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Influence of CGRP (8-37), but not adrenomedullin (22-52), on the haemodynamic responses to lipopolysaccharide in conscious rats

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1 The functional involvement of the vasodilator peptides, adrenomedullin (ADM) and calcitonin gene-related peptide (CGRP), in the haemodynamic sequelae of continuous infusion of lipopolysaccharide (LPS) was assessed in conscious, male, Long Evans rats, by the use of peptide antagonists.

2 It was demonstrated that ADM (22-52) at a dose of 500 nmol kg^{-1} h^{-1} caused significant inhibition of the effects of ADM (1 nmol kg^{-1}), without affecting responses to CGRP (0.1 or 1 nmol kg^{-1}).

3 Even when the regional vasodilator responses to LPS infusion were enhanced (by pre-treatment with dexamethasone and the endothelin antagonist, SB 209670, or by pretreatment with SB 209670 and the AT_1-receptor antagonist, losartan), ADM (22-52) had no significant cardiovascular effects. In contrast, the $CGRP_1$-receptor antagonist, CGRP (8-37), caused small, but significant, inhibitions of the hypotensive and renal and mesenteric vasodilator effects of LPS, but only 6 h after onset of infusion in the presence of dexamethasone and SB 209670.

4 The results indicate that, in this model of endotoxaemia, the marked regional vasodilatations seen in the presence of dexamethasone and SB 209670 do not involve ADM, but do involve CGRP, albeit only to a small extent.

Keywords: Adrenomedullin; adrenomedullin (22-52); calcitonin gene-related peptide, calcitonin gene-related peptide (8-37) endotoxaemia

Abbreviations: ADM, adrenomedullin; CGRP, calcitonin gene-related peptide; LPS, lipopolysaccharide

Introduction

The regional and cardiac haemodynamic consequences of continuous, low dose infusion of lipopolysaccharide (LPS) in conscious rats are complex (Gardiner *et al*., 1995b). In part, this is due to temporal variation in the extent of involvement of opposing vasodilator and vasoconstrictor mechanisms. For example, we have reported that the LPS-induced activation of the renin-angiotensin system precedes the recruitment of endothelin, but, subsequently, both these vasoconstrictor systems act in concert to oppose the vasodilatation attributable, partly, to the marked expression of inducible nitric oxide synthase (iNOS), which is maximal around 6 h after the onset of LPS infusion (Gardiner *et al*., 1995b; 1996c).

In a series of experiments, involving various combinations of antagonists of AT_1-receptors, ET_A- and ET_B-receptors, and V_1-receptors, together with the iNOS inhibitor, aminoguanidine, or dexamethasone (to suppress iNOS and cyclo-oxygenase 2 expression), (Gardiner *et al*., 1995b; 1996a,b,c) we have observed substantial, residual vasodilator responses during LPS infusion, the mechanism(s) of which is unknown. Speculatively, we have suggested the dexamethasone-resistant vasodilator response to LPS could be due to adrenomedullin (ADM) (Gardiner *et al*., 1996a), because the production and release of this peptide is increased in endotoxaemia (Sugo *et al*., 1995), and is enhanced by dexamethasone (Minamino *et al*., 1995). To address this possibility we needed to administer an antagonist of the cardiovascular actions of ADM.

Recently, Eguchi *et al*. (1994) reported that human ADM (22-52) is an inhibitor of the cellular actions of ADM, but it is not well-established that it is an effective antagonist of the *in vivo* cardiovascular actions of exogenous ADM. For example, Champion *et al*. (1997a) found that, in the perfused hindlimb vascular bed of the cat, ADM (22-52) did not influence vasodilator responses to ADM, but inhibited the responses to the structurally similar peptide, calcitonin gene-related peptide (CGRP). However, there is evidence to suggest that the mechanisms underlying the cardiovascular effects of ADM fragments may differ between rats and cats (Nossaman *et al*., 1996; Champion *et al*., 1997a,b) and, in a more recent study (Dogan *et al*., 1997), it was shown that co-infusion of ADM (22-52) with ADM inhibited the regional cerebral vasodilator effects of ADM in rats. Therefore, our first objective was to determine an effective antagonistic dose of ADM (22-52) against the regional systemic vasodilator effects of exogenous ADM *in vivo* (Gardiner *et al*., 1995a), before assessing the influence of that dose of ADM (22-52) on the vasodilator responses to LPS infusion under different conditions.

In other models of endotoxaemia there is evidence that CGRP may contribute to the haemodynamic sequelae (e.g., Arden *et al*., 1994; Wang *et al*., 1995, 1996), particularly since the $CGRP_1$-receptor antagonist, human CGRP (8-37), reverses, albeit transiently, the marked hypotension caused by bolus injection of LPS (Hüttemeier *et al*., 1993). It could be argued, however, that the influence of CGRP (8-37) was due to antagonism of the actions of ADM, rather than CGRP (e.g. Nuki *et al*., 1993; Ikeda *et al*., 1996; Mazzocchi *et al*., 1996), although we (Gardiner *et al*., 1995a) and others (e.g., Elhawary *et al*., 1995; Pinto *et al*., 1996; Nandha *et al*., 1996; Hjelmqvist *et al*., 1997) have found that CGRP (8-37) has no influence on the actions of ADM. Since it is feasible that CGRP contributes to the vasodilator effects of LPS infusion in conscious rats, our second objective was to determine whether or not the dose of ADM (22-52) used had any influence on cardiovascular

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responses to exogenous CGRP, and, thereafter, to determine if a dose of CGRP (8-37), known to be without effect on responses to exogenous ADM (Gardiner *et al*., 1995a), had any influence on the cardiovascular responses to LPS infusion under various conditions (see Methods). Some of the results have been presented to the British Pharmacological Society (Gardiner *et al*., 1997, 1998).

Methods

All experiments were carried out on male, Long Evans rats (350–450 g) bred in the Biomedical Services Unit in Nottingham. Surgery was performed under anaesthesia (sodium methohexitone, Brietal, Lilly, 40–60 mg kg^{-1} i.p., supplemented as required). Seven–14 days before intravascular catheterization, pulsed Doppler probes were sutured around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor flow to the hindquarters). The day before experiments began, i.v. catheters (right jugular vein) and an i.a. catheter (distal abdominal aorta *via* the ventral caudal artery) were implanted; all details of these procedures have been published (Gardiner *et al*., 1995a,b). In conscious, unrestrained animals, with free access to food and water, the following six experiments were run:-

1 *Assessment of the antagonistic effects of ADM (22-52)* in vivo

Based on our previous work (Gardiner *et al*., 1995a) we gave bolus injections of ADM at a dose of 1 nmol kg^{-1} in the absence or presence of ADM (22-52) infused at 50 nmol kg^{-1} h^{-1} ($n=3$), 275 nmol kg^{-1} h^{-1} ($n=6$) or 500 nmol kg^{-1} h^{-1} ($n=11$). Animals were randomized to receive ADM in the absence or presence of ADM (22-52) as the first challenge, with at least 5 h between the two events. ADM was given 10 min after the onset of infusion of ADM (22-52).

To ensure that any antagonistic action of ADM (22-52) was selective, we also assessed responses to CGRP at 0.1 nmol kg^{-1} ($n=8$) or 1 nmol kg^{-1} ($n=6$) in the absence and presence of the highest dose of ADM (22-52) (as above).

2 *Influence of ADM (22-52) on responses to LPS in the presence of the ET_A-, ET_B-receptor antagonist, SB 209670, and dexamethasone*

In order to optimize conditions for detecting an involvement of ADM (see Introduction), animals ($n=7$) were given SB 209670 (300 μg kg^{-1} bolus, 300 μg kg^{-1} h^{-1} infusion) and dexamethasone (12.5 μg kg^{-1} h^{-1}) (Gardiner *et al*., 1996a) starting 1 h before the onset of LPS infusion (at 150 μg kg^{-1} h^{-1}). Six hours after the onset of LPS infusion ADM (22-52) was infused for 20 min at 500 nmol kg^{-1} h^{-1}. As a time control for this experiment, and for that involving CGRP (8-37) (see

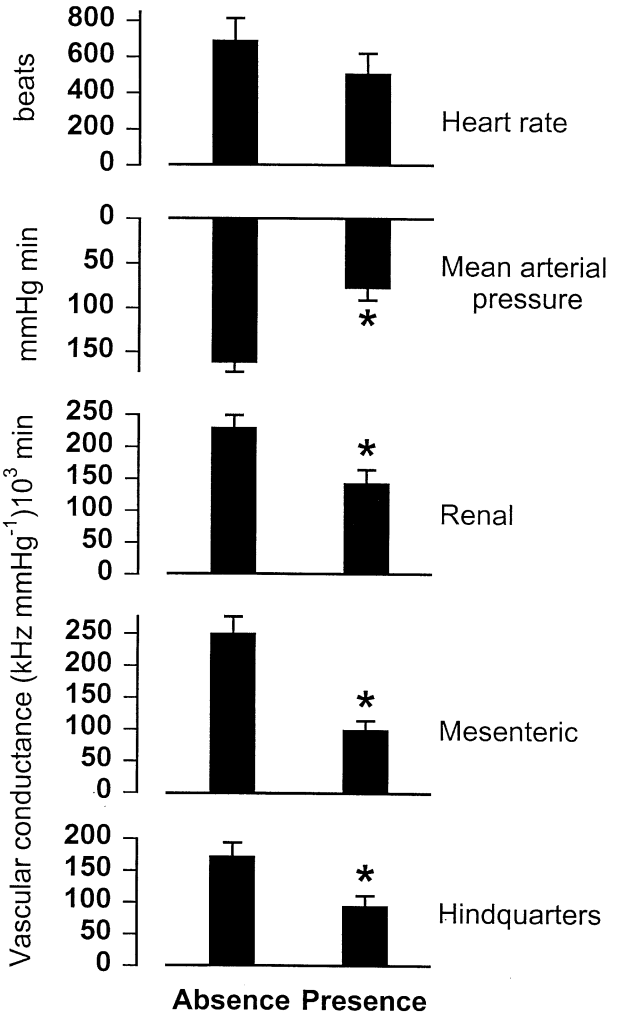

Figure 1 Integrated (areas over or under curves; $t=0–15$ min) cardiovascular responses to adrenomedullin (1 nmol kg^{-1}) in the absence or in the presence of adrenomedullin (22-52) (20 min after the start of infusion at 500 nmol kg^{-1} h^{-1}) in the same conscious, Long Evans rats ($n=11$). Values are mean and vertical bars show s.e.mean; $*P<0.05$ versus corresponding value in the absence of adrenomedullin (22-52).

Table 1 Cardiovascular variables in conscious, Long Evans rats

	Heart rate (beats min^{-1})	*Mean blood pressure* (mmHg)	*Doppler shift* *Renal* (kHz)	*Mesenteric* (kHz)	*Hindquarters* (kHz)	*Vascular conductance* *Renal*	*Mesenteric* ([kHz $mmHg^{-1}$]10^3)	*Hindquarters*
Control	313±9	106±2	5.2±0.2	4.7±0.4	3.2±0.3	49±3	45±4	31±3
ADM (22–52) 275 nmol kg^{-1} h^{-1}	321±9	107±1	5.4±0.2	5.6±0.5*	3.1±0.3	51±2	53±5*	29±3
Control	319±7	103±3	5.5±0.3	5.9±0.6	3.3±0.2	53±3	58±5	33±3
ADM (22–52) 500 nmol kg^{-1} h^{-1}	330±7	102±3	5.8±0.3	7.2±0.8*	3.6±0.2	58±3	71±8*	36±3

Control values were recorded in the resting state and those showing the effects of ADM (22–52) were recorded 10 min after the onset of infusion of the peptide at 275 nmol kg^{-1} h^{-1} ($n=6$) or 500 nmol kg^{-1} h^{-1} ($n=11$). Values are means±s.e.mean; $*P<0.05$ versus control value (Wilcoxon test).

experiment 4), animals ($n=7$) were given LPS in the presence of SB 209670 and dexamethasone (as above), and 6 h after the onset of LPS infusion saline (0.4 ml h^{-1}) was infused for 20 min.

3 *Influence of ADM (22-52) on responses to LPS in the presence of SB 209670 and losartan*

Animals ($n=3$) were given SB 209670 (as above) and losartan (10 mg kg^{-1} bolus) 1 h before onset of LPS infusion (as above). Six hours after onset of LPS infusion, ADM (22-52) was infused (as above).

As a time control for this experiment and that involving CGRP (8-37) (experiment 6), animals ($n=6$) received a saline infusion (0.4 ml h^{-1}) instead of ADM (22-52), or CGRP (8-37).

4 *Influence of CGRP (8-37) on responses to LPS in the presence of SB 209670 and dexamethasone*

This experiment was to match that involving ADM (22-52) (experiment 2). The protocol was identical to that in experiment 2, except that, 6 h after the onset of LPS infusion animals ($n=9$) were given CGRP (8-37) (6 μmol kg^{-1} h^{-1}) for 20 min (Gardiner *et al*., 1990; 1995a).

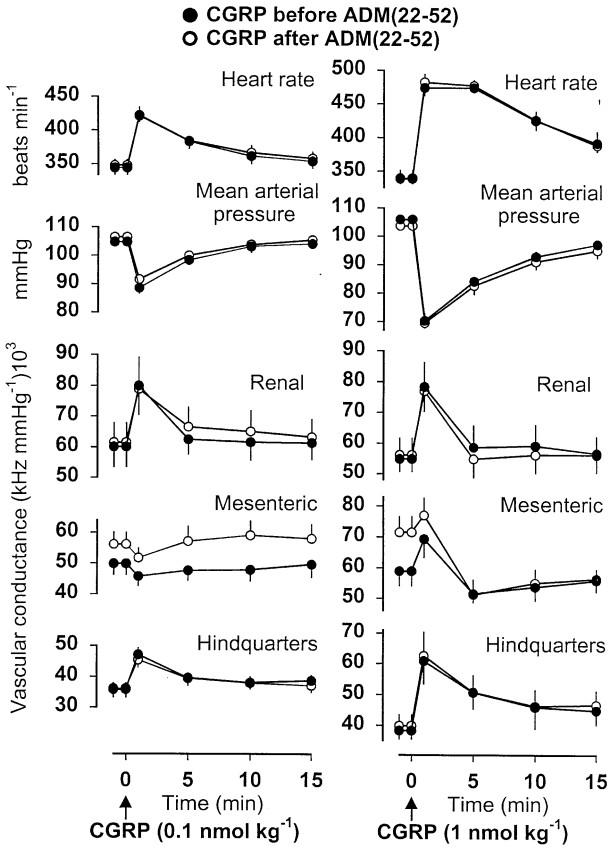

Figure 2 Cardiovascular responses to CGRP at 0.1 nmol kg^{-1} ($n=8$) or 1 nmol kg^{-1} ($n=6$) in the absence or presence of ADM (22-52) (500 nmol kg^{-1} h^{-1}) in conscious Long Evans rats. The mesenteric vasoconstrictor effect of the higher dose of CGRP was enhanced in the presence of ADM (22-52), possibly due to the mesenteric vasodilator influence of the latter. Values are mean and vertical bars show s.e.mean.

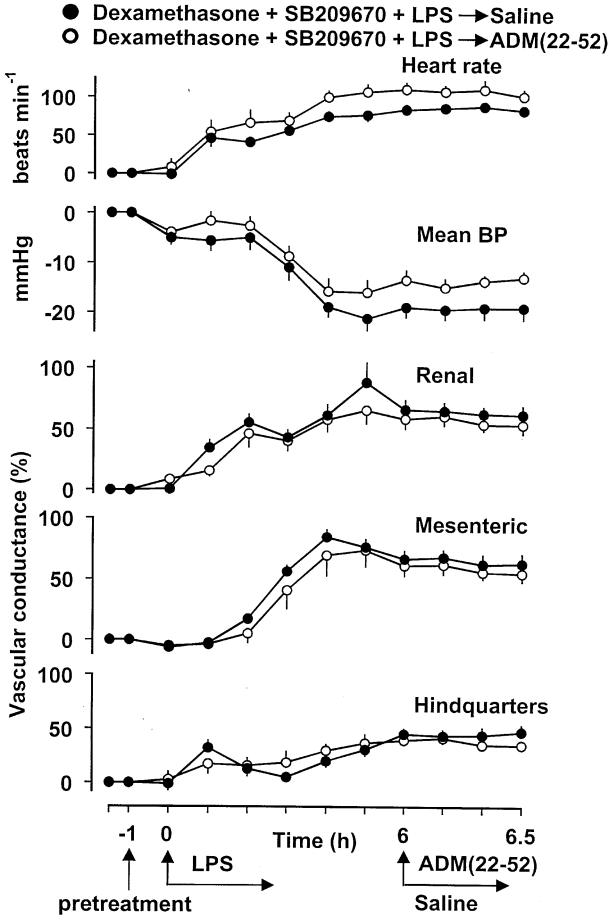

Figure 3 Cardiovascular responses to infusion of LPS (150 μg kg^{-1} h^{-1}) in the presence of dexamethasone and SB 209670 in conscious Long Evans rats. At 6 h, the animals were infused with ADM (22-52) (500 nmol kg^{-1} h^{-1}) ($n=7$) or saline ($n=7$). The tachycardia, hypotension and renal, mesenteric and hindquarters vasodilatations were not affected significantly by ADM (22-52). Values are mean and vertical bars show s.e.mean.

Table 2 Changes in cardiovascular variables in conscious, Long Evans rats

	Δ *Heart rate* (beats min^{-1})	Δ *Mean blood pressure* (mmHg)	Δ *Doppler shift* *Renal* (%)	*Mesenteric* (%)	*Hindquarters* (%)	Δ *Vascular conductance* *Renal* (%)	*Mesenteric* (%)	*Hindquarters* (%)
Group A	+86±14	−17±3	+35±5	+44±7	+36±8	+64±10	+74±8	+65±13
Group B	+100±13	−47±2	+21±1	+84±5	+5±5	+119±8	+232±8	+89±2
Group C	+114±19	−28±3	+29±11	+83±14	+26±16	+83±20	+159±26	+80±29

Group A = infusion of LPS for 6 h after pretreatment with dexamethasone and SB 209670 ($n=9$). Group B = infusion of LPS for 6 h after pretreatment with losartan and SB 209670 ($n=3$). Group C = infusion of LPS for 6 h after pretreatment with SB 209670 ($n=6$). Values are mean ± s.e.mean.

5 *Influence of CGRP (8-37) on responses to LPS in the presence of SB 209670*

Animals ($n=6$) were given SB 209670 and LPS (doses and timing as in experiment 5), and then CGRP (8-37) (as in experiment 4).

6 *Influence of CGRP (8-37) on responses to LPS in the presence of SB 209670 and losartan*

Here, we followed the same protocol as in experiment 3, except that animals ($n=3$) were given CGRP (8-37) rather than ADM (22-52).

Since Hüttemeier *et al*. (1993) reported that the involvement of CGRP in the cardiovascular responses to LPS was particularly marked around 40 min after the administration of LPS, a separate group ($n=3$) of rats was given CGRP (8-37), starting 40 min after the onset of infusion of LPS in the presence of SB 209670 and losartan.

As a time control for experiments 4, 5 and 6, animals ($n=6$) were given saline and, starting 1 h later, were infused with saline for 6 h (instead of LPS) before receiving CGRP (8-37) (6 μg kg^{-1} h^{-1}) for 20 min.

Data analysis

Cardiovascular variables were recorded continuously for at least 30 min before experiments were begun to ensure animals were in a steady state. Where the protocols involved infusions over 6 h or more, recordings were made continuously over the first 1 h and thereafter every hour for 6 h until the next intervention, when recordings were, again, made continuously. Average values for heart rate, mean arterial blood pressure, and renal, mesenteric and hindquarters Doppler shift were noted and values for regional vascular conductances were calculated from the mean arterial blood pressure and Doppler shift data (Gardiner *et al*., 1995a,b). Within-group analysis was by Friedman's test, and between-group analysis was by the Kruskal-Wallis test; a P value <0.05 was taken as significant.

Materials

LPS (*E. coli* serotype 0127 B8) was obtained from Sigma (U.K.). Adrenomedullin and human adrenomedullin (22-52)

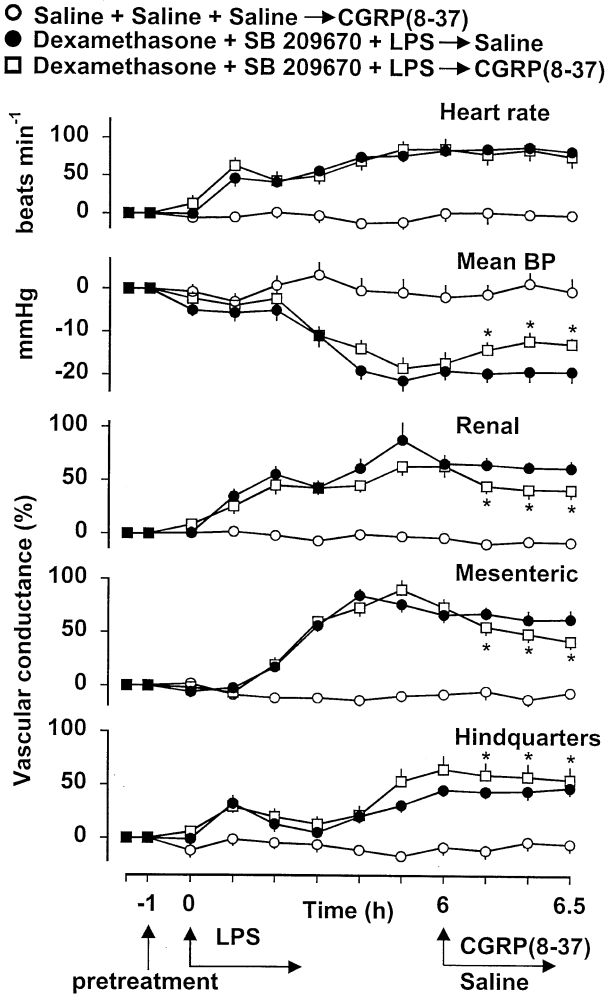

Figure 4 Cardiovascular responses to infusion of LPS (150 μg kg^{-1} h^{-1}) in the presence of dexamethasone and SB 209670 in conscious Long Evans rats. At 6 h, animals were infused with CGRP (8-37) (6 μmol kg^{-1} h^{-1}) ($n=9$) or saline ($n=7$; data as in Figure 3). CGRP (8-37) caused small, but significant inhibitions of the hypotension and renal, mesenteric and hindquarters vasodilatations. In animals receiving saline infusions ($n=6$), CGRP (8-37) at 6 h had no effects. Values are mean and vertical bars show s.e.mean. *$P<0.05$ versus the 6 h value (Friedman's test).

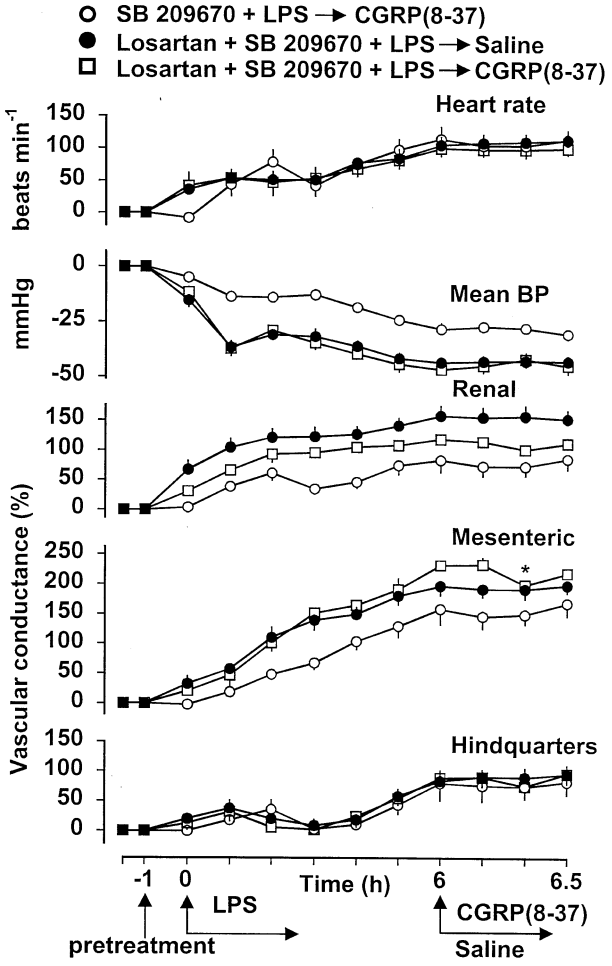

Figure 5 Cardiovascular responses to infusion of LPS (150 μg kg^{-1} h^{-1}) in conscious Long Evans rats pretreated with SB 209670, and infused with CGRP (8-37) (6 μmol kg^{-1} h^{-1}) at 6 h ($n=6$), or pretreated with losartan and SB 209670, and infused with CGRP (8-37) at 6 h ($n=3$), or pretreated with losartan and SB 209670 and infused with saline at 6 h ($n=6$). Values are mean and vertical bars show s.e.mean. *$P<0.05$ versus the 6 h value (Friedman's test).

were obtained from the Peptide Research Institute (Osaka, Japan), CGRP (8-37) was a gift from Celltech (Slough, U.K.), losartan potassium was a gift from Dr R.D. Smith (Du Pont, U.S.A.) and SB 209670 ([(±1S,2R,3S)-2-carboxymethoxy-4-methoxyphenyl) - 1 - (3,4 - methylenedioxyphenyl) - 5 - (prop - 1-yloxy)indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SKB, U.S.A.). All substances were dissolved in sterile, isotonic saline (NaCl 154 mmol l^{-1}); in the case of peptides, the saline had 1% bovine serum albumin (Sigma, U.K.) added. All infusions were given at 0.4 ml h^{-1} and bolus injections were given in 0.1 ml, flushed in with 0.1 ml.

Results

Assessment of the antagonistic effects of ADM (22-52) in vivo

ADM, at a dose of 1 nmol kg^{-1}, caused tachycardia, hypotension and renal, mesenteric and hindquarters vasodilatation, as reported previously (Gardiner *et al*., 1995a) (Figure 1).

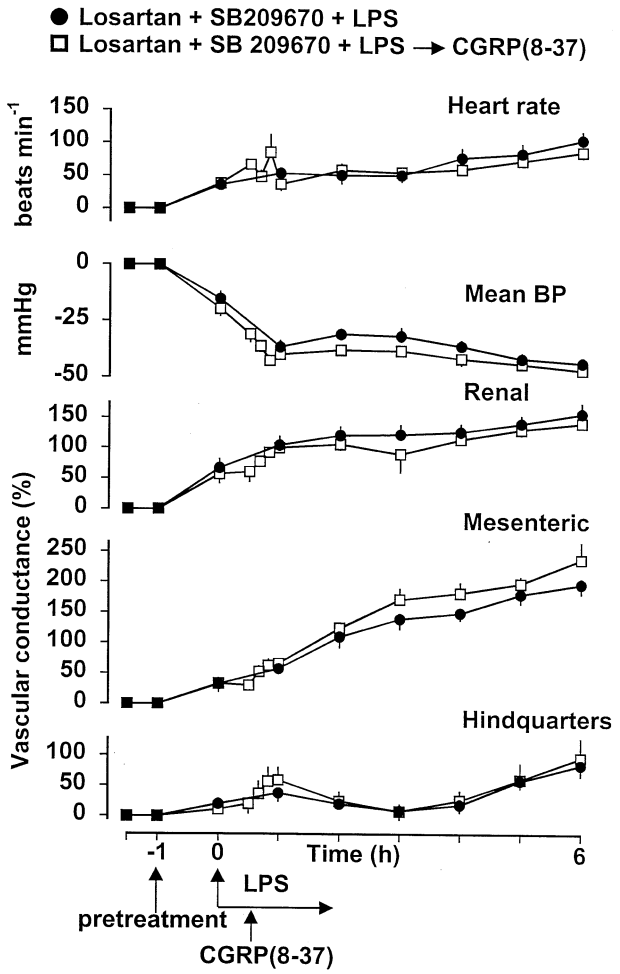

Figure 6 Cardiovascular responses to infusion of LPS (150 μg kg^{-1} h^{-1}) in conscious Long Evans rats ($n=3$) pretreated with losartan and SB 209670, and given CGRP (8-37) starting 40 min after the onset of LPS infusion. For comparison, the data from animals infused with LPS following pretreatment with losartan and SB 209670 (from Figure 5) are included. Values are mean and vertical bars show s.e.mean. CGRP (8-37) did not reverse the hypotension or vasodilatations associated with LPS infusion.

ADM (22-52) at a dose of 50 nmol kg^{-1} h^{-1} had no consistent effects itself, and did not influence responses to ADM (data not shown). ADM (22-52) at 275 and 500 nmol kg^{-1} h^{-1} caused dose-dependent increases in mesenteric Doppler shift and vascular conductance, but no other cardiovascular variables were changed significantly (Table 1).

Responses to ADM were unaffected by ADM (22-52) at 275 nmol kg^{-1} h^{-1} (data not shown) but at 500 nmol kg^{-1} h^{-1}, ADM (22-52) caused significant inhibition of the hypotensive and renal, mesenteric and hindquarters vasodilator effects of ADM (Figure 1).

Cardiovascular responses to CGRP at 0.1 and 1 nmol consisted of dose-dependent tachycardia, hypotension and renal and hindquarters vasodilatations, with biphasic changes in the mesenteric vascular bed (Figure 2). None of these effects of CGRP were inhibited by ADM (22-52); indeed, the latter enhanced the mesenteric vasoconstrictor effects of CGRP at a dose of 1 nmol (Figure 2).

Influence of ADM (22-52) on responses to LPS in the presence of SB 209670 and dexamethasone

In the presence of SB 209670 and dexamethasone, LPS infusion caused a biphasic fall in mean arterial blood pressure, together with significant increases in renal, mesenteric and hindquarters vascular conductances (Figure 3). Administration of ADM (22-52) 6 h after the onset of LPS infusion had no significant effect on any cardiovascular variable (Figure 3).

Influence of ADM (22-52) on responses to LPS in the presence of SB 209670 and losartan

ADM (22-52) had no significant effects on any cardiovascular variable during infusion of LPS in the presence of SB 209670 and losartan (data not shown).

Influence of CGRP (8-37) on responses to LPS in the presence of SB 209670 and dexamethasone

When CGRP (8-37) was administered 6 h after the onset of LPS infusion in the presence of SB 209670 and dexamethasone, there were significant inhibitions of the hypotension and renal, mesenteric and hindquarters vasodilatations (Figure 4).

Influence of CGRP (8-37) on responses to LPS in the presence of SB 209670

CGRP (8-37) had no significant effect on cardiovascular variables when administered 6 h after the onset of LPS infusion in the presence of SB 209670 (Figure 5).

Influence of CGRP (8-37) on responses to LPS in the presence of SB 209670 and losartan

When CGRP (8-37) was administered 6 h after the onset of LPS infusion in the presence of SB 209670 and losartan, the only significant change was a reduction in the mesenteric vascular conductance, which did not coincide with the onset of infusion and was not sustained (Figure 5). Moreover, even when CGRP (8-37) was administered 40 min after the onset of LPS infusion under these conditions, (i.e., when Hüttemeier *et al*. (1993) reported the largest effect), CGRP (8-37) did not reverse the hypotension or reduce any of the vasodilatations (Figure 6).

Discussion

In the present work we found no evidence for a functional involvement of ADM in the cardiovascular responses to LPS infusion in conscious rats. Moreover, only in the presence of SB 209670 and dexamethasone were the hypotensive and renal and mesenteric vasodilator responses to LPS inhibited by the CGRP-receptor antagonist, CGRP (8-37), consistent with a modest role for CGRP under those specific conditions.

We demonstrated an antagonistic effect of ADM (22-52) on the cardiovascular responses to exogenous ADM, thereby corroborating the *in vivo* observations of Eguchi *et al*. (1994), and the *in vivo* observations of Dogan *et al*. (1997). In the latter study, co-infusion of ADM (22-52) at a dose of 5 μg kg^{-1} min^{-1} (i.e. 1.4 nmol kg^{-1} min^{-1}) with ADM at a dose of 0.5 μg kg^{-1} min^{-1} (i.e., 80 pmol kg^{-1} min^{-1}) completely prevented the ADM-induced increase in regional cerebral blood flow (Dogan *et al*., 1997). In our study, we used higher doses of ADM (22-52) (i.e., 4.5 and 8.3 nmol kg^{-1} min^{-1}), but examined the effects against a high bolus dose of ADM (1 nmol kg^{-1}). It is feasible that with larger doses of ADM (22-52), we would have been able to demonstrate more effective antagonism. However, at the doses used, ADM (22-52) itself had a selective mesenteric vasodilator effect. Others have shown that, in the isolated mesenteric vascular bed of the cat (Santiago *et al*., 1995), or the pulmonary vascular bed of the rat (Nossaman *et al*., 1996), ADM (1-52) and ADM (15-52), but not ADM (22-52), cause vasodilatation. Those findings were taken to suggest that the amino acids, 15-52, and the six-membered ring structure (within the fragment 15-22) were necessary for the vasodilator action of ADM. Consistent with those *in vitro* observations, it has been shown that, following i.v. administration in anaesthetized rats, ADM (1-52) and ADM (15-52) caused decreases in blood pressure, whereas ADM (22-52) had no measurable effect at bolus doses of up to 300 nmol kg^{-1} (Champion *et al*., 1996). Dogan *et al*. (1997) also reported no effect of i.v. infusion of ADM (22-52) on blood pressure or regional cerebral blood flow. We also found no effect of ADM (22-52) on arterial blood pressure, but the mesenteric vasodilator action we observed is not compatible with the assertions of Santiago *et al*. (1995) or Nossaman *et al*. (1996). It is possible that the mesenteric vasodilator response to ADM (22-52) was due to an agonistic action, but this seems unlikely because ADM did not cause selective effects in the mesenteric vascular bed, but, rather, clear tachycardia, hypotension and increases in flow and conductance in renal, mesenteric and hindquarters vascular beds (see Gardiner *et al*., 1995a).

It is apparent that the mechanisms underlying the vasodilator actions of ADM and its fragments are complex, being species-dependent (e.g., Nossaman *et al*., 1996) and influenced by the choice of vasoconstrictor used to induce tone *in vitro* (Heaton *et al*., 1995). Thus, it is perhaps not surprising that, when investigated at a regional level *in vivo*, hitherto unreported effects of ADM (22-52) are observed. The lack of any mesenteric vasodilator effect in the presence of LPS (Figure 3) may have been due to the pre-existing elevation in mesenteric vascular conductance masking such an effect. Although it is feasible that the effects of ADM (22-52) on the mesenteric vascular bed influenced the subsequent responses to ADM, the fact that ADM (22-52) at a dose of 275 nmol kg^{-1} h^{-1} also increased mesenteric flow and conductance, yet did not influence responses to ADM, argues against this. Moreover, ADM (22-52) at a dose of 500 nmol kg^{-1} h^{-1} caused significant inhibition of the hypotensive and renal and hindquarters vasodilator responses to ADM without, itself, influencing those variables. This antagonistic action of ADM (22-52) was selective, inasmuch as responses to CGRP were unaffected. These data, together with our earlier findings (Gardiner *et al*., 1995a) indicate that, in the conscious rat, the cardiovascular effects of ADM or CGRP are mediated through specific receptors for these peptides. The apparent ability of ADM (22-52) to inhibit the depressor and regional vasodilator effects of ADM without causing significant suppression of the tachycardic effects of the latter, could be due to ADM (22-52) tending to increase heart rate (see Table 1).

Considering the reasonably clear evidence obtained in support of the significant, selective antagonistic action of ADM (22-52) against the vascular effects of exogenous ADM, our failure to detect a significant effect of ADM (22-52) on the cardiovascular responses to LPS, under any condition, is interpreted most simply as a lack of a functional contribution of ADM to haemodynamic status in our endotoxaemic model. We chose to examine the possible involvement of ADM at the 6 h time-point, since it was at that juncture that we had uncovered unidentified vasodilator mechanisms (see Introduction). Clearly, we cannot dismiss the possibility that at other stages during an LPS infusion, ADM may play a role. Indeed, in a very recent publication, Wang *et al*. (1998) reported that, in rats with sepsis induced by caecal ligation and puncture, there was an increase in plasma ADM levels by 2 h accompanied by increased ADM, mRNA in small intestine, left ventricle and thoracic aorta. However, these effects were also apparent up to 20 h after the onset of sepsis, so it is not likely that any activation of ADM production during LPS infusion is transient.

To our knowledge, the only evidence for an involvement of ADM in pathological states, such as endotoxaemia and cirrhosis, comes from measurements of plasma levels of the peptide, and correlations of those levels with the severity of the condition (e.g., Samson, 1998; Guevara *et al*., 1998; Fernandez-Rodriguez *et al*., 1998). On the basis of this indirect evidence it has been suggested that ADM may contribute to the vasodilatation associated with these conditions, although it should be noted that the correlation between plasma ADM and systemic vascular resistance index is not always significant (Fernandez-Rodriguez *et al*., 1998). Thus, collectively, there is no hard evidence to support a role for ADM in pathological vasodilatation. However, perhaps a more cautious interpretation of our results is that, for reasons to do with the dose of ADM (22-52) employed, and/or the possibility that ADM exerts local effects not influenced by ADM (22-52), it is possible that an influence of ADM went undetected in the present experiments. Interestingly, in a paper published whilst the present report was in preparation, Wang *et al*. (1998) cite unpublished findings of theirs showing that administration of anti-ADM antibodies, 1.5 h after caecal ligation and puncture, prevents the development of an hyperdynamic circulation. However, as mentioned above, in the model studied by Wang *et al*. (1998), ADM levels were elevated for up to 20 h, so it is not likely we missed an involvement of ADM in the cardiovascular responses to LPS infusion before 6 h.

Since we were able to discern an influence of CGRP (8-37) on the cardiovascular responses to LPS in the presence of SB 209670 and dexamethasone, it appears our experimental approach is not fundamentally flawed, but it is notable that it was only in this condition we were able to detect a functional role for CGRP. This surprised us, because both dexamethasone (Wang *et al*., 1991) and angiotensin II (Kawasaki *et al*., 1998) have been reported to inhibit CGRP release, so we had

expected the protocol in which we assessed the influence of CGRP (8-37) on the responses to LPS in the presence of SB 209670 and losartan would reveal the greatest contribution from CGRP to the haemodynamic changes. Table 2 summarizes the cardiovascular changes in the three groups infused with LPS for 6 h, just before CGRP (8-37) was administered. It is clear that the hypotension and renal, mesenteric and hindquarters vasodilatations were particularly marked in the group receiving SB 209670, losartan and LPS, so any pressor and/or vasoconstrictor action of CGRP (8-37) should have been readily apparent in this group.

An important difference between the vasodilatations which occur in our experimental model in the presence of SB 209670 (with, or without losartan) and LPS, and those seen in the presence of dexamethasone, SB 209670 and LPS is that in the former condition, but not in the latter, there is likely to be a major contribution from nitric oxide (Gardiner *et al*., 1995b). Thus, it is feasible that nitric oxide (and possibly cyclo-oxygenase 2 products) in some way inhibits, or masks the release, or action, of CGRP; this proposition, however, differs from the findings of Wang *et al*. (1995), who concluded prostaglandins mediated CGRP release in endotoxaemia.

Our failure to detect an effect of CGRP (8-37) when it was administered 6 h after the onset of LPS infusion in the absence of dexamethasone, raised the possibility that we had missed an earlier involvement of CGRP (*c.f*. Hüttemeier *et al*., 1993). However, even when we administered CGRP (8-37) 40 min after the onset of LPS infusion in the presence of SB 209670 and losartan it had no effect. From these results we conclude that the substantial effect of CGRP (8-37) on the early hypotensive response to bolus injection of LPS in anaesthetized rats is peculiar to that condition. It remains to be demonstrated that administering antagonists of ADM and/or CGRP have any beneficial therapeutic effect in patients with endotoxaemia and high circulating levels of these peptides.

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