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Inhibition by phosphoramidon of the regional haemodynamic effects of proendothelin-2 and -3 in conscious rats

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1 Regional haemodynamic studies were carried out in conscious, Long Evans rats, chronically-instrumented with pulsed Doppler flow probes and intravascular catheters.

2 In the first experiment, proendothelin-2 and -3 (0.1 and 1.0 nmol kg^{-1}, i.v. boluses) were found to cause dose-dependent pressor, bradycardic, and renal and, particularly, mesenteric vasoconstrictor effects. The hindquarters showed an initial vasodilatation (which was not dose-dependent) followed by a vasoconstriction (which was dose-related). The pressor and renal and mesenteric vasoconstrictor effects of proendothelin-3 were greater than those of proendothelin-2.

3 In the second experiment, it was demonstrated that phosphoramidon (10 μmol kg^{-1}, i.v. bolus) abolished the pressor, bradycardic, and hindquarters vasoconstrictor effects of proendothelin-2 (1.0 nmol kg^{-1}), and inhibited significantly the renal and mesenteric vasoconstrictor actions of this peptide. Phosphoramidon had similar effects on the responses to proendothelin-3 (1.0 nmol kg^{-1}), although a slight pressor effect of this peptide remained in the presence of phosphoramidon.

4 In the third experiment, it was found that phosphoramidon had no significant effect on the pressor or vasoconstrictor responses to endothelin-2 or -3 (0.1 nmol kg^{-1}).

5 Collectively, the results indicate that the haemodynamic effects of proendothelin-2 and -3 *in vivo* in conscious rats are probably due to their conversion to endothelin-2 and -3, respectively, by an enzyme(s) that is inhibited by phosphoramidon. There appears to be no obvious difference between proendothelin-2, proendothelin-3 and proendothelin-1 in this respect.

Keywords: Proendothelin-2; proendothelin-3; phosphoramidon; haemodynamics

Introduction

No doubt as a result of the rate at which the field has developed, there are many unresolved issues relating to the biology of endothelin. This is particularly true in regard to the enzymatic processes whereby endothelin-1 is produced from its precursor, proendothelin-1 (Yanagisawa *et al.*, 1988). Although several different 'endothelin-1-converting enzyme' (ECE-1) systems have been identified, it seems most likely that a membrane-bound, phosphoramidon-sensitive metalloprotease is the major ECE-1 of endothelial cells (Matsumura *et al.*, 1990b; Okada *et al.*, 1990). However, the conversion of exogenous proendothelin-1 to endothelin-1 by the membrane fraction of porcine aortic vascular smooth muscle cells is not phosphoramidon-sensitive under normal conditions (Matsumura *et al.*, 1991b), in contrast to the membrane-bound ECE-1 of the vascular smooth muscle of bovine carotid artery (Hioki *et al.*, 1991). Matsumura *et al.* (1991b) suggested their observations were due to a 'masking' of the action of phsophoramidon-sensitive ECE-1 by the presence of a phosphoramidon-insensitive ECE-1, and by degradation of proendothelin-1 and/or endothelin-1. However, it does appear that there is a difference between the endothelial and vascular smooth muscle ECE-1 systems, because Ikegawa *et al.* (1991) observed inhibition by phosphoramidon of spontaneous production of endothelin-1 from intact, porcine, aortic endothelial cells, but not from intact vascular smooth muscle cells. Despite this difference, the production of endothelin-1 from exogenous proendothelin-1 by both cell types was sensitive to phosphoramidon.

The picture is even more complex when the activity of ECE-1 against other substrates is considered. Thus, some biochemical studies indicate that the membrane-bound ECE-1 of bovine endothelial cells converts proendothelin-3 to endothelin-3, but at a rate only 1/9 that of the conversion of proendothelin-1 to endothelin-1 (Okada *et al.*, 1990). In contrast, the soluble ECE-1 of the same cells does not appear to convert proendothelin-3 to endothelin-3 (Takada *et al.*, 1991), although the same group (Okada *et al.*, 1991) subsequently reported that both membrane-bound and soluble forms of ECE-1 from bovine endothelial cells were inactive against proendothelin-2 and proendothelin-3. ECE-1 from renal epithelial cell lines has also been found to be inactive against proendothelin-2 and -3 (Takada *et al.*, 1992), although it was acknowledged that enzyme systems other than those studied might be important *in vivo*.

In vivo, the pressor effects of exogenous proendothelin-1 can be markedly inhibited by phosphoramidon (Matsumura *et al.*, 1990a; Fukuroda *et al.*, 1990; McMahon *et al.*, 1991; Gardiner *et al.*, 1991; Le Monnier de Gouville & Cavero, 1991; Pollock & Opgenorth, 1991), and this effect is associated with a reduction in the regional vasoconstrictor effects of proendothelin-1 (Gardiner *et al.*, 1991; Pollock & Opgenorth, 1991). As pointed out elsewhere (Gardiner *et al.*, 1991), *in vivo* administration of phosphoramidon might have differential influences on the production and degradation of endothelin-1 (Vijayaraghavan *et al.*, 1990; Sokolovsky *et al.*, 1990), and hence the overall changes could be multifactorial in origin. However, with bolus administration of exogenous proendothelin-1, inhibition of the degradation of the resulting endothelin-1 is not likely to contribute greatly to the effects of phosphoramidon, since the neutral endopeptidase inhibitor, SQ 28,603, which is more potent than phosphoramidon at inhibiting degradation of endothelin-1 (Dickinson *et al.*, 1991), has no significant effects on the haemodynamic actions of exogenous endothelin-1 (Gardiner *et al.*, 1992a). Interestingly, SQ 28,603 does inhibit the pressor effects of proendothelin-1 (Gardiner *et al.*, 1992a)

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indicating this compound may suppress ECE-1 activity, unlike kelatorphan, captopril or enalaprilat (McMahon *et al.*, 1991; Pollock & Opgenorth, 1991). The results with SQ 28,603 are consistent with ECE-1 being a form of neutral endopeptidase, and this is in line with the observations of McMahon *et al.* (1991) showing that thiorphan causes dose-dependent inhibition of the pressor effects of proendothelin-1. However, these findings go against those of Pollock & Opgenorth (1991) who reported that thiorphan was without effect on responses to proendothelin-1. But examination of the results of Pollock & Opgenorth (1991) shows that baseline values in the presence of thiorphan were elevated (see their Table 1), and this may have masked an inhibitory effect of thiorphan on responses to proendothelin-1. Nevertheless, the *in vivo* studies are generally consistent with the proposition that most, if not all, the cardiovascular effects of exogenous proendothelin-1 are dependent upon endothelin-1 produced by the activity of a phosphoramidon-sensitive ECE-1, that may be differentially expressed in different vascular beds (Gardiner *et al.*, 1991; Hisaki *et al.*, 1991).

In line with the apparent selectivity of ECE-1 for proendothelin-1 as a substrate, Télémaque & D'Orléans-Juste (1991) and D'Orléans-Juste *et al.* (1991) have reported that, in the vas deferens of the rat and in the intact guinea-pig, proendothelin-3 is biologically inactive. However, these findings are not easy to reconcile with our observations showing that, in conscious rats, proendothelin-3 exerts clear pressor and vasoconstrictor effects (Gardiner *et al.*, 1992c), similar in pattern to those seen with proendothelin-1 (Gardiner *et al.*, 1991; 1992c).

Since we had previously demonstrated inhibition of the haemodynamic effects of proendothelin-1 by phosphoramidon in conscious rats (Gardiner *et al.*, 1991), and considering the unresolved questions outlined above, the present work was carried out to determine whether or not the *in vivo* effects of proendothelin-3 were susceptible to inhibition by phosphoramidon. We also assessed the regional haemodynamic effects of proendothelin-2 and the effects of phosphoramidon on these. Finally, we investigated the effects of phosphoramidon on haemodynamic responses to endothelin-2 and endothelin-3, to ensure that any putative effects of phosphoramidon on responses to proendothelin-2 or -3 were not due to inhibition of the action of the peptides produced from them.

Methods

Male, Long Evans rats (400–450 g) were used in all experiments. Under sodium methohexitone anaesthesia (60 mg kg^{-1}, i.p., supplemented as required), pulsed Doppler flow probes (Haywood *et al.*, 1981) were sutured around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor flow to the hindquarters). The probe wires were led subcutaneously to emerge at the back of the neck where they were anchored by a suture. Following closure of incisions, animals were given ampicillin (Penbritin, Beecham, 7 mg kg^{-1}, i.m.) and returned to individual home cages with free access to food and water.

At least 7 days later, animals were anaesthetized (sodium methohexitone, 40 mg kg^{-1}, i.p., supplemented as necessary), and the signals from the pulsed Doppler probes were checked. Any animal without good quality signals (signal: noise $>$ 20:1) from all 3 probes was excluded from the study; those with 3 acceptable flow signals had an intra-arterial (distal abdominal aorta via the ventral caudal artery) and intravenous (right jugular) catheters implanted. The catheters ran subcutaneously to emerge at the same site as the probe wires. The latter were soldered into a micro-connector (Microtech Inc., Boothwyn, USA) that was held in a clamp fitted to a harness worn by the rat. The catheters ran through a flexible spring connected to the harness; the spring was supported by a counterbalanced lever system to allow the rat free movement. Animals were returned to their home cages with free access to water and food, and experiments did not commence until at least 24 h later. The following protocols were run:

Experiment 1 Cardiovascular responses to proendothelin-2 and proendothelin-3

Animals ($n = 8$) were given bolus i.v. injections of proendothelin-2 and proendothelin-3 (0.1 and 1.0 nmol kg^{-1}). The experiment ran over 2 days with animals receiving 2 injections a day. They received the peptides in random order, but the higher doses were given after the lower doses, because of the prolonged effects of the former.

Experiment 2 Effects of phosphoramidon on cardiovascular responses to proendothelin-2 and proendothelin-3

Animals ($n = 7$) were challenged with i.v. bolus injections of proendothelin-2 or proendothelin-3 (1.0 nmol kg^{-1}) in the absence, or 5 min after, i.v. bolus injection of phosphoramidon (10 μmol kg^{-1}; Gardiner *et al.*, 1991). Animals were given proendothelin-2 and proendothelin-3 in random order on separate days. Responses to either peptide in the absence and presence of phosphoramidon were measured on the same day (at least 5 h apart). In pilot experiments we determined that repeated doses of proendothelin-2 or -3, separated by 5 h, evoked similar responses.

Experiment 3 Effects of phosphoramidon on responses to endothelin-2 and endothelin-3

Animals ($n = 6$) were challenged with i.v. bolus injections of endothelin-2 or endothelin-3 (0.1 nmol kg^{-1}) before, or 5 min after, phosphoramidon. Animals were randomized to receive endothelin-2 or -3 on separate days, with the exposures to each peptide in the absence and presence of phosphoramidon being separated by at least 5 h.

Continuous recordings were made (on a Gould ES 1000 system) of phasic and mean systemic arterial blood pressure, instantaneous heart rate, and phasic and mean Doppler shift signals (Crystal Biotech VF1 system). The phasic Doppler shift signals were recorded only to ensure there were no technical problems (we have encountered circumstances when the mean Doppler signal looked acceptable although the phasic Doppler signal showed loss of pulsatility). Measurements of mean arterial blood pressure, heart rate and mean renal, mesenteric and hindquarters Doppler shift signals were made at selected time points. This was done by averaging (by eye) over 20 s epochs. Percentage changes in mean Doppler shift signals were taken as indices of changes in flow (Haywood *et al.*, 1981; Gardiner *et al.*, 1989a; 1990a,b,c; 1991; 1992a,b,c). Vascular conductances were calculated by dividing mean Doppler shift by mean blood pressure and expressing changes as percentages (Gardiner *et al.*, 1991).

Data analysis

Within-group analysis was carried out by use of Friedman's test (Theodorsson-Norheim, 1987). Comparison of responses in the same groups of animals in different experimental protocols was made on areas under or over curves (AUC and AOC, respectively, $t = 0–60$ min) by Wilcoxon's rank sums test. A P value < 0.05 was taken as significant.

Peptides

Endothelin-2, endothelin-3, proendothelin-2 (human, 1–37) and proendothelin-3 (human, 1–41 amide) were obtained from the Peptide Institute (Osaka, Japan) through their UK agents (Scientific Research Associates). The peptides were dissolved initially in aqueous acetic acid (0.1%) and stored in

100 µl aliquots at − 80°C. Before use the stock solution was diluted in saline containing 1% bovine serum albumin (Sigma, U.K.). Phosphoramidon (sodium salt, Sigma, U.K.) was dissolved in isotonic saline. All bolus injections were given in 100 µl and flushed in with 150 µl isotonic saline. Injection of vehicle solutions in these volumes had no consistent haemodynamic effects.

Results

Cardiovascular responses to proendothelin-2 and proendothelin-3

The low dose (0.1 nmol kg^{-1}) of proendothelin-2 had no significant effects on mean arterial blood pressure (MAP), but there was a transient tachycardia (Figure 1, Table 1). Renal blood flow did not change significantly, although there was a slight decrease in mesenteric blood flow and hindquarters flow showed an initial increase (Figure 1, Table 1). There was no significant decrease in renal vascular conductance, although there was a slight, but significant, mesenteric vasoconstriction, and a hindquarters vasodilatation followed by a delayed vasoconstriction (Figure 1, Table 1).

Proendothelin-3 (0.1 nmol kg^{-1}) caused a slight, but significant, rise in MAP, but this was not significantly different from the effect of proendothelin-2, and there was also a similar initial tachycardia (Figure 1, Table 1). Proendothelin-3 had no significant effect on renal blood flow, and the initial rise in hindquarters flow it caused was not different from that seen with proendothelin-2 (Figure 1, Table 1). However, the reduction in mesenteric blood flow caused by proendothelin-3 was significantly greater than that seen with proendothelin-2, as was the mesenteric vasoconstriction (Figure 1,Table 1), whereas the renal vasoconstriction and hindquarters vasodilatation were not different from the effects of proendothelin-2 (Figure 1, Table 1).

The high dose (1 nmol kg^{-1}) of proendothelin-2 caused a clear, and prolonged, rise in MAP accompanied by a sustained bradycardia (Figure 1, Table 1). There was a slight reduction in renal blood flow, a more marked reduction in mesenteric blood flow, and an initial rise followed by a non-significant reduction in hindquarters flow (Figure 1, Table 1). Renal and mesenteric vascular conductances were

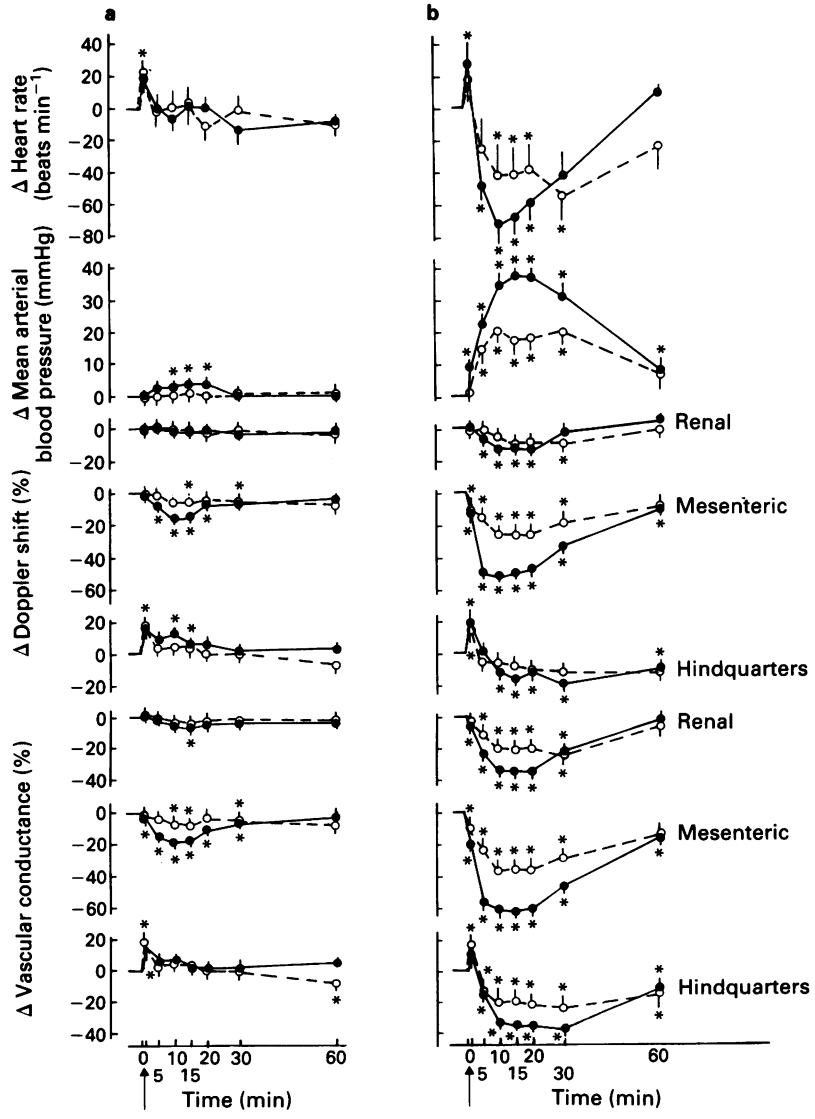

Figure 1 Cardiovascular changes in the same conscious, Long Evans rats in response to bolus i.v injections of proendothelin-2 (○) and proendothelin-3 (●) at doses of 0.1 nmol kg^{-1} (a) or 1.0 nmol kg^{-1} (b). Values are mean and vertical bars show s.e.mean ($n = 8$).
*$P < 0.05$ versus original baseline. Statistics for differences between responses to proendothelin-2 and proendothelin-3 are given in the text.

Table 1 Integrated (area under or over curve (AUC, AOC) for 0–60 min) cardiovascular response to bolus i.v. injections of proendothelin (ProEt)-2 or -3 in the same conscious, Long Evans rats ($n = 8$)

		ProEt-2 (0.1 nmol kg^{-1})	*ProEt-3* (0.1 nmol kg^{-1})	*ProEt-2* (1 nmol kg^{-1})	*ProEt-3* (1 nmol kg^{-1})
MAP (mmHg min)	AUC	NS	159 ± 62	970 ± 156	1552 ± 166†
HR (beats)	AUC	530 ± 264	336 ± 113	NS	208 ± 91
	AOC	NS	NS	2569 ± 391	2789 ± 506
Doppler shift (%min)					
Renal	AOC	NS	NS	465 ± 122	448 ± 107
Mesenteric	AOC	432 ± 65	528 ± 79†	1113 ± 106	1964 ± 167†
Hindquarters	AUC	263 ± 167	441 ± 100	114 ± 47	69 ± 19
	AOC	NS	NS	NS	853 ± 74
Vascular conductance (%min)					
Renal	AOC	NS	259 ± 79	1067 ± 95	1291 ± 148†
Mesenteric	AOC	448 ± 83	613 ± 69†	1657 ± 118	2631 ± 176†
Hindquarters	AUC	369 ± 198	489 ± 132	61 ± 29	28 ± 15
	AOC	419 ± 140	NS	1192 ± 225	1736 ± 100

Values are mean ± s.e.mean.
At the low dose, the predominant heart rate response was a tachycardia whereas at the high dose there was a marked, subsequent bradycardia. In the hindquarters vascular bed an initial vasodilatation was followed by a vasoconstriction; hence values for AUC and AOC are included.
†$P < 0.05$: significant difference from the effects of the corresponding dose of ProEt-2 (Wilcoxon's ranks sums test).
NS indicates no significant change in that variable.

decreased, and there was an initial dilatation followed by a constriction in the hindquarters vascular bed (Figure 1, Table 1).

The pattern of cardiovascular response to proendothelin-3 (1 nmol kg^{-1}) was similar to that above, although the rise in MAP and reduction in mesenteric blood flow were greater with proendothelin-3 than with proendothelin-2, as were the renal and mesenteric vasoconstrictions (Figure 1, Table 1). With proendothelin-3, the reductions in heart rate and renal flow, and the initial rise and subsequent fall in hindquarters flow and vascular conductance were not different from those seen with proendothelin-2 (Figure 1, Table 1).

Effects of phosphoramidon on cardiovascular response to proendothelin-2 and proendothelin-3

The responses to proendothelin-2 and to proendothelin-3 (1 nmol kg^{-1}) in the animals in this experiment were generally similar to those seen in the first experiment (Figure 2, Table 2). As before, the pressor effect of proendothelin-3 was greater than that of proendothelin-2. However, in this experiment the bradycardic affect of proendothelin-3 was significantly greater than that of proendothelin-2 (Figure 2, Table 2). There was no significant difference between the effects of proendothelin-2 and proendothelin-3 in respect of the reductions in renal blood flow or hindquarters blood flow (Figure 2, Table 2). However, the reduction in mesenteric blood flow evoked by proendothelin-3 was greater than that elicited by proendothelin-2. Proendothelin-3 caused more marked renal vasoconstriction and mesenteric vasoconstriction than did proendothelin-2, but the hindquarters vasoconstrictor responses to the peptides were not significantly different (Figure 2, Table 2).

Phosphoramidon had no significant haemodynamic effects itself, but it abolished the pressor, bradycardic and hindquarters vasoconstrictor effects of proendothelin-2, and reduced significantly its renal and mesenteric vasoconstrictor actions (Figure 2, Table 2).

In the presence of phosphoramidon, the pressor influence of proendothelin-3 was markedly reduced, and this effect was accompanied by an abolition of the bradycardic and hindquarters vasoconstrictor response and significant reductions of the renal and mesenteric vasoconstrictor responses (Figure 2, Table 2).

Effects of phosphoramidon on cardiovascular responses to endothelin-2 and endothelin-3

The pressor and vasoconstrictor response to endothelin-2 and -3 were as described elsewhere (Gardiner *et al.*, 1990a,b,c); phosphoramidon had no significant effects on these responses (data not shown).

Discussion

The present study has shown that proendothelin-2 and -3 have dose-dependent haemodynamic effects in conscious rats. These effects are inhibited by phosphoramidon at a dose that has no effects on the pressor or vasoconstrictor response to exogenous endothelin-2 or -3. Hence, these results are consistent with the *in vivo* haemodynamic actions of exogenous proendothelin-2 and -3 being due to their conversion into endothelin-2 and -3, respectively, by phosphoramidon-sensitive enzyme(s). In this regard, proendothelin-2 and -3 show no marked differences from proendothelin-1 (Matsumura *et al.*, 1990a,b; Fukuroda *et al.*, 1990; McMahon *et al.*, 1991; Gardiner *et al.*, 1991; Le Monnier de Gouville & Cavero, 1991; Pollock & Opgenorth, 1991).

These results are particularly striking against the background (see Introduction) of biochemical and cardiovascular studies showing that phosphoramidon-sensitive, ECE-1 does not act on proendothelin-2 or -3. Of course, it is feasible that the failure to demonstrate phosphoramidon-sensitive conversion of proendothelin-2 and -3 to endothelin-2 and -3, respectively, in the *in vitro* systems examined (Okada *et al.*, 1990; 1991; Takada *et al.*, 1992) is a reflection of the inability of such systems to represent the *in vivo* condition. However, this argument cannot pertain to the observations of D'Orléans-Juste *et al.* (1991) who found proendothelin-3, in doses as high as 20 nmol kg^{-1}, had no cardiovascular effects *in vivo*. It is possible the fundamental difference between the results of D'Orléans-Juste *et al.* (1991) and ours is due to a species difference (since they studied anaesthetized guinea-pigs), but this seems unlikely because Télémaque & D'Orléans-Juste (1991) also found proendothelin-3 was inactive in rat tissues, albeit *in vitro*. The inactivity of the proendothelin-3 used by Télémaque & D'Orléans-Juste *et al.* (1991) cannot be due to their material not being the authentic peptide, because they

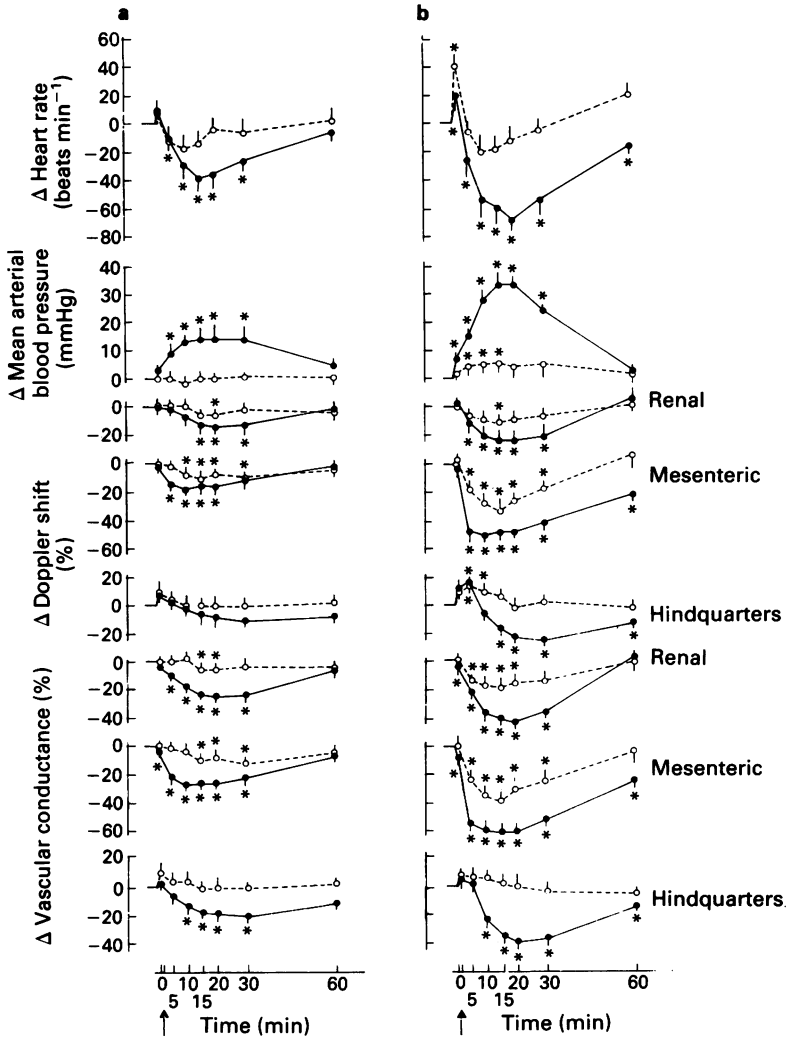

Figure 2 Cardiovascular changes in the same conscious, Long Evans rats in response to bolus injections of proendothelin-2 (1.0 nmol kg^{-1}, a) or proendothelin-3 (1.0 nmol kg^{-1}, b) before (●) and 5 min after i.v. bolus injection of phosphoramidon (10 μmol kg^{-1}, ○). Values are mean and vertical bars show s.e.mean ($n = 7$).
*$P < 0.05$ versus original baseline. Statistics for differences between responses before and after administration of phosphoramidon are given in the text.

Table 2 Integrated (area under or over curve (AUC, AOC) for 0–60 min) cardiovascular responses to bolus i.v. injections of proendothelin-2 or -3 (1 nmol kg^{-1}) before and 5 min after phosphoramidon (10 μmol kg^{-1}) in the same conscious, Long Evans rats ($n = 7$)

		Proendothelin-2	*Proendothelin-2 after phosphoramidon*	*Proendothelin-3*	*Proendothelin-3 after phosphoramidon*
MAP (mmHg min)	AUC	665 ± 132	NS*	1201 ± 66†	517 ± 129*
HR (beats)	AUC	NS	NS	108 ± 48	727 ± 181*
	AOC	1539 ± 418	NS*	2612 ± 371†	NS*
Doppler shift (%min)					
Renal	AOC	661 ± 197	316 ± 67*	1087 ± 267	523 ± 174*
Mesenteric	AOC	791 ± 179	520 ± 84*	2258 ± 200†	1046 ± 212*
Hindquarters	AUC	NS	NS	117 ± 27	399 ± 121
	AOC	NS	NS	991 ± 181	NS*
Vascular conductance (%min)					
Renal	AOC	1093 ± 210	300 ± 74*	1804 ± 170†	831 ± 260*
Mesenteric	AOC	1141 ± 219	537 ± 106*	2758 ± 174†	1325 ± 259*
Hindquarters	AUC	NS	NS	NS	NS
	AOC	981 ± 249	NS*	1666 ± 158	NS*

Values are mean ± s.e.mean.
†$P < 0.05$: significant difference from the effects of proendothelin-2.
*$P < 0.05$: significant difference from the effects seen in the absence of phosphoramidon (Wilcoxon's ranks sums test).
NS indicates no significant change in that variable.

obtained it from the same source as we did (Peptide Institute). While we have no explanation for this important disparity, we shall discuss our results on the assumption they are correct, not the least because we have found that two separate batches of proendothelin-3 had clear haemodynamic effects in several different groups of experimental animals (Gardiner *et al.*, 1992c and present study).

In the conscious rat, the cardiovascular effects of proendothelin-2 were, in several respects (pressor and renal and mesenteric vasoconstrictor actions) less than those of proendothelin-3. While this could have been due to more effective conversion of the latter to the active peptide, it is possible this was on account of the forms of peptide used (proendothelin-3 was amidated, but proendothelin-2 was not). However, it is notable that the pattern of response to proendothelin-2 and -3, with a particularly marked mesenteric vasoconstriction in both cases, was similar to that seen with proendothelin-1 (Gardiner *et al.*, 1991; 1992a), and is consistent with substantial ECE activity in this vascular bed (Hisaki *et al.*, 1991). In general, the pattern of response to proendothelin-2 and -3 was similar to that seen with endothelin-2 and -3 (Gardiner *et al.*, 1990a,b,c), consistent with these peptides being responsible for the effects of proendothelin-2 and -3, respectively. However, as noted elsewhere for proendothelin-1 (Gardiner *et al.*, 1991), proendothelin-2 and -3 exerted more marked hindquarters vasoconstrictor effects than endothelin-2 and -3. This is consistent with the endothelins causing more marked stimulation of those factors mediating the vasodilatation that opposes their vasoconstrictor influences. While it appears that endothelium-derived nitric oxide (NO) is not a major contributor to the hindquarters vasodilator effects of endothelin-1 (Gardiner *et al.*, 1989b), putative interactions between NO and endothelins should always be borne in mind. This is particularly true in the light of the recent finding that manipulating the availability of sulphydryl groups has marked effects on phosphoramidon-sensitive ECE-1 activity (Matsumura *et al.*, 1991a), since there is good evidence that NO interacts with sulphydryl groups (Moncada *et al.*, 1991); thus, NO might indirectly influence ECE activity.

The regionally differentiated effects of proendothelin-2 and -3 are consistent with the local conversion of these precursors into the active peptides, as suggested for proendothelin-1 (Gardiner *et al.*, 1991), although it does not follow that the same enzyme system(s) is responsible for the conversion of all three proendothelins. Recently, Modin *et al.* (1991) reported that phosphoramidon inhibited the pressor effects of proendothelin-1, but not the elevation in plasma endothelin-1. These results are entirely consistent with local, rather than systemic, conversion of proendothelins being responsible for their biological effects (Gardiner *et al.*, 1991; Le Monnier de Gouville & Cavero, 1991; Watanabe *et al.*, 1991). Indeed, the lack of change in plasma endothelin-1 levels under conditions in which its cardiovascular influences clearly are diminished illustrates the inability of the plasma level of endothelin-1 (and, presumably, -2 and -3) to provide information of functional significance.

The fact that phosphoramidon abolished the pressor effects of proendothelin-2, but not those of proendothelin-3, could have been due to the difference in the magnitudes of the response to these peptides in the absence of phosphoramidon. However, the latter had substantial inhibitory effects on the haemodynamic actions of both proendothelin-2 and -3, and there was no evidence that phosphoramidon was a less effective inhibitor of the effects of these peptides than of the actions of proendothelin-1 (Gardiner *et al.*, 1991). As in that instance, there was particularly marked inhibition of the hindquarters vasoconstrictor actions of proendothelin-2 and -3 by phosphoramidon. Thus, it seems likely that this effect was a major determinant of the ability of phosphoramidon to inhibit the pressor actions of the proendothelins. While a greater contribution of the hindquarters, rather than renal and/or mesenteric, vascular bed to haemodynamic status may seem surprising, it is consistent with the finding that the hindquarters vascular bed makes a particular contribution to the maintenance of hypertension following chronic inhibition of NO synthesis (Gardiner *et al.*, 1992b), for example.

In conclusion, proendothelin-2 and -3 have marked haemodynamic effects *in vivo* in conscious rats, and these effects appear to be largely-dependent on a phosphoramidon-sensitive ECE.

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