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Effects of the dual metallopeptidase inhibitor, MDL 100,240, on regional haemodynamic responses to vasoactive peptides in conscious rats

[1]S.M. Gardiner, P.A. Kemp, *F. Brunner-Ferber & T. Bennett

School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and *Clinical Pharmacology, Hoechst Marion Roussel, Romainville Cedex, France

1 The effects of combined inhibition of neutral endopeptidase 24.11 and angiotensin-converting enzyme, with the dual metallopeptidase inhibitor, MDL 100,240 (3 mg kg^{-1} bolus, 3 mg kg^{-1}, h^{-1} infusion), on baseline haemodynamics and on responses to a variety of vasoactive peptides were studied in conscious Long Evans rats (350–450 g: $n=9$) chronically instrumented for the assessment of regional haemodynamics.

2 The experiments ran over 4 consecutive days. On the first 2 days the animals received the vehicle for MDL 100,240, and were given bolus i.v. injections of angiotensin I (AI; 250 pmol kg^{-1}), angiotensin II (AII; 125 pmol kg^{-1}), bradykinin (BK: 3 nmol kg^{-1}) and endothelin-1 (ET-1; 250 pmol kg^{-1}) on one day and AI (as above), atrial natriuretic peptide (ANP: 500 pmol kg^{-1}) and big endothelin-1 (big ET-1; 500 pmol kg^{-1}) on the other day in a random manner. On the third and fourth experimental days the vasoactive peptides were given in the same order as before, but in the presence of MDL 100,240.

3 Thirty minutes after onset of administration of vehicle, on the first or second experimental day, there were no consistent cardiovascular changes. However, at the same time following onset of MDL 100,240 administration on the third experimental day, there was a significant, but slight, reduction in mean arterial blood pressure (MAP; -5 ± 2 mmHg) together with tachycardia (41 ± 12 beats min^{-1}) and increases in renal and mesenteric flows (17 ± 3 and 13 ± 4%, respectively) and vascular conductances (23 ± 4 and 19 ± 5%, respectively). The mesenteric vasodilator effect of MDL 100,240 was still present on the fourth experimental day before administration of the drug on that day, but otherwise the pattern of response to MDL 100,240 was similar to that on the previous day.

4 In the presence of vehicle, AI caused hypertension, bradycardia, and reductions in renal mesenteric and hindquarters vascular conductances; all these effects were abolished by MDL 100,240.

5 In the presence of vehicle, AII caused effects similar to those of AI. MDL 100,240 did not affect the pressor, bradycardic or hindquarters vasoconstrictor effects of AII. However, in the presence of MDL 100,240, the overall renal and mesenteric vasoconstrictor effects of AII were enhanced, probably because of the renal and mesenteric vasodilatation caused by MDL 100,240.

6 In the presence of vehicle, BK had a slight pressor effect, accompanied by tachycardia and transient increases in conductances in renal, mesenteric and hindquarters vascular beds. In the presence of MDL 100,240 BK caused marked hypotension, but an attenuated tachycardia; renal, mesenteric and hindquarters vasodilator responses were enhanced.

7 In the presence of vehicle, ANP caused slight hypotension and tachycardia, together with reductions in renal and mesenteric vascular conductances, and transient increases in hindquarters conductance. MDL 100,240 enhanced the hypotensive effect of ANP and promoted a delayed hindquarters vasoconstriction.

8 Big ET-1, in the presence of vehicle, caused a marked and prolonged increase in MAP, accompanied by bradycardia and reductions in renal, mesenteric and hindquarters vascular conductances. Although MDL 100,240 significantly attenuated the magnitude of the pressor effect of big ET-1, its bradycardic and renal, mesenteric and hindquarters haemodynamic actions were not reduced significantly.

9 In the presence of vehicle, ET-1 caused an initial hypotension, tachycardia and vasodilatation in the hindquarters, but reductions in renal and mesenteric vascular conductances; thereafter there was a rise in MAP and bradycardia with vasoconstriction in all three vascular beds. MDL 100,240 had no effect on the initial hypotensive, tachycardic or hindquarters haemodynamic effects of ET-1. Moreover the subsequent pressor and bradycardic actions of ET-1 were unchanged, but its renal and mesenteric vasoconstrictor effects were enhanced, possibly because of the dilatation caused by MDL 100,240 in these vascular beds.

10 Overall, the results are consistent with MDL 100,240 exerting important haemodynamic actions in conscious rats through inhibition of angiotensin-converting enzyme and neutral endopeptidase.

Keywords: Dual metallopeptidase inhibitor; angiotensin I; angiotensin II; bradykinin; ANP; big endothelin-1; endothelin-1

Introduction

Angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP) are responsible for the production and/or degradation of a variety of vasoactive peptides. Principally, ACE influences angiotensin II (AII) formation and bradykinin (BK) degradation, whereas NEP is responsible for the degradation of the natriuretic peptides (ANP, BNP, CNP), substance P, endorphins, as well as BK (Littlewood *et al*., 1988; Erdös & Skidgel, 1989; Rubanyi & Polokoff, 1994). It is also

[1] Author for correspondence.

possible that NEP, or a related enzyme, is involved in the conversion of big endothelin (ET) to ET-1 (see Gardiner *et al*., 1992a). Recently, several drugs which are dual metallopeptidase inhibitors (i.e., they inhibit both ACE and NEP) have been produced (e.g., Flynn *et al*., 1993; Fournié-Zaluski *et al*., 1994; French *et al*., 1994; Bralet *et al*., 1994; Marie *et al*., 1995; Trippodo *et al*., 1995; Vera *et al*., 1995), but their *in vivo* characteristics have not been fully established, at least as far as their influence on responses to exogenous vasoactive peptides in normal rats is concerned.

French *et al*. (1994) demonstrated that the dual metallopeptidase inhibitor, MDL 100,240, at a dose of 3 mg kg^{-1}, i.p., caused inhibition ($\sim$ 80%) of the pressor effect of AI for at least 2 h in pithed rats. In anaesthetized rats, MDL 100,240 (10 mg kg^{-1}, i.v.) had no effect on mean arterial pressure, but caused what was originally a non-depressor infusion of ANP to evoke a significant hypotension, and also converted a non-depressor effect of BK into a dramatic hypotension. However, the regional haemodynamic effects of these interventions are unknown.

In previous studies we have shown that the ACE inhibitor, captopril, had no acute effects on basal regional haemodynamics in normal rats, but caused suppression of the effects of AI and enhancement of responses to BK (Gardiner *et al*., 1989; 1993a). Furthermore, we found that the NEP inhibitor, SQ 28,603, had no effects on basal haemodynamics in conscious rats, even at a dose of 50 mg kg^{-1} (Gardiner *et al*., 1992a). However, under those conditions, SQ 28,603 enhanced some responses to ANP and inhibited some effects of big ET-1, but had no influence on responses to AI, AII or ET-1 (Gardiner *et al*., 1992a). It is not known what effects combined inhibition of ACE and NEP have in this experimental paradigm, so we investigated the effects of MDL 100,240 on haemodynamic responses to AI, AII, BK, ANP, big ET-1 and ET-1 in conscious rats.

Methods

All experiments were carried out on male, Long Evans rats (350–450 g) bred in the Biomedical Services Unit in Nottingham. Under sodium methohexitone anaesthesia (Brietal, Lilly, 40–60 mg kg^{-1}, i.p., supplemented as required), pulsed Doppler probes were implanted around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor hindquarters flow). Seven to 14 days later, animals were anaesthetized (as above) and had catheters implanted in the abdominal aorta via the caudal artery to allow monitoring of arterial blood pressure and heart rate, and the right external jugular vein for i.v. administration of drugs. Experiments were begun 24 h after catheter implantation.

Pilot experiments

In 3 animals we assessed responses to bolus injections of AI (250 pmol kg^{-1}), AII (125 pmol kg^{-1}), big ET-1 (500 pmol kg^{-1}) and ANP (500 pmol kg^{-1}) in the presence of vehicle (1% $NaHCO_3$, 1% ethanol, infused at 0.4 ml h^{-1}, following 0.2 ml bolus) in the morning or in the presence of MDL 100,240 (10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion) in the afternoon of the same day. However, assessment of responses to AI on subsequent days showed that the inhibitory effects of this dose of MDL 100,240 were apparent for at least 72 h. Even with MDL 100,240 at a dose of 3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion, some degree of inhibition of the effects of AI was still present the following day.

Full experiments

In the light of the pilot experiments and in an attempt to avoid the complication of carry-over of the effects of MDL 100,240, each animal went through a 4-day experimental protocol, receiving challenges in the presence of vehicle on the first two days, and in the presence of MDL 100,240 (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion) on the last two days. Of the vasoactive peptides to be studied, big ET-1 and ET-1 have very prolonged effects, whereas AI, AII, BK and ANP are relatively short acting. Therefore, we used only one dose of each peptide, and animals were treated randomly to receive AI (250 pmol kg^{-1}), ANP (500 pmol kg^{-1}) and big ET-1 (500 pmol kg^{-1}) on one day, and AI (250 pmol kg^{-1}), AII (125 pmol kg^{-1}), BK (3 nmol kg^{-1}) and ET-1 (250 pmol kg^{-1}) on the other day, beginning 30 min after the onset of infusion of vehicle or MDL 100,240. Bolus injections were separated by periods of up to 90 min to allow variables to return to baseline. On each day the vehicle or MDL 100,240 was infused throughout the period during which the bolus doses of peptides were given, i.e. 3–4 h.

Data analysis

Responses to challenges were assessed by use of Friedman's test (Theodorsson-Norheim, 1987). Comparison of responses to challenges in the presence of vehicle and MDL 100,240 was by Wilcoxon's matched-pairs signed ranks test applied to areas under or over curves. The areas were calculated from the response over the time course indicated in the appropriate figures, with the last baseline value before the administration of the peptide taken as the reference point from which the areas of triangles and trapezoids were calculated. A P value <0.05 was taken as significant. Details regarding experimental recording procedures have been published (Gardiner *et al*., 1992a; 1993a).

Drugs and peptides

MDL 100,240 ([4S-[4α,7α(R*), 12bβ]]-7-[[2-(acetylthio)-1-oxo-3-phenylpropyl]amino]-1,2,3,4,6,7,8,12b-octahydro-6-oxo-pyrido[2,1-a][2]benzazepine-4-carboxylic acid) was obtained from Marion Merrell Dow (U.S.A.) and was dissolved in vehicle (1% $NaHCO_3$, 1% ethanol; French *et al*., 1994). AI, AII, BK and ANP were obtained from Bachem (U.K.), and big ET-1 and ET-1 from Peptide Institute (Osaka, Japan). All peptides were dissolved in sterile saline containing 1% BSA (Sigma U.K.) and injected in a volume of 0.1 ml.

Results

Resting haemodynamics

Resting cardiovascular variables before administration of vehicle on day 1 and day 2 were very similar (Table 1). Thirty minutes after the onset of vehicle administration the only consistent change was a small fall in hindquarters vascular conductance (Table 1). It is likely this change was due to the animals adapting to the recording conditions.

On the third experimental day, before administration of MDL 100,240, resting cardiovascular variables were similar to those on the first two days (Table 1). However, 30 minutes after the onset of infusion of MDL 100,240 there was a significant, albeit modest, fall in mean arterial blood pressure (MAP), and increases in heart rate and renal and mesenteric flows and conductances (Table 1). These effects of MDL 100,240 were still apparent on the following day, but only the increase in mesenteric vascular conductance was significant (Table 1). The pattern of response to MDL 100,240 on the fourth experimental day was similar to that on the third day (Table 1), except that there was no additional mesenteric vasodilatation.

Responses to AI

In the presence of vehicle (on days 1 and 2), AI had similar marked pressor and bradycardic effects, accompanied by substantial reductions in renal and mesenteric vascular conductance and a smaller constriction in the hindquarters vascular bed (Figure 1). In the presence of MDL 100,240 on days

Table 1 Cardiovascular variables in the same group of conscious, Long Evans rats on four consecutive experimental days, before and 30 minutes after administration of vehicle (day 1 and day 2) or MDL 100,240 (day 3 and day 4)

	Day 1		*Day 2*		*Day 3*		*Day 4*	
	−Vehicle a	*+Vehicle* b	*−Vehicle* c	*+Vehicle* d	*−MDL* e	*+MDL* f	*−MDL* g	*+MDL* h
Heart rate (beats min^{-1})	351±5	333±4[a]	348±9	337±5	331±6	372±12[e]	334±3[f]	362±4[c,g]
Mean blood pressure (mmHg)	103±2	104±2	101±3	105±2[c]	103±2	98±2[e]	99±2	96±2[e]
Renal Doppler shift (kHz)	5.7±0.5	6.0±0.6	5.8±0.4	5.7±0.4	5.8±0.5	6.8±0.7[e]	6.2±0.6	7.0±0.7[e,g]
Mesenteric Doppler shift (kHz)	6.4±0.4	6.5±0.5	6.9±0.5	6.9±0.5	7.0±0.6	7.7±0.5[e]	7.9±0.6	8.0±0.5[e]
Hindquarters Doppler shift (kHz)	4.4±0.3	3.6±0.4[a]	3.9±0.3	3.6±0.3[a]	3.9±0.4	3.8±0.4	4.1±0.4	4.2±0.3
Renal vascular conductance ([kHz $mmHg^{-1}$] 10^{-3})	55±5	57±6	57±4	54±4	57±4	70±6[e]	63±5	73±7[e,g]
Mesenteric vascular conductance ([kHz $mmHg^{-1}$] 10^{-3})	63±4	62±4	68±4	65±4	68±6	79±5[e]	80±5[e]	83±4[e]
Hindquarters vascular conductance ([kHz $mmHg^{-1}$] 10^{-3})	43±3	34±3[a]	39±3	34±3[a,c]	38±4	39±4	42±4	43±3

Values are mean±s.e.mean ($n=9$): superscripts indicate $P<0.05$ versus value in corresponding column.

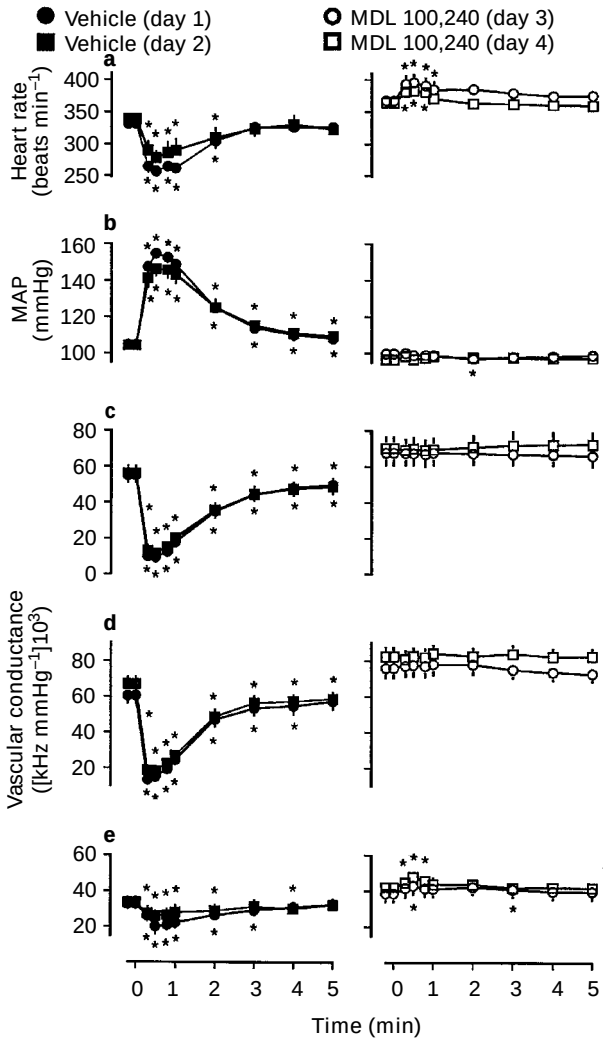

Figure 1 Cardiovascular responses ((a) heart rate, (b) mean arterial pressure (MAP), (c) renal, (d) mesenteric and (e) hindquarters vascular conductance) to bolus injection of AI (250 pmol kg^{-1}) in the same conscious, Long Evans rats (n = 9) on day 1 and day 2, in the presence of vehicle and on day 3 and day 4 in the presence of MDL 100,240. Values are mean and vertical lines show s.e.mean; $^{*}P<0.05$ versus baseline (Friedman's test).

3 and 4, all these effects of AI were abolished (Figure 1), but there was a slight tachycardia and increase in hindquarters vascular conductance, probably due to the alerting effect of the injection.

Responses to AII

In the presence of vehicle, AII caused effects similar to those of AI (Figure 2). In the presence of MDL 100,240, the renal and mesenteric vasoconstrictor effects of AII were significantly enhanced, but this was probably due to the renal and mesenteric vasodilatation caused by MDL 100,240 because the nadirs of the effects of AII were unchanged (Figure 2).

Responses to BK

In the presence of vehicle, the dose of BK used had a slight pressor effect accompanied by tachycardia, and transient increases in renal, mesenteric and hindquarters conductances (Figure 3). In the presence of MDL 100,240, BK caused marked hypotension, but a slightly reduced tachycardia; there were falls in renal and mesenteric flows initially (data not shown), but, because of the profound hypotension, the increases in vascular conductance were significantly enhanced, as

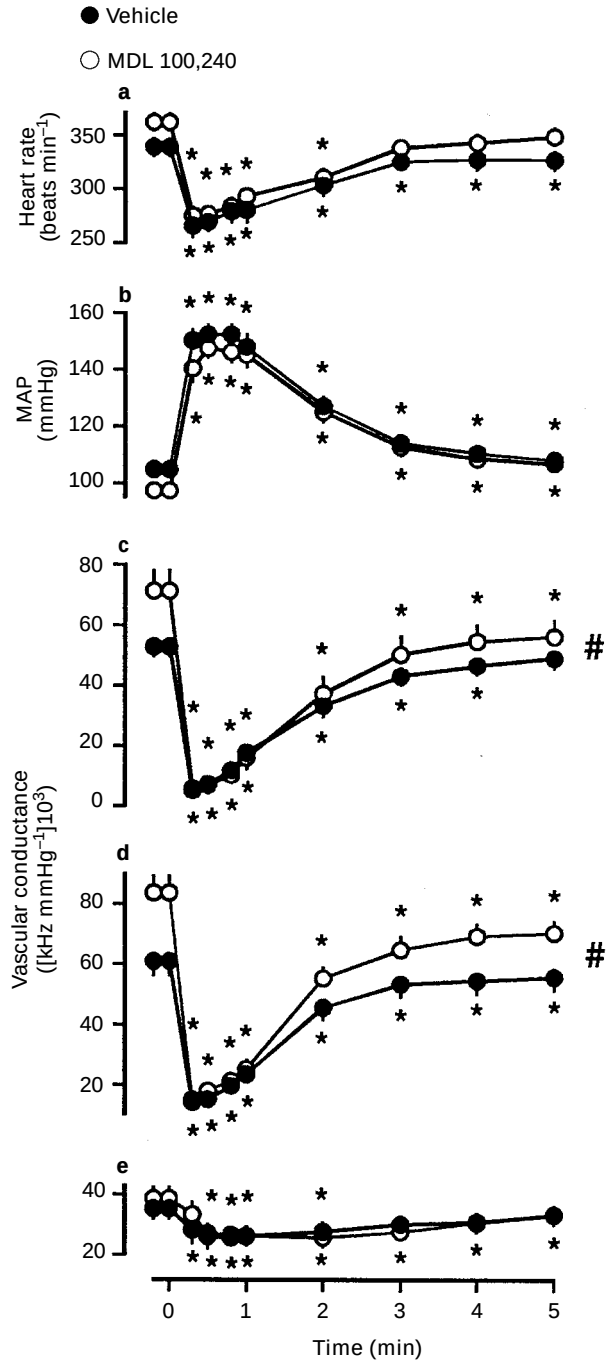

Figure 2 Cardiovascular responses ((a) heart rate, (b) MAP, (c) renal, (d) mesenteric and (e) hindquarters vascular conductances) to bolus injection of AII (125 pmol kg^{-1}) in the same conscious Long Evans rats ($n=9$) in the presence of vehicle or MDL 100,240. Values are mean and vertical lines show s.e.mean; *$P<0.05$ versus baseline (Friedman's test); #$P<0.05$ for differences between integrated responses (Wilcoxon's test).

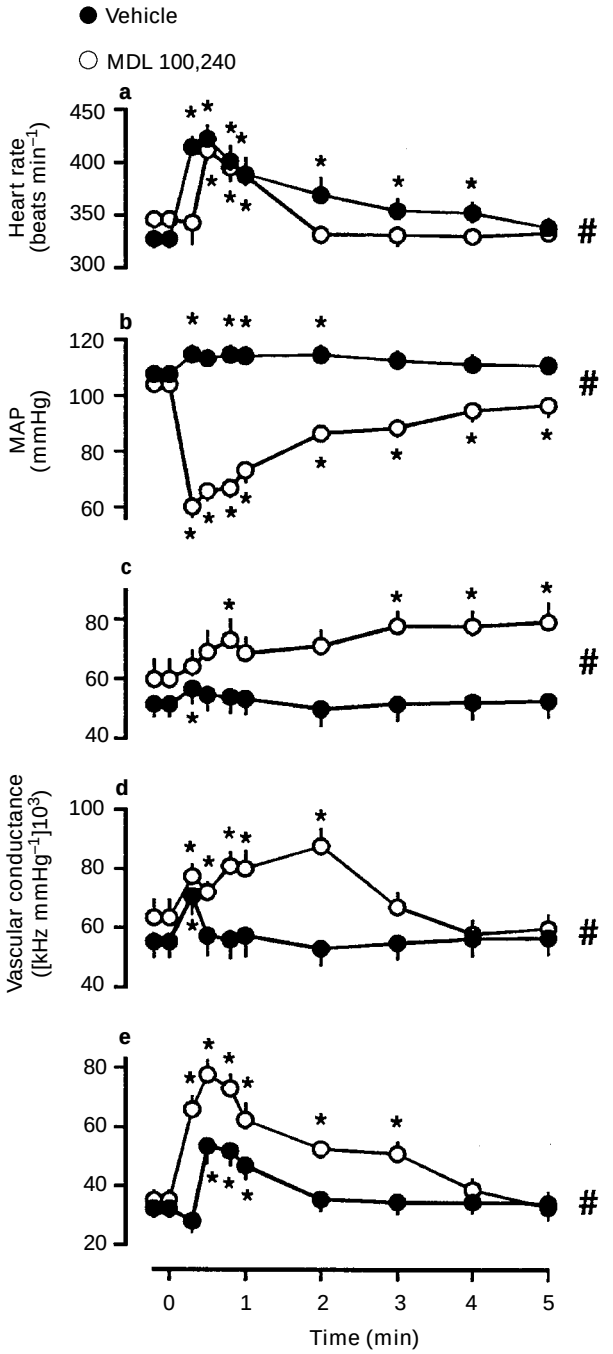

Figure 3 Cardiovascular responses ((a) heart rate, (b) MAP, (c) renal, (d) mesenteric and (e) hindquarters vascular conductance) to a bolus injection of BK (3 nmol kg^{-1}) in the same conscious Long Evans rats ($n=9$) in the presence of vehicle or MDL 100,240. Values are mean and vertical lines show s.e.mean; *$P<0.05$ versus baseline (Friedman's test); #$P<0.05$ for differences between integrated responses (Wilcoxon's test).

was the hindquarters vasodilatation (Figure 3). Most variables had returned towards baseline within 5 minutes, although the BK-induced increase in renal vascular conductance in the animals treated with MDL 100,240 lasted for 10–15 minutes.

Responses to ANP

In the presence of vehicle, ANP caused a slight, transient hypotension and tachycardia, accompanied by reductions in renal and mesenteric vascular conductances; hindquarters vascular conductance showed an initial increase only (Figure 4). MDL 100,240 enhanced the hypotensive effects of ANP, and promoted a hindquarters vasoconstrictor response to this peptide (Figure 4).

Responses to big ET-1

In the presence of vehicle, big ET-1 caused a marked and prolonged increase in MAP and a bradycardia, accompanied by reductions in renal, mesenteric and hindquarters vascular conductances (Figure 5). In the presence of MDL 100,240, the pressor effect of big ET-1 was significantly attenuated and appeared to be slower in onset, but neither the bradycardia nor the overall regional vasoconstrictions were significantly affec-

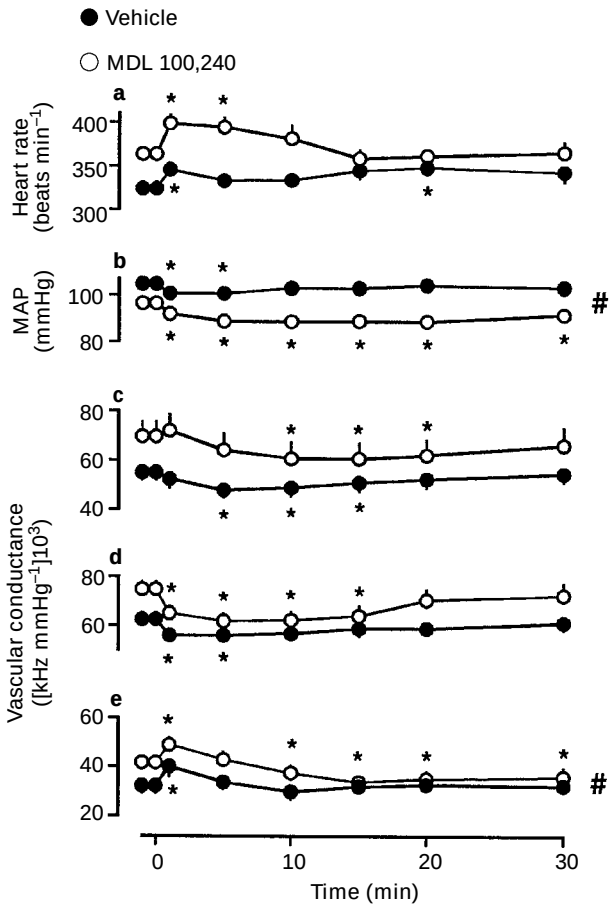

Figure 4 Cardiovascular responses ((a) heart rate, (b) MAP, (c) renal, (d) mesenteric and (e) hindquarters vascular conductance) to bolus injection of ANP (500 pmol kg^{-1}) in the same conscious Long Evans rats ($n=9$) in the presence of vehicle or MDL 100, 240. Values are mean and vertical lines show s.e.mean; $*P<0.05$ versus baseline (Friedman's test); $\#P<0.05$ for differences between integrated responses (Wilcoxon's test).

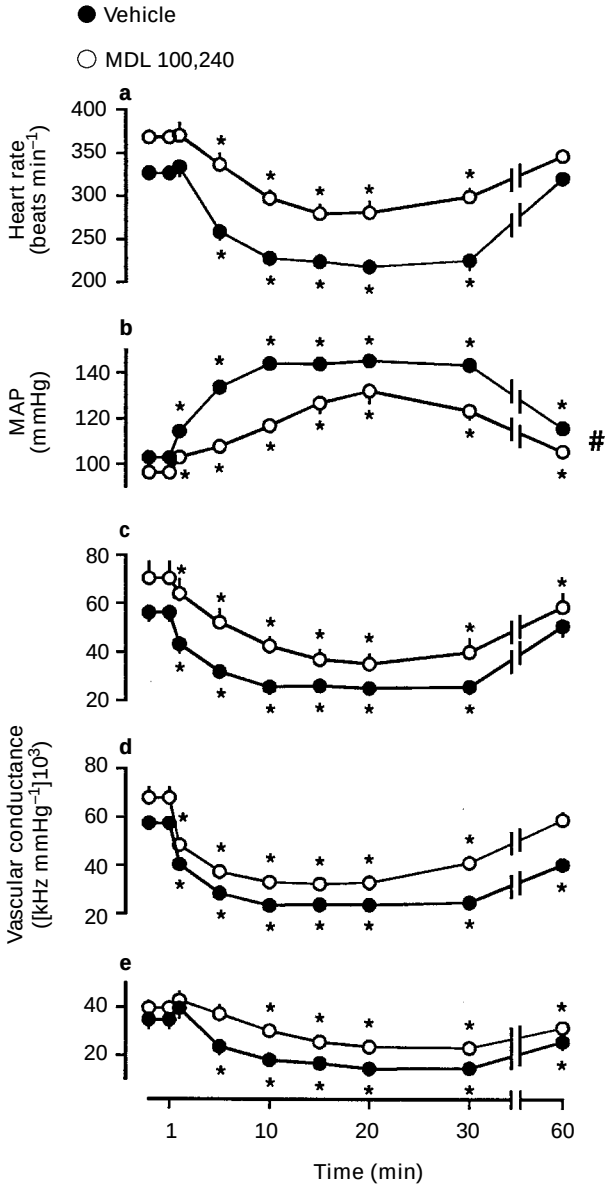

Figure 5 Cardiovascular responses ((a) heart rate, (b) MAP, (c) renal, (d) mesenteric and (e) hindquarters vascular conductance) to a bolus injection of big ET-1 (500 pmol kg^{-1}) in the same conscious Long Evans rats ($n=9$) in the presence of vehicle or MDL 100,240. Values are mean and vertical lines show s.e.mean; $*P<0.05$ versus baseline (Friedman's test); $\#P<0.05$ for differences between integrated responses (Wilcoxon's test).

ted, although the reduction in hindquarters vascular conductance was delayed (Figure 5).

Responses to ET-1

In the presence of vehicle, ET-1 caused an initial hypotension, tachycardia, an increase in hindquarters vascular conductance, and decreases in renal and mesenteric vascular conductances (Figure 6). Thereafter there was a prolonged rise in MAP associated with bradycardia, and sustained reductions in renal and mesenteric vascular conductances, but only slight hindquarters vasoconstriction (Figure 6). In the presence of MDL 100,240, the initial hypotension, tachycardia and increase in hindquarters vascular conductance were unchanged. However, the renal and mesenteric vasoconstrictor effects of ET-1 were enhanced, probably because of the change in baseline (see responses to AII).

Discussion

The major findings of the present work are that MDL 100,240 (at a dose of 3 mg kg^{-1} bolus and 3 mg kg^{-1} h^{-1} infusion) caused a slight fall in MAP, together with tachycardia and hyperaemic dilatation in the renal and mesenteric vascular beds. In addition, it abolished the pressor and regional vasoconstrictor effects of AI, markedly increased the hypotensive and vasodilator effects of BK, caused some enhancement of the hypotensive and hindquarters haemodynamic effects of ANP, and some inhibition of the pressor effects of big ET-1.

In the absence of MDL 100,240, the vasoactive peptides studied showed a pattern of regional haemodynamic selectivity consistent with our previous observations with AI (Gardiner *et al*., 1993a), AII (Gardiner *et al*., 1993b), BK (Gardiner *et al*., 1992b), ANP (Gardiner *et al*., 1992a), ET-1 (Gardiner *et al*., 1990) and big ET-1 (Gardiner *et al*., 1992a).

The finding that MDL 100,240 had significant haemodynamic effects in conscious rats differs from the results of French *et al*. (1994) showing a lack of effect of MDL 100,240 on MAP in anaesthetized rats. Since the dose of MDL 100,240 used here clearly influenced responses to exogenous AI, BK, ANP and big ET-1, we cannot determine which actions were responsible for its effect on resting haemodynamics. The active metabolite of MDL 100,240 has been shown (French *et al*., 1994) to inhibit ACE and NEP with similar potency *in vitro* (K_i for ACE = 0.11 nM; K_i for

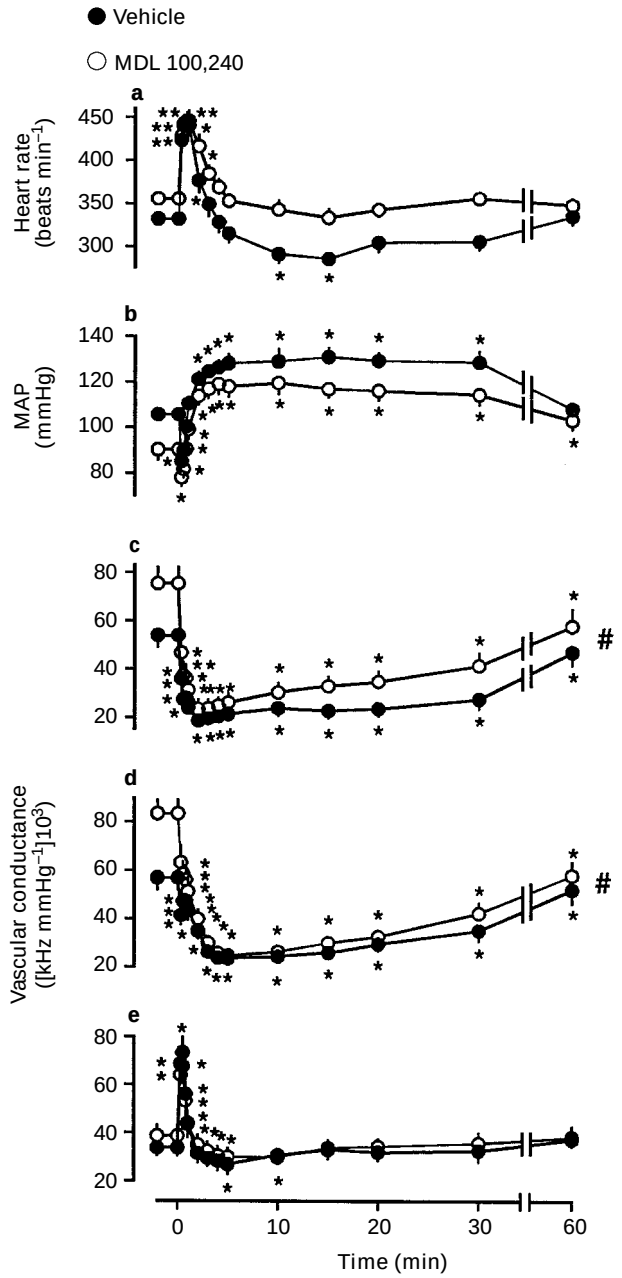

Figure 6 Cardiovascular responses ((a) heart rate, (b) MAP, (c) renal, (d) mesenteric and (e) hindquarters vascular conductance) to bolus injection of ET-1 (250 pmol kg^{-1}) in the same conscious Long Evans rats ($n=9$) in the presence of vehicle or MDL 100,240. Values are mean and vertical lines show s.e.mean; *$P<0.05$ versus baseline (Friedman's test); #$P<0.05$ for differences between integrated responses (Wilcoxon's test).

NEP = 0.08 nM). Thus, it is likely that both enzymes were inhibited equally in the present experiments. Enhancement of the effects of ANP, together with inhibition of the production of AII and ET could explain the fall in MAP and the renal and mesenteric vasodilatation caused by MDL 100,240. It is notable that the effects of the latter on resting haemodynamics contrast with the lack of influence of captopril, or the NEP inhibitor, SQ 28,603, separately (Gardiner *et al*., 1989; 1992a; 1993a).

French *et al*. (1994) observed that MDL 100,240 (3 mg kg^{-1} i.p.) caused about 80% inhibition of the pressor effect of AI (maximum Δ 63 $\pm$ 4 mmHg) in pithed rats. In the present study, MDL 100,240, given i.v. as a primed infusion (3 mg kg^{-1}, 3 mg kg^{-1} h^{-1}), completely abolished all pressor and vasoconstrictor effects of AI at a dose sufficient to raise MAP by 40 – 50 mmHg. It is feasible that i.p. injection of MDL 100,240, as given by French *et al*. (1994), would be less effective than the primed i.v. infusion, as given here. Interestingly, in the light of our findings, and those of French *et al*. (1994), on the thioester prodrug, MDL 100,240, it is notable that the same group (French *et al*., 1995), when investigating the antihypertensive effect of the active metabolite, MDL 100,173, used a much higher dose (20 mg kg^{-1} i.v.) than would appear necessary to inhibit ACE and NEP. French *et al*. (1995) did not study the effects of lower doses of the drug in that context.

Because we studied only one dose of MDL 100,240 we cannot comment on the possibility that, like captopril (Gardiner *et al*., 1993a), it might be more effective at enhancing the actions of BK than suppressing the effects of AI, although this is particularly likely because NEP, as well as ACE, is involved in the degradation of BK. The inhibitory effect of MDL 100,240 on ACE and NEP probably accounts for its remarkable ability to augment the depressor and vasodilator effects of BK. Since there was such a marked fall in MAP in response to BK in the presence of MDL 100,240, it is possible that a component of the enhanced renal and mesenteric vasodilatation was an autoregulatory response to the fall in flow. Moreover, it is feasible that, under those circumstances, the hypotensive effect of BK was contributed to by a reduction in cardiac output (Gardiner *et al*., 1993a). In this regard it is notable that, in the presence of MDL 100,240, some animals showed an initial, marked bradycardic response to BK (see also Waldron *et al*., 1982), possibly due to augmentation of its stimulant action on cardiac afferent fibres (Nerdrum *et al*., 1986; Staszewska-Woolley & Woolley, 1989; Geppetti *et al*., 1990).

Although MDL 100,240 enhanced the depressor effect of ANP and inhibited the pressor action of big ET-1, it did so in the absence of a significant change in regional haemodynamic effects of these peptides, in contrast to SQ 28,603 (Gardiner *et al*., 1992a). However, the latter compound was given at a much higher dose (50 mg kg^{-1}) so we cannot comment on the relative effectiveness of the compounds. Since at the dose used ANP causes a fall in MAP in spite of regional vasoconstriction, it is likely that a reduction in cardiac output secondary to a fall in venous return underlies its depressor effect (see also Gardiner *et al*., 1988; 1992a), and the vasoconstrictions are counterregulatory. From the present results it appears that MDL 100,240 enhances the action of ANP on cardiac output.

Significant inhibition of the pressor action of big ET-1 by MDL 100,240, in the absence of significant suppression of any of its regional vasoconstrictor actions is surprising. However, it is feasible that the non-significant attenuation of each regional vasoconstrictor response to big ET-1 by MDL 100,240, when cumulated, represented a significant reduction in the overall fall in total peripheral conductance. It should be noted also that MDL 100,240 caused an enhancement of the renal and mesenteric vasoconstrictor effects of ET-1, so against this background, it could be argued MDL 100,240 caused a relative diminution of the vasoconstrictor effects of big ET-1. The failure of MDL 100,240 to reduce the bradycardic effect of big ET-1, in spite of attenuating its pressor action, indicates that the reduction in heart rate was not simply a baroreflex response to the rise in MAP evoked by big ET-1. A similar phenomenon was seen with SQ 28,603 (Gardiner *et al*., 1992a) and remains to be explained. It is feasible that the change in heart rate was due to a central action of big ET-1 which was not affected by MDL 100,240.

The apparent enhancement of the renal and mesenteric vasoconstrictor effects of AII and ET-1 by MDL 100,240 occurred in conditions in which the latter compound had caused an increase in conductance in both vascular beds, and hence could have been due to this baseline shift. This proposition is consistent with the finding that the pressor effects of AII and ET-1 were unchanged by MDL 100,240.

In summary, MDL 100,240 causes regional haemodynamic effects in conscious, normotensive rats in line with its ability to influence the cardiovascular actions of AI, BK, ANP and big ET-1. Whether or not its regional haemodynamic actions are amplified in the presence of hypertension (see French *et al*., 1995), and the extent of involvement of cardiac haemodynamic changes, are examined in the accompanying paper.

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