L-NMMA (30 mg kg^{-1}) on four consecutive days in the same group of Long Evans rats (●, $n=8$); on the fourth day, bosentan (30 mg kg^{-1}) was given 15 min before L-NMMA. A second group of rats (○, $n=6$) was given bosentan 15 min before their first and only exposure to L-NMMA. *$P<0.05$ between responses in the two groups on the first experimental day; significant differences between integrated responses (areas under or over curves) in the same group on the four experimental days are indicated by:-†$P<0.05$ versus Day 1, $^{\circ}$ $P<0.05$ versus Day 2, $^{\Delta}$ $P<0.05$ versus Day 3. Values are mean with s.e.mean.

Table 1 Resting cardiovascular variables in the same group of conscious, Long Evans rats (n = 8), prior to administration of L-NMMA on four consecutive experimental days

	Day 1	*Day 2*	*Day 3*	*Day 4*
Heart rate (beats min^{-1})	334±6	339±9	339±6	336±4
Mean blood pressure (mmHg)	103±2	105±2	105±1	102±2
Renal Doppler shift (kHz)	6.2±0.5	6.0±0.6	6.3±0.7	5.6±0.4
Mesenteric Doppler shift (kHz)	7.3±0.9	7.4±1.0	8.3±1.1	7.7±0.8
Hindquarters Doppler shift (kHz)	4.2±0.3	4.1±0.2	3.9±0.1	4.2±0.3
Renal vascular conductance ((kHz mmHg^{-1}) 10^3)	61±5	58±6	61±7	55±4
Mesenteric vascular conductance ((kHz mmHg^{-1}) 10^3)	72±9	71±10	80±10	76±9
Hindquarters vascular conductance (kHz mmHg^{-1}) 10^3)	42±3	39±2	37±1	42±3

Values are mean±s.e.mean.

actions of ET mediated by ET_B-receptors, thereby causing Thompson *et al.* (1995) to overestimate the involvement of ET in the pressor and vasoconstrictor actions of L-NAME. However, the picture is complicated by the fact that ET_B-receptors can mediate pressor and vasoconstrictor responses to ET-1, and its analogues, in conscious rats (Gardiner *et al.*, 1994c). Moreover, since the dose of bosentan used in the present study does not block all the effect of exogenous ET (Gardiner *et al.*, 1994b), it is feasible that an action of ET on receptors other than ET_A- or ET_B-receptors (Gardiner *et al.*, 1994b,c) contributes to the haemodynamic responses to L-NMMA.

Thompson *et al.* (1995) reported also that BQ-610 abolished the bradycardic effect of L-NAME. This is surprising considering that the L-NAME-induced reduction in heart rate is largely a reflex response to the rise in arterial blood pressure (Widdop *et al.*, 1992). However, in the light of the latter finding our observation that bosentan attenuated the pressor, but not the bradycardic, effect of L-NMMA is equally puzzling.

In summary, although bosentan caused significant inhibition of the cardiovascular effects of L-NMMA, substantial responses remained. Hence, considering previous findings indicating a lack of involvement of sympathoadrenal and renin-angiotensin systems in the acute cardiovascular responses to NOS inhibitors (Gardiner *et al.*, 1990c; 1994a), it appears that loss of endothelial NO-mediated vasodilator tone is the major factor involved in the haemodynamic responses to i.v. administration of L-NMMA in conscious rats. However, we do not know what would happed to the response to L-NMMA if the effects of the sympathoadrenal and renin-angiotensin systems and ET were blocked simultaneously.

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