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Coeliac haemodynamic effects of endothelin-1, endothelin-3, proendothelin-1 [1–38] and proendothelin-3 [1–41] in conscious rats

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1 Conscious, chronically-instrumented, Long Evans rats were given bolus doses of endothelin-1, endothelin-3 (both at 0.01 and 0.1 nmol kg^{-1}), proendothelin-1 [1–38] and proendothelin-3 [1–41] (both 0.1 and 1 nmol kg^{-1}) in order to compare their effects on coeliac haemodynamics, because it has been reported that, in conscious dogs, endothelin-1 has paradoxical, prolonged hyperaemic vasodilator effects in this vascular bed. Measurements were made also of mesenteric and hindquarters haemodynamics for comparison. In a separate experiment, endothelin-1 (0.1 nmol kg^{-1}) was given before and 20 min after the onset of an infusion of mecamylamine (50 μmol $kg\ h^{-1}$) to ensure that the responses measured were not confounded by rapid reflex changes in autonomic activity.

2 None of the peptides caused any increases in coeliac flow or any sustained rises in coeliac vascular conductance, although such changes were clear-cut in the hindquarters vascular bed following the higher dose of endothelin-1 and endothelin-3. In animals treated with mecamylamine the regional haemodynamic effects of the higher dose endothelin-1 were not different from those in animals with intact baroreflexes.

3 Although the lower dose of both endothelin-1 and endothelin-3 caused less marked coeliac than mesenteric vasoconstriction, this difference was not apparent with the higher dose of the peptides, or with proendothelin-1 [1–38]. However, proendothelin-3 [1–41] had less marked coeliac and hindquarters vasoconstrictor effects than proendothelin-1 [1–38], in spite of both peptides causing similar changes in mesenteric haemodynamics. These differences could have been due to regional differences in the conversion of proendothelin-1 [1–38] and proendothelin-3 [1–41] to endothelin-1 and endothelin-3, respectively. Our findings contradict the proposition that exogenous proendothelin-3[1–41] is not converted into endothelin-3 *in vivo*.

4 Since, in contrast to endothelin-1 and endothelin-3, proendothelin-1 [1–38] and proendothelin-3 [1–41] caused only small hindquarters vasodilatations, but large vasoconstrictions, it is feasible that the vasodilator responses to exogenous endothelin-1 and endothelin-3 are pharmacological phenomena, and that, *in vivo*, the production of endothelins from proendothelins exerts widespread vasoconstrictor effects.

Keywords: Haemodynamics; endothelin-1; endothelin-3; proendothelin-1 [1–38]; proendothelin-3 [1–41]; conscious rats

Introduction

Although endothelin-1 was originally described as the most potent vasoconstrictor peptide yet discovered (Yanagisawa *et al.*, 1988), it was soon found to cause marked, initial vasodilatations *in vivo*, particularly in the hindquarters and carotid vascular beds (Wright & Fozard, 1988; Gardiner *et al.*, 1989). These effects were observed in anaesthetized, areflexic rats (Wright & Fozard, 1988) and in conscious, intact animals (Gardiner *et al.*, 1989). It has been claimed that endothelin-3 has greater vasodilator effects than endothelin-1 (Warner *et al.*, 1989), although we have not been able to confirm that in conscious rats (Gardiner *et al.*, 1990a,b). Furthermore, although both endothelin-1 and endothelin-3 have been found to elicit mesenteric vasodilator effects *in vitro* (De Nucci *et al.*, 1988; Warner *et al.*, 1989), such responses have not been seen *in vivo* (Wright & Fozard, 1988; Gardiner *et al.*, 1989; 1990a,b). However, recently Bigaud & Vatner (1990) described a substantial, and persistent, coeliac vasodilator effect of endothelin-1, in association with mesenteric and iliac vasoconstrictions in conscious dogs. Therefore, in the present work one objective was to investigate the coeliac haemodynamic effects of endothelin-1 and endothelin-3 in conscious rats. In the same animals we made simultaneous measurements of mesenteric and hindquarters blood flows to ensure that the responses we had described earlier were reproducible, and also to act as comparisons for the changes in coeliac haemodynamics. Since there is a possibility that rapid autonomic reflex changes could influence the regional haemodynamic responses to vasoactive agents *in vivo*, we carried out an additional experiment in a separate group of rats in which we assessed regional haemodynamic responses to endothelin-1 (0.1 nmol kg^{-1}) before and after administration of mecamylamine (Wright & Fozard, 1990).

There is increasing evidence that the *in vivo* haemodynamic effects of proendothelin-1 [1–38] are due to its conversion to endothelin-1 (Kimura *et al.*, 1989; Kashiwabara *et al.*, 1989; D'Orléans-Juste *et al.*, 1990; Matsumura *et al.*, 1990; Fukuroda *et al.*, 1990; Douglas & Hiley, 1991; Gardiner *et al.*, 1991) and that this conversion occurs locally rather than in the systemic circulation (Gardiner *et al.*, 1991). If this is the case, then administration of proendothelin-1 [1–38] might produce haemodynamic effects that more closely resemble the actions of endogenous endothelin-1 than does administration of exogenous endothelin-1. It is not known if a similar argument can be advanced for proendothelin-3 [1–41], since *in vitro* data indicate this peptide is not cleaved by the same

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enzyme that cleaves proendothelin-1 [1–38] (Okada *et al.*, 1990), and there are no data on the *in vivo* regional haemodynamic effects of proendothelin-3 [1–41] in conscious rats. Therefore, another objective was to assess the effects of proendothelin-1 [1–38] and proendothelin-3 [1–41] on coeliac, mesenteric and hindquarters haemodynamics in the same animals that received endothelin-1 and endothelin-3, to allow a comparison of the responses to all these peptides.

Methods

Male, Long Evans rats (350–450 g) were anaesthetized (sodium methohexitone, 60 mg kg^{-1}, i.p., supplemented as required) and had miniaturized pulsed Doppler probes (Haywood *et al.*, 1981) sutured around the coeliac and superior mesenteric arteries (Gardiner *et al.*, 1990c) and the distal abdominal aorta, below the level of the ileocaecal artery, to monitor hindquarters blood flow (Gardiner *et al.*, 1989; 1990a,b). The probe wires ran subcutaneously and emerged at the back of the neck where they were anchored by a suture. Animals were given ampicillin (Penbritin, Beecham: 7 mg kg^{-1}, i.m.) and were then returned to their individual home cages with free access to food (Biosure, GLP grade diet 41B (M)) and water. At least 7 days later, animals were briefly re-anaesthetized (sodium methohexitone, 40 mg kg^{-1}, i.p.) and had intravenous (right jugular vein) and intraarterial (distal abdominal aorta via ventral caudal artery) catheters implanted. Following a recovery period of at least 24 h, experiments were begun and they were completed over the following 3 days.

In the first experiment, all animals ($n = 9$) received 2 doses of endothelin-1 and endothelin-3 (0.01 and 0.1 nmol kg^{-1}), and 2 doses of proendothelin-1 [1–38] and proendothelin-3 [1–41 amide] (0.1 and 1.0 nmol kg^{-1}). The order of administration of the peptides was randomised, but the lower doses were always given before the higher doses because the effects of the latter were so prolonged. We did not give endothelin-1 or endothelin-3 at a dose of 1.0 nmol kg^{-1} because in pilot experiments it caused cardiovascular deterioration. No animal received more than 3 doses of peptides on any day, and at least 2 h elapsed between doses.

In the second experiment, animals ($n = 7$) were challenged with endothelin-1 (0.1 nmol kg^{-1}) in the absence and in the presence of mecamylamine, to block ganglionic transmission. In pilot experiments we gave mecamylamine at the dose used by Wright & Fozard (1990) (0.25 mg kg^{-1} over 15 min), but found this did not abolish reflex heart rate changes in our conscious rats. Therefore, in the full experiment, we gave a 10 fold higher dose (i.e. 50 μmol kg^{-1} h^{-1}) and started the infusion of mecamylamine 20 min before challenging with endothelin-1. The experiments in the absence and presence of mecamylamine were carried out on separate days.

In the first experiment, for 30 min before any peptide was injected, continuous measurements were made of mean systemic arterial blood pressure (MAP), instantaneous heart rate, and mean coeliac, mesenteric and hindquarters Doppler shift signals. Percentage changes in the latter were taken as indices of changes in flow, and % changes in regional vascular conductance were calculated from mean Doppler shift signals and mean systemic arterial blood pressure (Gardiner *et al.*, 1990a,b).

In the second experiment there were significant differences in resting cardiovascular variables in the absence and presence of mecamylamine (see Results). In order not to obscure any possible differences between responses to endothelin-1 in the two conditions, we compared changes in the form of the absolute values rather than percentages.

The results given in the text refer to group means (± s.e.mean) derived from average values obtained over periods of 20 s immediately before any intervention and the 20 s periods around the time points at which measurements were made.

Peptides

Endothelin-1, endothelin-3, proendothelin-1 [1–38] and proendothelin-1 [1–41] amide were obtained from the Peptide Institute (Osaka) through their UK agents (Scientific Marketing Associates). In the text, for brevity, proendothelin-1 [1–38] and proendothelin-3 [1–41 amide] are referred to as proendothelin-1 and proendothelin-3, respectively.

The peptides were dissolved in a small volume of glacial acetic acid, diluted with isotonic saline (157 mmol l^{-1} NaCl) containing 1% bovine serum albumin (Sigma) and 100 μl aliquots were frozen at − 80°C. All injections were given in 100 μl and flushed in with 150 μl saline. Peptide vehicle alone in these volumes had no consistent cardiovascular effects. Mecamylamine hydrochloride (Sigma) was dissolved in isotonic saline and infused at 0.3 ml h^{-1}.

Data analysis

Changes relative to pre-injection baseline values were assessed by Friedman's test (Theordorsson-Norheim, 1987). Comparisons between responses to different peptides were made by Wilcoxon's ranks sums test applied to areas under or over curves (AUC or AOC, respectively) derived from the raw data using a Fortran programme (Gardiner *et al.*, 1990e). Baseline variables in the absence and presence of mecamylamine were compared by Student's t test for paired samples. A P value <0.05 was taken as significant.

Results

Experiment 1

The baseline values for all protocols in experiment 1 are given in Table 1.

Table 1 Average resting cardiovascular variables before administration of endothelin-1, endothelin-3, proendothelin-1 [1–38] or proendothelin-3 [1–41] in the same conscious, Long Evans rats ($n = 9$)

		Endothelin-1	*Endothelin-3*	*Proendothelin-1 [1–38]*	*Proendothelin-3 [1–41]*
Heart rate (beats min^{-1})		337 ± 10	338 ± 7	328 ± 16	336 ± 12
Mean arterial blood pressure (mmHg)		106 ± 1	105 ± 1	108 ± 2	107 ± 2
Doppler shift (kHz)	Coeliac	9.0 ± 1.0	9.2 ± 1.1	8.6 ± 1.0	8.4 ± 1.2
	Mesenteric	6.0 ± 0.5	5.8 ± 0.6	5.9 ± 0.7	5.6 ± 0.7
	Hindquarters	4.2 ± 0.2	4.4 ± 0.2	3.8 ± 0.3	3.8 ± 0.2
Vascular conductance ([kHz $mmHg^{-1}$]10^3)	Coeliac	86 ± 11	87 ± 10	80 ± 8	78 ± 10
	Mesenteric	57 ± 5	55 ± 5	55 ± 7	53 ± 6
	Hindquarters	40 ± 3	42 ± 2	35 ± 3	37 ± 2

Values are mean ± s.e.mean.

Responses to endothelin-1

The lower dose (0.01 nmol kg^{-1}) of endothelin-1 caused a transient tachycardia (AUC, 90 ± 19 units) followed by a slight bradycardia (AOC, 44 ± 17 units) but no change in MAP (Figure 1). However, there was a significant, although slight, reduction in coeliac blood flow (AOC, 46 ± 6 units) and a more marked reduction in mesenteric blood flow (AOC, 107 ± 13 units) (Figure 1). These changes were associated with a slight coeliac (AOC, 39 ± 7 units) and a greater mesenteric (AOC, 97 ± 12 units) vasoconstriction (Figure 1). In contrast, there was a transient rise in blood flow (AUC, 81 ± 11 units) and vascular conductance (AUC, 92 ± 15 units) in the hindquarters (Figure 1).

The higher dose (0.1 nmol kg^{-1}) of endothelin-1 caused tachycardia (AUC, 116 ± 16 units) followed by bradycardia (AOC, 144 ± 25 units) in company with a fall (AOC, 43 ± 4 units) and subsequent rise (AUC, 48 ± 11 units) in MAP (Figure 2). Reductions in coeliac and mesenteric flows (AOC, 83 ± 15 and 117 ± 9 units, respectively) and vascular conductances (AOC, 101 ± 13 and 119 ± 13 units, respectively) occurred, but in contrast to the responses to the low dose of endothelin-1, there were no significant differences between the responses in the coeliac and mesenteric vascular beds, although there was a slight, initial vasodilatation in the former that was absent in the latter (Figure 2). There was a substantial rise in hindquarters flow (AUC, 139 ± 26 units) in association with a large vasodilatation (AUC, 211 ± 31 units) that was succeeded by a slight, and transient vasoconstriction (AOC, 48 ± 10 units) (Figure 2).

Responses to endothelin-3

The lower dose (0.01 nmol kg^{-1}) of endothelin-3 elicited responses that were not different from those following the lower dose of endothelin-1 (heart rate: tachycardia AUC, 63 ± 22 units, bradycardia AOC, 55 ± 16 units; coeliac flow AOC, 49 ± 15 units; mesenteric flow AOC, 66 ± 13 units; hindquarters flow AUC, 69 ± 15 units; coeliac conductance AOC, 44 ± 15 units; mesenteric conductance AOC, 60 ± 11 units; hindquarters conductance AUC, 78 ± 18 units, Figure 1).

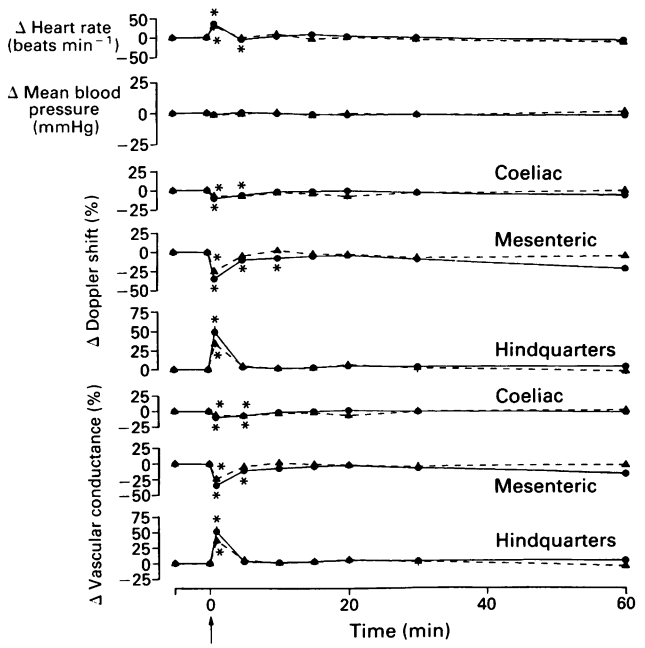

Figure 1 Cardiovascular changes following an i.v. bolus injection (at arrow) of endothelin-1 (0.01 nmol kg^{-1}; ●—●) or endothelin-3 (0.01 nmol kg^{-1}; ▲---▲) in the same conscious, Long Evans rats ($n = 9$). Values are mean, and vertical bars show s.e.mean; where the bars are not visible they lie within the symbols. $*P < 0.05$ versus baseline.

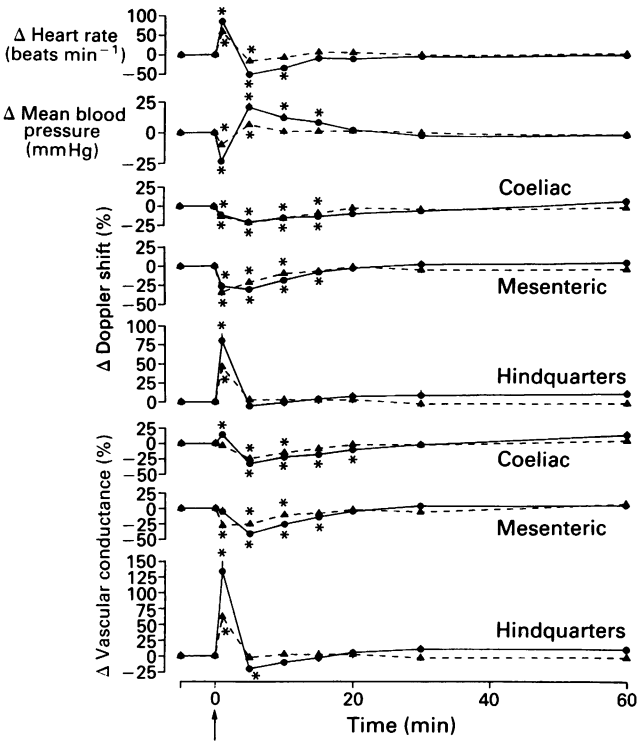

Figure 2 Cardiovascular changes following an i.v. bolus injection (at arrow) of endothelin-1 (0.1 nmol kg^{-1}; ●—●) or endothelin-3 (0.1 nmol kg^{-1}; ▲---▲) in the same conscious, Long Evans rats ($n = 9$). Values are mean, and vertical bars show s.e.mean; where the bars are not visible they lie within the symbols. $*P < 0.05$ versus baseline. The statistics for differences between the integrated responses to endothelin-1 and endothelin-3 are given in the text.

Although the general pattern of cardiovascular response to the higher dose (0.1 nmol kg^{-1}) of endothelin-3 was qualitatively similar to that of endothelin-1 (Figure 2), the later bradycardic effect of endothelin-3 (AOC, 59 ± 17 units) was less than that to endothelin-1, while the initial tachycardic response to the former (AUC, 102 ± 14 units) was not different from that of the latter (Figure 2). However, the early fall (AOC, 27 ± 6 units) and subsequent rise (AUC, 20 ± 5 units) in MAP following endothelin-3 were both less than those following endothelin-1 (Figure 2), although the reductions in coeliac and mesenteric blood flows and vascular conductances were not different following endothelin-3 (coeliac flow AOC, 73 ± 13; mesenteric flow AOC, 110 ± 12 units; coeliac conductance AOC, 75 ± 14 units; mesenteric conductance AOC 108 ± 11 units) and endothelin-1 (Figure 2). But both the increase in hindquarters flow and the rise in vascular conductance were significantly less with endothelin-3 (AUC, 75 ± 19 and 103 ± 23 units, respectively) than with endothelin-1, and there was no subsequent hindquarters vasoconstriction with endothelin-3 (Figure 2).

Responses to proendothelin-1

The lower dose (0.1 nmol kg^{-1}) of proendothelin-1 caused a slight rise (AUC, 47 ± 17 units) followed by a fall (AOC, 114 ± 23 units) in heart rate and a gradual, but small, pressor effect (AUC, 41 ± 13 units) (Figure 3). There were slight, but persistent, reductions in coeliac flow (AOC, 95 ± 13 units) and mesenteric flow (AOC, 73 ± 18 units) and conductances (AOC, 122 ± 15 and 101 ± 17 units, respectively) that were not different (Figure 3). There was an initial increase and a subsequent decrease in hindquarters flow (AUC, 34 ± 14 units; AOC, 76 ± 17 units) and vascular conductance (AUC, 33 ± 14 units; AOC, 96 ± 23 units) (Figure 3).

The higher dose (1.0 nmol kg^{-1}) of proendothelin-1 caused no significant tachycardia, but a marked and sustained reduction in heart rate (AOC, 571 ± 58 units) and increase in MAP (AUC, 204 ± 7 units) (Figure 4). These effects were accompanied by substantial, and similar, falls in coeliac flow (AOC, 240 ± 25 units) and mesenteric flow (AOC, 252 ± 27 units) and vascular conductances (AOC, coeliac 326 ± 18 units; mesenteric 333 ± 21 units) (Figure 4). There were early, slight, and transient increases in hindquarters flow (AUC, 27 ± 7 units) and conductance (AUC, 19 ± 6 units) but, thereafter, both showed prolonged reductions (AOC, flow 238 ± 27 units; conductance 319 ± 20 units) (Figure 4).

Responses to proendothelin-3

The lower dose (0.1 nmol kg^{-1}) of proendothelin-3 had no effect on MAP but there was a slight increase (AUC, 38 ± 12 units) followed by a decrease (AOC, 104 ± 15 units) in heart rate (Figure 3). These effects were not different from those following proendothelin-1. There were modest reductions in coeliac and mesenteric flows (AOC, 119 ± 14 and 70 ± 9 units, respectively) and vascular conductances (AOC, coeliac 124 ± 14 units; mesenteric 73 ± 12 units) following proendothelin-3 that were similar to those after proendothelin-1 (Figure 3). However, proendothelin-3 had no significant effects on hindquarters haemodynamics (Figure 3).

The higher dose (1.0 nmol kg^{-1}) of proendothelin-3 caused a slight, initial tachycardia (AUC, 42 ± 9 units), but subsequently there was a bradycardia (AOC, 292 ± 37 units) in association with a rise in MAP (AUC, 116 ± 18 units (Figure 4). The bradycardic and pressor effects of proendothelin-3 were significantly less than those of proendothelin-1. Proendothelin-3 caused reductions in coeliac and mesenteric blood flows (AOC, coeliac 152 ± 13 units; mesenteric 236 ± 16 units) and vascular conductances (AOC, coeliac 209 ± 8 units; mesenteric 284 ± 21 units). The effects of proendothelin-3 on coeliac haemodynamics were significantly less than those of proendothelin-1 (Figure 4).

There was a tendency towards an increase in hindquarters flow (AUC, 27 ± 13 units) and in vascular conductance (26 ± 17 units) following proendothelin-3, but the changes did not reach significance, although were not significantly different from those following proendothelin-1 (Figure 4). In contrast, the later reduction in hindquarters flow (AOC, 11 ± 21 units) and vascular conductance (AOC, 175 ± 24 units) were significantly less than those following proendothelin-1 (Figure 4).

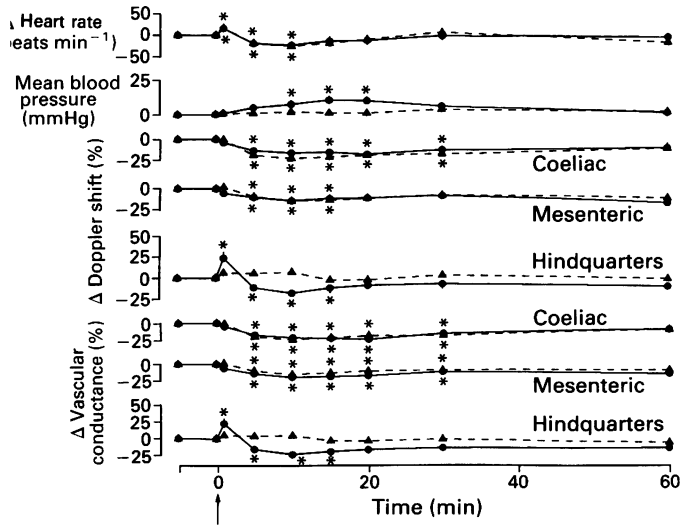

Figure 3 Cardiovascular changes following an i.v. bolus injection (at arrow) of proendothelin-1 [1–38] (0.1 nmol kg^{-1}; ●—●) or proendothelin-3 [1–41 amide] (0.1 nmol kg^{-1}; ▲---▲) in the same conscious, Long Evans rats ($n = 9$). Values are mean, and vertical bars show s.e.mean; where the bars are not visible they lie within the symbols. *$P < 0.05$ versus baseline.

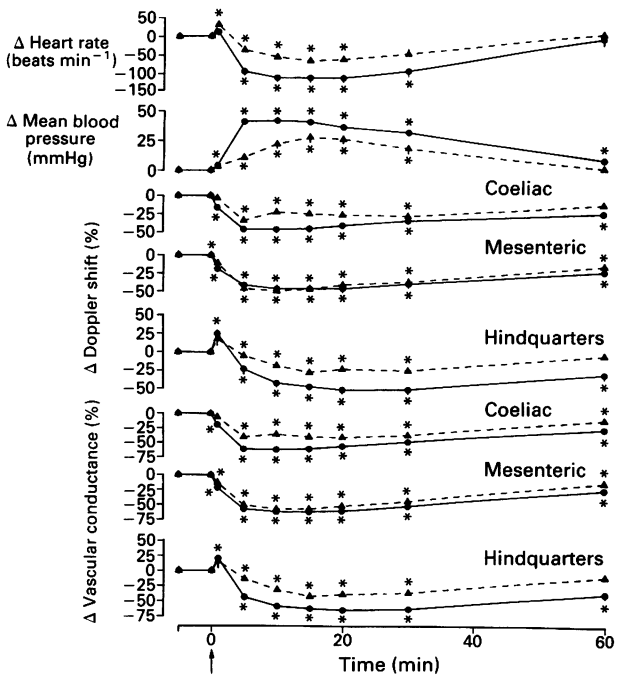

Figure 4 Cardiovascular changes following an i.v. bolus injection (at arrow) of proendothelin-1 [1–38] (1.0 nmol kg^{-1}; ●—●) or proendothelin-3 [1–41 amide] (1.0 nmol kg^{-1}; ▲---▲) in the same conscious, Long Evans rats ($n = 9$). Values are mean, and vertical bars show s.e.mean; where the bars are not visible they lie within the symbols. *$P < 0.05$ versus baseline. The statistics for differences between the integrated responses to proendothelin-1 [1–38] and proendothelin-3 [1–41] amide are given in the text.

Experiment 2

The baseline values in the absence and presence of mecamylamine are shown in Table 2. In the absence of mecamylamine haemodynamic variables were not different from those in experiment 1 (see Table 1), and the cardiovascular responses to endothelin-1 (0.1 nmol kg^{-1}) were similar to those seen in experiment 1 (compare Figures 2 and 5).

In the presence of mecamylamine it was difficult to assess true baseline values for cardiovascular variables, since any motor activity of the animals was accompanied by marked swings in MAP and blood flows.

However, on average, there was resting hypotension and bradycardia and reductions in mesenteric and hindquarters blood flows, together with a mesenteric vasoconstriction (Table 2, Figure 5). Administration of endothelin-1 in the presence of mecamylamine caused an initial reduction in MAP (– 22 ± 1 mmHg) that was not different from that seen in the absence of mecamylamine (– 19 ± 2 mmHg) (Figure 5). Although the subsequent increase in MAP was significantly greater in the former than in the latter condition, the absolute level of MAP reached was not different (Figure 5). The changes in regional vascular conductances elicited by endothelin-1 under the two conditions were not significantly different (Figure 5). There were no significant changes in heart rate following administration of endothelin-1 in the presence of mecamylamine (Figure 5).

Discussion

Elsewhere we have shown that reflex effects are not responsible for the changes in cardiac function (other than heart rate)

Table 2 Average resting cardiovascular variables before and 20 min after onset of mecamylamine infusion (50 μmol kg^{-1} h^{-1}) in the same conscious, Long Evans rats ($n = 7$)

		Before mecamylamine	*During mecamylamine*
Heart rate (beats min^{-1})		334 ± 7	284 ± 7*
Mean arterial blood pressure (mmHg)		106 ± 2	83 ± 1*
Doppler shift (kHz)	Coeliac	8.1 ± 0.8	7.0 ± 0.5
	Mesenteric	6.1 ± 0.5	3.5 ± 0.2*
	Hindquarters	3.8 ± 0.4	2.7 ± 0.4*
Vascular conductance ([kHz $mmHg^{-1}$]10^3)	Coeliac	77 ± 8	85 ± 7
	Mesenteric	57 ± 4	42 ± 3*
	Hindquarters	36 ± 4	33 ± 5

Values are mean ± s.e.mean. *$P < 0.05$ versus before mecamylamine (paired t test).

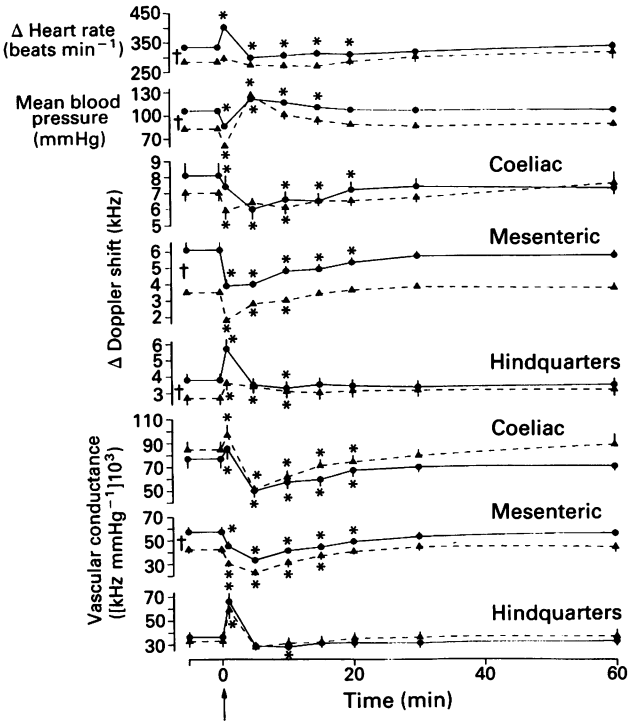

Figure 5 Cardiovascular responses to an i.v. bolus injection (at arrow) of endothelin-1 (0.01 nmol kg^{-1}) in the same conscious, Long Evans rats ($n = 7$) in the absence (●—●) or presence (▲---▲) of mecamylamine (50 μmol kg^{-1} h^{-1} begun 20 min before administration of endothelin-1). Values are mean and vertical bars show s.e.mean; where the bars are not visible they lie within the symbols. *$P < 0.05$ versus baseline; †$P < 0.05$ for resting values in the absence and presence of mecamylamine.

following administration of endothelin-1 (Gardiner *et al.*, 1990d). In addition, the results of the second experiment in the present study indicate that rapid reflex changes in autonomic activity were not confounding the observations by attenuating or potentiating the direct regional haemodynamic effects of endothelin-1. Thus, there is no reason to assume that any of our findings were not due, straightforwardly, to the cardiovascular effects of the peptides studied.

Our main objective was to obtain data on the coeliac haemodynamic effects of endothelin-1 and endothelin-3 in conscious rats, since Bigaud & Vatner (1990) have reported unusual, prolonged vasodilator responses to endothelin-1 in this vascular bed in conscious dogs, and *in vitro* data indicate endothelin-3 is a more potent vasodilator than endothelin-1 (Warner *et al.*, 1989). However, it was clear that under none of our experimental conditions did either peptide elicit increases in coeliac blood flow, although, at the higher dose, endothelin-1 caused a transient increase in coeliac vascular conductance (Figure 2), and the reductions in flow and vascular conductance in this vascular bed following the lower dose of endothelin-1 and endothelin-3 were less than those in the mesenteric vascular bed. Our failure to observe increases in coeliac blood flow was clearly not due to an inability to detect hyperaemia since, in the same animals, we confirmed the occurrence of marked increases in hindquarters flow in response to both endothelin-1 and endothelin-3 (Gardiner *et al.*, 1990a,b). Furthermore, in other studies we have demonstrated clear increases in coeliac blood flow in response to peptides such as neuromedin U-25, rat α-calcitonin gene-related peptide and vasopressin (Gardiner *et al.*, 1990c).

In the present experiments the initial hypotensive and hindquarters hyperaemic vasodilator effects of endothelin-1 were greater than those of endothelin-3. Moreover, only the former peptide caused coeliac vasodilatation. Hence, in conscious rats there is no evidence that endothelin-3 is a more potent vasodilator agent than endothelin-1 (Warner *et al.*, 1989).

The lower dose of exogenous endothelin-1 and endothelin-3 had less marked coeliac, than mesenteric, vasoconstrictor effects, and no hindquarters vasoconstrictor action. However, the higher dose of both peptides caused similar vasoconstrictions in coeliac and mesenteric beds, without reducing hindquarters flow. One possible explanation of these differences is that bolus doses of endothelin-1 or endothelin-3 cause marked stimulation of vasodilator mechanisms that oppose their vasoconstrictor action, as clearly seen in the hindquarters. Thus, the same could be true to a lesser extent in the coeliac and mesenteric vascular beds, even though overt increases in flow did not occur. If this were the case, it is feasible that the more marked vasoconstrictor effects of endothelin-1 and endothelin-3 in the coeliac and mesenteric, than in the hindquarters, vascular beds was due to more effective stimulation of vasodilator mechanisms in the latter. However, another possibility is that rapid enzymatic degradation of exogenous endothelin-1 and endothelin-3 (Sokolovsky *et al.*, 1990; Vijayaraghavan *et al.*, 1990) in the hindquarters vascular bed prevents them exerting more marked effects at that site.

An additional objective was to compare the profiles of effect of proendothelin-1 and proendothelin-3, and to consider them against those of endothelin-1 and endothelin-3. The first point of note is that neither proendothelin-1 nor proendothelin-3 evoked increases in coeliac flow or vascular conductance. But, although in several instances proendothelin-1 had greater vasoconstrictor effects than proendothelin-3, the latter, nevertheless, did exert marked coeliac, mesenteric and hindquarters haemodynamic effects. Hence, the lack of conversion of proendothelin-3 to endothelin-3 *in vitro* by enzymes that act upon proendothelin-1 (Okada *et al.*, 1990; 1991) is likely to be a feature of the isolated system studied in those experiments. Our findings differ from those reported recently by D'Orléans-Juste *et al.* (1991), in anaesthetized guinea-pigs. These workers observed that doses of proendothelin-3 up to 20 nmol kg^{-1} were without effects on

MAP in their experiments, whereas endothelin-1, proendothelin-1 and endothelin-3 at doses of 2 nmol kg^{-1} caused rises in MAP of about 25 mmHg. In recent experiments in animals instrumented for measurement of renal, mesenteric and hindquarters haemodynamics, we have confirmed the pressor and vasoconstrictor effets of proendothelin-3 (Gardiner, S.M. *et al.*, unpublished observations). Furthermore, in those same experiments, we found that proendothelin-2 exerted pressor and vasoconstrictor effects. These observations support our proposition (see above) that *in vitro* assessment of enzyme activity may produce incomplete results, since Okada *et al.* (1991) have reported that the enzyme that converts proendothelin-1 to endothelin-1 is inactive against proendothelin-2.

From the present results it appears that proendothelin-1 can exert similar vasoconstrictor effects on coeliac, mesenteric and hindquarters vascular beds, but proendothelin-3, while having similar mesenteric vasoconstrictor effects to proendothelin-1, has less marked coeliac and hindquarters vasoconstrictor effects. Hence the mesenteric vascular bed may be endowed with enzyme systems that effectively convert both proendothelin-1 and proendothelin-3 to endothelin-1 and endothelin-3, respectively, whereas the local conversion of proendothelin-3 to endothelin-3 might be less in the coeliac and hindquarters vascular beds. In this context, it is notable that the mesenteric vasoconstrictor effets of proendothelin-1 are more resistant to phosphoramidon than are its renal or hindquarters vasoconstrictor actions (Gardiner *et al.*, 1991).

The present results with proendothelin-1 and proendothelin-3 indicate that activation of vasodilator mechanisms (except, perhaps in the hindquarters) may be a feature only of the pharmacology of exogenous endothelin-1 and endothelin-3 and that, *in vivo*, the local genesis of endothelin-1 or endothelin-3 from proendothelin-1 or proendothelin-3, respectively, might exert vasoconstrictor effects in all vascular beds.

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