

Effects of indomethacin on the regional haemodynamic responses to low doses of endothelins and sarafotoxin

¹Sheila M. Gardiner, Alix M. Compton & Terence Bennett

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

1 Regional haemodynamic responses to i.v. bolus injections of low doses (4 pmol and 40 pmol) of endothelin-1, -2, -3 and sarafotoxin-S6b were assessed in conscious, Long Evans rats in the absence and presence of indomethacin.

2 Both doses of endothelin-3 and sarafotoxin-S6b caused early renal vasodilatations that were not affected by indomethacin. Endothelin-1 caused an initial renal vasodilatation only in the presence of indomethacin, indicating that this peptide produced concurrent release of cyclo-oxygenase products that caused renal vasoconstriction. Neither dose of endothelin-2 produced an increase in renal conductance.

3 The 4 pmol dose of all four peptides caused mesenteric vasoconstrictions only. With the 40 pmol dose of the peptides, none caused early mesenteric vasoconstriction except in the presence of indomethacin. Thus, in this vascular bed the primary vasoconstrictor effects of the peptides (seen with the 4 pmol dose) were offset, following the 40 pmol dose, by release of vasodilator cyclo-oxygenase products. Indomethacin alone caused significant vasoconstriction only in the mesenteric vascular bed, indicating that in this region of the circulation, vasodilator prostanoids might be involved also in the tonic control of vascular conductance.

4 All four peptides at both doses caused early hindquarters vasodilatation. However, only the initial hypotensive and hindquarters vasodilator effects of the 40 pmol dose of sarafotoxin-S6b were attenuated by indomethacin. Under these conditions the hindquarters vasodilator effects of sarafotoxin-S6b were similar to those of the other peptides, indicating that the more marked effects of sarafotoxin-S6b in the absence of indomethacin were contributed to by vasodilator cyclo-oxygenase products in the hindquarters.

Introduction

In vitro and *in vivo* data indicate that endothelin-1 (Et-1) and endothelin-3 (Et-3) can exert vasodilator effects, possibly through release of eicosanoids and/or endothelium-derived relaxing factor (De Nucci *et al.*, 1988; Warner *et al.*, 1989a,b; Rakugi *et al.*, 1989; Rae *et al.*, 1989; Thiemeermann *et al.*, 1989; Lidbury *et al.*, 1989; Herman *et al.*, 1989). Although differential release of endogenous vasodilators by Et-1 and Et-3 could explain their differential pressor effects (Inoue *et al.*, 1989), pretreatment with indomethacin does not have predictable effects on the initial hypotensive effects of Et-1 in pithed or anaesthetized rats (De Nucci *et al.*, 1988; Walder *et al.*, 1989; Winquist *et al.*, 1989). However, it is feasible that indomethacin pretreatment could modify the regional haemodynamic effects of Et-1 or Et-3 without influencing the changes in systemic arterial blood pressure induced by these peptides. In the present work we investigated this possibility. In addition, we extended the experiments to include a comparison of the responses to endothelin-2 (Et-2) (Inoue *et al.*, 1989) and to sarafotoxin-S6b (S6b) (Takasaki *et al.*, 1988a; Kloog *et al.*, 1988) in the absence and presence of indomethacin, since there is a substantial structural homology between Et-1, Et-2 and Et-3 and S6b (Takasaki *et al.*, 1988a,b; Lee & Chiappinelli, 1988; Kloog *et al.*, 1988).

Methods

All experiments were carried out on male Long Evans rats 3–4 months old (380–420 g). The procedures were as described previously (Gardiner *et al.*, 1988). Under sodium methohexitone anaesthesia (60 mg kg⁻¹ i.p., supplemented as necessary) pulsed Doppler probes (Haywood *et al.*, 1981) were sutured around left renal and superior mesenteric arteries and the distal abdominal aorta (to monitor hindquarters flow). At

least 7 days later, animals were briefly re-anaesthetized (sodium methohexitone, 40 mg kg⁻¹ i.p.) and had jugular venous catheters and a distal abdominal aortic catheter implanted. Experiments were begun the following day when animals were fully conscious, and ran over 2 days. On day 1 bolus doses (4 and 40 pmol) of Et-1, Et-2 and Et-3 and S6b were given in randomized order, but with the lower dose before the higher dose, and doses separated by at least 60 min. The following day, indomethacin was administered by primed infusion (5 mg kg⁻¹ and 5 mg kg⁻¹ h⁻¹) and, starting 30 min later, the bolus doses of peptides were given in the same order and with the same timing as on day 1. Mean arterial blood pressure (MBP), instantaneous heart rate (HR) and renal, mesenteric and hindquarters Doppler shift signals were recorded continuously. Percentage changes in the latter were calculated as indices of changes in regional blood flows (Haywood *et al.*, 1981), and % changes in vascular conductance were calculated from mean Doppler shift signals and MBP.

The initial hypotensive responses to the peptides were maximal about 15 s after administration of both doses. The subsequent pressor responses peaked at about 1 min with the 4 pmol dose and at about 2 min after the 40 pmol dose; these values are included in the tables. Data were subjected to two-way, non-parametric analysis of variance (Friedman's test) and Wilcoxon's ranks sum test.

Peptides and drugs

All peptides were obtained from the Peptide Institute, Osaka, Japan (through Scientific Research Associates, London) and dissolved in isotonic saline containing 1% bovine serum albumin. Administration of both doses of all peptides was in a volume of 0.1 ml; this volume of vehicle had no cardiovascular effects. No allowance was made for body weight in the peptide dose administered, since the maximum difference in injectate volumes would have been only 10 µl. Furthermore, since all animals served as their own controls, only intra-individual comparisons of peptide effects were carried out.

¹ Author for correspondence.

Indomethacin (Merck Sharp & Dohme Ltd) was dissolved in 10 mM sodium bicarbonate; the bolus dose was given in a volume of 0.34 ml over 10 min. The continuous infusion was given at 0.3 ml h⁻¹.

Results

Table 1 summarizes the values for cardiovascular variables before peptide administration on day 1 and before and 30 min after the onset of indomethacin administration (but before peptides were injected) on day 2. There were no significant differences for any of the variables except mesenteric flow and vascular conductance.

Responses to endothelin-1

The 4 pmol dose of Et-1 caused a significant initial fall in MBP and rise in HR in the presence of indomethacin; these effects and the subsequent increase in MBP were not different

from the corresponding changes in the absence of indomethacin (Table 2). In the latter condition there was an early mesenteric vasoconstriction and hindquarters vasodilatation that were not different from the responses seen in the absence of indomethacin (Table 2). The subsequent renal and mesenteric vasoconstrictions were similar also in the two conditions (Table 2).

Administration of the 40 pmol dose of Et-1 caused similar initial falls and subsequent rises in MBP and associated tachycardias and bradycardias in the absence and presence of indomethacin (Table 3). However, in the latter condition there was an initial renal vasodilatation and mesenteric vasoconstriction not seen in the absence of indomethacin (Table 3). The early hindquarters vasodilatation and the subsequent renal and mesenteric vasoconstrictions were not affected by indomethacin (Table 3).

Responses to endothelin-2

The 4 pmol dose of Et-2 did not reduce MBP significantly although there was a significant tachycardia (Table 2). The

Table 1 Cardiovascular variables in the same conscious Long Evans rats before peptide administration on day 1 and before and 30 min after the onset of indomethacin administration (i.e. before peptides were injected) on day 2

	Day 1	Day 2	
		Pre-indomethacin	Post-indomethacin
Heart rate (beats min ⁻¹)	319 ± 6	322 ± 8	302 ± 10
Mean blood pressure (mmHg)	106 ± 4	108 ± 3	111 ± 3
Doppler shift (kHz)			
Renal	9.8 ± 1.1	9.8 ± 0.7	9.5 ± 0.7
Mesenteric	7.6 ± 0.3	8.0 ± 0.5	6.8 ± 0.5†
Hindquarters	4.4 ± 0.4	4.2 ± 0.7	3.7 ± 0.7
Conductance (100 (kHz mmHg ⁻¹))			
Renal	92 ± 9	91 ± 5	85 ± 6
Mesenteric	72 ± 5	74 ± 6	62 ± 5*†
Hindquarters	42 ± 4	39 ± 7	35 ± 7

Values are means ± s.e.mean (n = 8).

* P < 0.05 versus day 1; † P < 0.05 versus pre-indomethacin (Wilcoxon test).

Table 2 Cardiovascular changes following bolus injection (4 pmol) of endothelin-1, -2, or -3 or sarafotoxin-S6b in the absence or presence of indomethacin, in conscious, Long Evans rats

	Endothelin-1		Endothelin-2		Endothelin-3		Sarafotoxin-S6b	
	Time after injection (min)							
	0.25	1.0	0.25	1.0	0.25	1.0	0.25	1.0
Δ Heart rate (beats min ⁻¹)								
- Indomethacin	33(9)*	19(9)	31(10)*	16(7)*	35(7)*	19(8)*	36(13)*	13(11)
+ Indomethacin	29(8)*	19(12)	36(9)*	9(7)	49(5)*	33(11)*	44(7)*	7(12)
Δ Mean blood pressures (mmHg)								
- Indomethacin	-2(3)	9(2)*	-4(2)	3(1)	-7(2)*	1(1)	-4(3)	8(1)*
+ Indomethacin	-6(2)*	5(1)*	-2(3)	1(1)	-12(2)*	1(2)	-11(4)*	3(2)
Δ Renal flow (%)								
- Indomethacin	-2(3)	-13(2)*	0(1)	-7(2)*	4(2)*	-3(3)	3(2)	-11(2)*
+ Indomethacin	-1(2)	-13(3)*	1(2)	-3(2)	1(1)	-4(2)	1(2)	-8(2)*
Δ Mesenteric flow (%)								
- Indomethacin	-18(5)*	-24(2)*	-14(3)*	-20(3)*	-17(6)*	-25(5)*	-24(3)*	-31(2)*
+ Indomethacin	-19(3)*	-28(3)*	-7(3)*	-8(2)*	-24(4)*	-26(4)*	-26(5)*	-26(3)*
Δ Hindquarters flow (%)								
- Indomethacin	34(6)*	35(8)*	27(3)*	20(3)*	25(6)*	19(7)*	29(5)*	22(4)*
+ Indomethacin	36(7)*	39(12)*	24(5)*	15(7)*	52(8)*	31(9)*	40(12)*	44(20)*
Δ Renal conductance (%)								
- Indomethacin	0(7)	-20(2)*	3(3)	-9(2)*	12(4)*	-3(3)	7(4)	-17(2)*
+ Indomethacin	5(2)*	-17(4)*	2(3)	-4(2)*	13(2)*	-5(2)*	12(3)*	-10(1)*
Δ Mesenteric conductance (%)								
- Indomethacin	-16(8)	-29(1)*	-11(3)*	-22(2)*	-12(7)*	-25(6)*	-21(3)*	-36(2)*
+ Indomethacin	-15(3)*	-31(3)*	-6(3)*	-9(2)*†	-14(6)*	-27(3)*	-18(6)*	-28(3)*
Δ Hindquarters conductance (%)								
- Indomethacin	37(6)*	25(9)*	31(5)*	17(3)*	34(8)*	18(8)*	34(7)*	14(4)*
+ Indomethacin	44(9)*	34(13)*	26(7)*	14(8)*	71(9)*	30(11)*	57(18)*	40(20)*

Values are means ± s.e.mean (n = 8).

* P < 0.05 versus baseline (Friedman's test).

† P < 0.05 for corresponding values in the absence and presence of indomethacin (Wilcoxon's test).

Table 3 Cardiovascular changes following bolus injection (40 pmol) of endothelin-1, -2, or -3 or sarafotoxin-S6b in the absence or presence of indomethacin, in conscious, Long Evans rats

	<i>Endothelin-1</i>		<i>Endothelin-2</i>		<i>Endothelin-3</i>		<i>Sarafotoxin-S6b</i>	
	0.25	2.0	0.25	2.0	0.25	2.0	0.25	2.0
Δ Heart rate (beats min ⁻¹)								
– Indomethacin	87(13)*	–44(9)*	34(15)	–24(9)*	68(13)*	–23(7)*	82(9)*	–27(11)
+ Indomethacin	62(10)*	–48(5)*	34(10)*	–39(7)*	54(13)*	–26(6)*	63(11)*	–26(19)
Δ Mean blood pressure (mmHg)								
– Indomethacin	–22(2)*	25(3)*	–9(4)	13(2)*	–13(3)*	13(2)*	–28(2)*	20(3)*
+ Indomethacin	–18(3)*	21(3)*	–7(3)	13(4)*	–15(4)*	9(2)*	–13(3)*†	14(4)*
Δ Renal flow (%)								
– Indomethacin	–18(4)*	–44(2)*	–7(4)	–29(2)*	8(2)*	–23(4)*	–13(5)	–33(4)*
+ Indomethacin	–5(4)	–46(4)*	–4(2)	–28(4)*	3(3)	–24(4)*	2(3)	–31(3)*
Δ Mesenteric flow (%)								
– Indomethacin	–19(4)*	–39(5)*	–17(7)*	–29(4)*	–10(5)	–29(8)*	–8(7)	–37(5)*
+ Indomethacin	–33(4)*	–44(3)*	–29(4)*	–33(4)*	–31(3)*	–43(4)*	–26(5)*	–42(3)*
Δ Hindquarters flow (%)								
– Indomethacin	55(7)*	–3(8)	47(9)*	10(4)*	47(5)*	1(8)	60(5)*	3(4)
+ Indomethacin	61(9)*	16(8)*	37(4)*	6(3)	37(7)*	8(4)	43(10)*	21(9)
Δ Renal conductance (%)								
– Indomethacin	4(4)	–55(2)*	3(8)	–37(3)*	24(7)*	–31(4)*	20(7)*	–43(4)*
+ Indomethacin	15(4)*†	–54(3)*	3(4)	–35(5)*	22(8)*	–30(4)*	16(4)*	–39(4)*
Δ Mesenteric conductance (%)								
– Indomethacin	3(6)	–51(4)*	–10(7)	–37(3)*	4(9)	–37(7)*	27(12)	–46(4)*
+ Indomethacin	–19(4)*†	–53(3)*	–24(4)*†	–39(6)*	–19(6)*†	–47(4)*	–15(7)†	–49(3)*
Δ Hindquarters conductance (%)								
– Indomethacin	98(11)*	–21(7)*	64(17)*	–2(4)	69(6)*	–10(7)	120(11)*	–13(5)
+ Indomethacin	96(14)*	–2(6)	48(9)*	–5(3)	61(10)*	–1(4)	64(13)*†	9(10)

Values are means \pm s.e.mean ($n = 8$).

* $P < 0.05$ versus baseline (Friedman's test).

† $P < 0.05$ for corresponding values in the absence and presence of indomethacin (Wilcoxon's test).

initial hindquarters vasodilatation and mesenteric vasoconstriction and the subsequent renal vasoconstriction were not affected by indomethacin, although the later mesenteric vasoconstriction was greater in the absence than in the presence of indomethacin (Table 2).

The initial fall in MBP following the 40 pmol dose of Et-2 did not reach significance, although the subsequent rise did, but neither the MBP changes nor the associated increases and decreases in HR were affected by indomethacin (Table 3). However, in the presence of indomethacin there was an initial mesenteric vasoconstriction following Et-2 that was not seen in the absence of indomethacin (Table 2). The early hindquarters vasodilatation and later renal and mesenteric vasoconstrictions were unaffected by indomethacin (Table 2).

Responses to endothelin-3

The initial responses to the 4 pmol dose of Et-3 (falls in MBP, and mesenteric vascular conductance and rises in HR and renal and hindquarters vascular conductances) were not affected by indomethacin (Table 2). Moreover, the subsequent responses were not different in the two conditions (Table 2).

The 40 pmol dose of Et-3 caused initial hypotension and tachycardia and renal and hindquarters vasodilatations that were unaffected by indomethacin (Table 3). However, in the presence of indomethacin there was an initial mesenteric vasoconstriction in response to Et-3 that was not seen in the absence of indomethacin (Table 3). The later pressor, bradycardic and renal and mesenteric vasoconstrictor responses to Et-3 were unaffected by indomethacin (Table 3).

Responses to sarafotoxin-S6b

The initial fall in MBP following the 4 pmol dose of S6b was significant only in the presence of indomethacin, although the actual change in MBP was not different from that seen in the absence of indomethacin (Table 2). The associated regional haemodynamic changes (renal and hindquarters vasodilatation and mesenteric vasoconstriction) were not different in the

absence and presence of indomethacin (Table 2), neither were the later changes (renal and mesenteric vasoconstrictions, hindquarters vasodilatation) (Table 2).

The 40 pmol dose of S6b caused an early fall in MBP that was significantly attenuated by indomethacin (Table 3). This effect was accompanied by a reduction in the hindquarters vasodilator response to S6b, and by a mesenteric vasoconstriction not seen in the absence of indomethacin (Table 3). Thereafter, the pressor effects and regional haemodynamic changes (renal and mesenteric vasoconstrictions) evoked by S6b were not affected by indomethacin (Table 3).

Discussion

The present work has shown that the initial hypotensive effects of low bolus doses of Et-1, Et-2 and Et-3 and S6b were associated with different regional haemodynamic profiles that were differentially affected by indomethacin when changes in MBP were not. The later pressor and regional constrictor effects of the endothelins and S6b were not enhanced by indomethacin. These results also indicate that while endogenous cyclo-oxygenase products could influence the initial responses to endothelins and S6b, other mechanisms must also contribute to the hypotensive and vasodilator effects of these peptides. The initial hypotensive responses to the peptides were so rapid that it is not likely they were modified by baroreflex-mediated changes in autonomic neuronal outflow to the different vascular beds investigated. Moreover, the 4 pmol bolus dose of the peptides was chosen because it had borderline effects on MBP, but clear-cut regional haemodynamic actions.

Indomethacin alone caused significant mesenteric vasoconstriction, indicating that tonic release of cyclo-oxygenase products might be important in controlling conductance in this region of the circulation. However, there was no evidence that indomethacin influenced the initial hypotension or caused a significant change in the associated haemodynamic events following the 4 pmol dose of the peptides although, after this dose of Et-1, there was an early renal vasodilatation in the

presence of indomethacin that was not seen in its absence (Table 2). Furthermore, following the 40 pmol dose of Et-1 there was a significant difference between the renal conductance changes in the absence and presence of indomethacin (Table 3), with a significant renal vasodilatation occurring in the latter condition. In rats, arachidonic acid and several prostanoids, including prostaglandin E₂ (PGE₂) and PGD₂, have renal vasoconstrictor effects (Gerber & Nies 1979; Quilley *et al.*, 1989). Therefore, the most likely explanation of the present findings is that any initial renal vasodilator effects of Et-1 were masked by concurrent release of vasoconstrictor prostanoids. This contrasts with the picture in rabbits where Et-1 stimulates the release of renal cyclo-oxygenase products that are vasodilator and act to limit the renal vasoconstrictor effects of Et-1 (Rae *et al.*, 1989).

In the present work the early renal vasodilator responses to the 40 pmol dose of Et-3 and S6b were not affected by indomethacin and were not different from the effect seen with Et-1 in the presence of indomethacin (Table 3). These findings indicate that this vasodilator mechanism does not involve cyclo-oxygenase products. It is feasible that Et-1, Et-3 and S6b release an alternative relaxing factor from endothelial cells (De Nucci *et al.*, 1988) (see below), although it was not possible to determine the extent to which autoregulatory phenomena might have contributed to the early renal vasodilatation seen following Et-1 and -3 and S6b. However, at least in the case of Et-3, this cannot be the sole explanation of the vasodilatation since the increase in renal conductance was associated with a significant increase in flow when MBP fell. Whatever the mechanism responsible for the early renal vasodilatation following Et-1 or -3 or S6b, it is noteworthy that it was not seen following the 40 pmol dose of Et-2 used in the present experiments (Table 3).

The 4 pmol dose of all four peptides caused early mesenteric vasoconstriction only, and these effects were not enhanced by indomethacin (Table 2). Hence, there was no evidence for anything other than primary constrictor responses to this dose of the peptides in this vascular bed, but administration of the 40 pmol dose of the peptides revealed a more complex picture. In the absence of indomethacin, none of the peptides caused an initial mesenteric vasoconstriction (Table 3); indeed, there was a numerical increase in mesenteric conductance following administration of S6b that just failed to reach significance (Table 3). However, in the presence of indomethacin, the 40 pmol dose of all four peptides caused an initial reduction in mesenteric vascular conductance that was significantly different from the response in the absence of indomethacin (Table 3). As mentioned above, indomethacin alone caused mesenteric vasoconstriction but, of itself, this would be expected to enhance rather than reduce vasodilator responses (Myers & Honig, 1969). Thus, it is likely that, following the 40 pmol dose, all four peptides exerted mesenteric vasoconstrictor effects that were masked by release of vasodilator cyclo-oxygenase products. These results are consistent with recently published data obtained in systems other than conscious animals (De Nucci *et al.*, 1988; Warner *et al.*, 1989a,b; Thiemermann *et al.*, 1989; Lidbury *et al.*, 1989; Herman *et al.*, 1989).

Et-1, Et-2 and Et-3 and S6b at both doses caused obvious initial hindquarters vasodilatations. With the 4 pmol dose these effects were similar and not influenced by indomethacin. However, the hypotensive effect of the 40 pmol dose of S6b (which was numerically the greatest) was reduced in the presence of indomethacin, in association with a significant attenuation of the rise in hindquarters vascular conductance (Table 3). In the presence of indomethacin, the hindquarters

vasodilator effects of Et-1, Et-2 and Et-3 were unchanged and similar to the vasodilatation elicited by S6b. These results indicate that: (1) a component of the initial hypotensive and hindquarters vasodilator response to S6b was due to release of vasodilator cyclo-oxygenase products, and that this mechanism was not triggered by the same doses of the endothelins and (2) Et-1, Et-2, Et-3 and S6b can elicit marked hindquarters vasodilatation by mechanisms not sensitive to indomethacin.

Recently, Whittle *et al.* (1989) demonstrated that the hypotensive response to Et-1 in pentobarbitone-anaesthetized rats was reduced by 72% in the presence of N^G-monomethyl-L-arginine (L-NMMA), a compound that inhibits endothelial cell nitric oxide production (see Moncada *et al.*, 1989). However, we have been unable to antagonize the hypotensive and hindquarters vasodilator effects of Et-1 (40 pmol) with L-NMMA in conscious rats (Gardiner *et al.*, 1989), even with doses 5 fold higher than the highest used by Whittle *et al.* (1989). In fact, as expected (Myers & Honig, 1969), because of the hypertension and vasoconstriction caused by L-NMMA, the hypotension and hindquarters vasodilator responses to Et-1 are enhanced (Gardiner *et al.*, 1989). A similar phenomenon is observed when Et-1 is administered during infusion of arginine vasopressin, at a rate adjusted to give an increase in MBP and a decrease in hindquarters conductance the same as those seen following L-NMMA (Gardiner *et al.*, 1990). Thus, there is no evidence that endothelial cell nitric oxide production was contributing to the hypotension or hindquarters vasodilator responses to Et-1 in our present experimental protocols. However, the possible involvement of such a mechanism in the responses to Et-2, Et-3 or S6b has not been investigated. Furthermore, we can say nothing about the putative involvement of nitric oxide-mediated and/or indomethacin-sensitive processes in the responses to Et-1, Et-2, Et-3 or S6b at higher doses than those used here.

The finding that the non-significant fall in MBP elicited by Et-2 was accompanied by hindquarters vasodilatation similar to that following Et-1 or Et-3 (Tables 1 and 2) argues against the apparent difference between the effects of the peptides on MBP being simply a dose-dependent phenomenon. However, it is quite feasible that experiments conducted with higher doses of the peptides would reveal patterns of response different from those described here (for example, we have found that bolus doses of 400 pmol of Et-2 cause marked hypotension (Gardiner, Compton & Bennett, unpublished observations). In addition, with higher doses of the peptides, the patterns of interaction between vasoconstrictor and vasodilator mechanisms might vary from those observed in the current experiments.

In summary, the present work has shown that Et-1, Et-2, Et-3 and S6b have haemodynamic profiles of action that depend on the dose administered. The early renal vascular actions of the 4 and 40 pmol doses of Et-1, the initial hindquarters vasodilator effects of the 40 pmol dose of S6b, and the early actions of the 40 pmol dose of all four peptides on the mesenteric vasculature, were influenced by mechanisms inhibited by indomethacin. However, only in the case of the 40 pmol dose of S6b did indomethacin influence the initial hypotension. It is likely, therefore, that mechanisms other than the release of vasodilator cyclo-oxygenase products are responsible for the vasodilator effects of low doses of endothelins and S6b in conscious rats. Moreover, since the later pressor and regional vasoconstrictor effects of both doses of Et-1, Et-2, Et-3 and S6b were not enhanced by indomethacin, it appears that vasodilator prostanoids do not offset the later vasoconstrictor effects of low doses of these peptides.

References

DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal in the pulmonary

circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9797-9800.

- GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1988). Regional haemodynamic effects of depressor neuropeptides in conscious, unrestrained, Long Evans and Brattleboro rats. *Br. J. Pharmacol.*, **95**, 197–208.
- GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1989). The effect of N^G-monomethyl-L-arginine (L-NMMA) on the haemodynamic actions of endothelin-1 in conscious Long Evans rats. *Br. J. Pharmacol. (Proc. Suppl.)*, **98**, 626P.
- GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1989). Effects of N^G-monomethyl-L-arginine on the haemodynamic actions of endothelin-1. *Eur. J. Pharmacol.*, **171**, 237–240.
- GERBER, J.G. & NIES, A.S. (1979). The haemodynamic effects of prostaglandins in the rat: evidence for important species variation in renovascular responses. *Circ. Res.*, **44**, 406–410.
- HAYWOOD, J.R., SHAFFER, R., FASTENOW, C., FINK, G.D. & BRODY, M.J. (1981). Regional blood flow measurement with pulsed Doppler flowmeter in conscious rat. *Am. J. Physiol.*, **241**, H273–H278.
- HERMAN, F., MAGYAR, K., CHABRIER, P.-E., BRAQUET, P. & FILEP, J. (1989). Prostacyclin mediates antiaggregatory and hypotensive actions of endothelin in anaesthetized beagle dogs. *Br. J. Pharmacol.*, **98**, 38–40.
- INOUE, A., YANAGISAWA, M., KIMURA, S., KUSUYA, Y., MIYAUCHI, T., GOTO, K. & MASAKI, T. (1989). The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 2863–2867.
- KLOOG, Y., AMBAR, F., SOKOLOVSKY, M., KOCHVA, E., WOLLBERG, Z. & BDOLAH, A. (1988). Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain. *Science*, **242**, 268–278.
- LEE, C.Y. & CHIAPPINELLI, V.A. (1988). Similarity of endothelin to snake venom toxin. *Nature*, **335**, 303.
- LIDBURY, P.S., THIEMERMANN, C., THOMAS, G.R. & VANE, J.R. (1989). Endothelin-3: selectivity as an antiaggregatory peptide *in vivo*. *Eur. J. Pharmacol.*, **166**, 335–338.
- MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1989). Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. *Biochem. Pharmacol.*, **38**, 1709–1715.
- MYERS, H.A. & HONIG, C.R. (1969). Influence of initial resistance on magnitude of responses to vasomotor stimuli. *Am. J. Physiol.*, **216**, 1429–1436.
- QUILLEY, J., McGIFF, J.C. & NASJLETTI, A. (1989). Role of endoperoxides in arachidonic acid-induced vasoconstriction in the isolated perfused kidney of the rat. *Br. J. Pharmacol.*, **96**, 111–116.
- RAE, G.A., TRYBULEC, M., DE NUCCI, G. & VANE, J.R. (1989). Endothelin-1 releases eicosanoids from rabbit isolated perfused kidney and spleen. *J. Cardiovasc. Pharmacol.*, **13** (suppl. 5), S89–S92.
- RAKUGI, H., NAKAMURA, M., TABUCHI, Y., NAGANO, M., MIKAMI, H. & OGIHARA, H. (1989). Endothelin stimulates the release of prostacyclin from rat mesenteric arteries. *Biochem. Biophys. Res. Commun.*, **160**, 924–928.
- TAKASAKI, C., TAMIYA, N., BDOLAH, A., WOLLBERG, Z. & KOCHVA, E. (1988a). Sarafotoxins S6: several isotoxins from *Actraspis engadensis* (burrowing asp) venom that affect the heart. *Toxicol.*, **26**, 543–548.
- TAKASAKI, C., YANAGISAWA, M., KIMURA, S., GOTO, K. & MASAKI, T. (1988b). Similarity of endothelin to snake venom toxin. *Nature*, **335**, 303.
- THIEMERMANN, C., LIDBURY, P.S., THOMAS, G.R. & VANE, J.R. (1989). Endothelin-1 releases prostacyclin and inhibits *ex vivo* platelet aggregation in the anaesthetized rabbit. *J. Cardiovasc. Pharmacol.*, **13** (Suppl. 5), S138–S141.
- WALDER, C.E., THOMAS, G.R., THIEMERMANN, C. & VANE, J.R. (1989). The haemodynamic effects of endothelin-1 in the pithed rat. *J. Cardiovasc. Pharmacol.*, **13** (suppl. 5), S93–S97.
- WARNER, T.D., MITCHELL, J.A., DE NUCCI, G. & VANE, J.R. (1989a). Endothelin-1 and endothelin-3 release EDRF from isolated perfused arterial vessels of the rat and rabbit. *J. Cardiovasc. Pharmacol.*, **13** (suppl. 5), S85–S88.
- WARNER, T.D., DE NUCCI, G. & VANE, J.R. (1989b). Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. *Eur. J. Pharmacol.*, **159**, 325–326.
- WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & REES, D.D. (1989). Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br. J. Pharmacol.*, **98**, 646–652.
- WINQUIST, R.J., BUNTING, P.B., GARSKY, V.M., LUMMA, P.K. & SCHOFIELD, T.L. (1989). Prominent depressor response to endothelin in spontaneously hypertensive rats. *Eur. J. Pharmacol.*, **163**, 199–203.

(Received August 21, 1989
 Revised December 7, 1989
 Accepted January 12, 1990)