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Comparative effects of the dual metallopeptidase inhibitor, MDL 100,240 and of enalaprilat on regional and on cardiac haemodynamics in conscious, hypertensive, transgenic ((mRen-2)27) rats

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1 Heterozygous, male, hypertensive, transgenic ((mRen-2)27) rats (350–450 g) were instrumented for the measurement of regional or cardiac haemodynamics ($n = 16$, in both groups). Animals were given continuous i.v. infusions of the angiotensin-converting enzyme inhibitor, enalaprilat, or the dual metallopeptidase inhibitor, MDL 100,240 (both at 3 mg kg^{-1}, 3 mg kg^{-1} h^{-1}; $n = 8$ for regional and cardiac haemodynamics), for 32 h. Twenty four hours after the onset of infusion of enalaprilat or MDL 100,240, the bradykinin (B_2)-receptor antagonist, Hoe 140 (1 mg kg^{-1}, i.v.), was given and measurements were continued for a further 8 h, to assess any possible involvement of bradykinin.

2 Over the first 8 h of infusion, both enalaprilat and MDL 100,240 had significant antihypertensive effects, accompanied by similar regional vasodilatations. However, the blood pressure lowering effect of MDL 100,240 (-54 ± 9 mmHg) was greater than that of enalaprilat (-38 ± 4 mmHg), because the former caused a significantly greater reduction in cardiac index.

3 Between 8–24 h after the onset of infusion, there was a reduction in the effect of enalaprilat on blood pressure, because cardiac index rose, with no further increase in total peripheral conductance. In contrast, the antihypertensive effect of MDL 100,240 persisted, in spite of a recovery in cardiac index, because there was further vasodilatation, particularly in the mesenteric and hindquarters vascular beds.

4 There were no apparent haemodynamic changes associated with the injection of Hoe 140, and over the following 8 h, the difference between the haemodynamic effects of enalaprilat and MDL 100,240 persisted; there was little evidence of suppression of the effects of either drug.

5 These results are more consistent with the antihypertensive effects of enalaprilat or MDL 100,240 in transgenic ((mRen-2)27) rats being due to suppression of angiotensin II production, than due to inhibition of bradykinin degradation. The additional effects of MDL 100,240 may be accounted for by inhibition of the degradation of natriuretic peptides reducing cardiac output, initially, and decreasing vascular tone, subsequently. Alternatively, the additional increase in vascular conductance following treatment with MDL 100,240 may represent an autoregulatory response to the reduced pressure.

Keywords: MDL 100,240; dual metallopeptidase inhibitor; enalaprilat; transgenic rats

Introduction

The renin angiotensin system is importantly involved in several aspects of cardiovascular regulation. The metallopeptidase, angiotensin-converting enzyme (ACE), is responsible for the production of angiotensin II; in addition, ACE is involved in bradykinin degradation (Erdös, 1990). Another metallopeptidase, neutral endopeptidase (NEP), cleaves various short linear or cyclic peptides, such as the natriuretic peptides, angiotensin II and bradykinin (Roques *et al*., 1993). Inhibitors of NEP were developed as potential antihypertensive agents due to their ability to inhibit the breakdown of atrial natriuretic peptide, but their effectiveness is limited by the natural counter-regulatory effects of the renin-angiotensin system – an influence possibly exacerbated by the inhibition of angiotensin II degradation (Richards *et al*., 1992). Thus, combined inhibition of NEP and ACE is believed to be a more effective therapeutic strategy than inhibition of either enzyme alone (Seymour *et al*., 1991).

There is evidence that combined inhibition of ACE and NEP may lower blood pressure and improve renal function in experimental hypertensive models in which ACE or NEP inhibitors, alone, may be relatively ineffective (e.g., Seymour *et al*., 1991; Pham *et al*., 1993; 1995; French *et al*., 1995; Vera *et al*., 1995; Trippodo *et al*., 1995b). However, it is less clear if combined inhibition of ACE and NEP confers added advantage over ACE or NEP inhibitors in those models susceptible to either inhibitor alone. Moreover, in the models of experimental hypertension investigated so far, comparative studies of the effects of ACE and/or NEP inhibition have only been carried out over the very short-term (up to 2 h), and have not involved detailed assessment of haemodynamic changes. Therefore, the main objective of this study was to compare the regional and cardiac haemodynamic effects of prolonged infusions of the combined ACE and NEP inhibitor, MDL 100,240 (French *et al*., 1994), with those of the ACE inhibitor, enalaprilat. We chose enalaprilat because of its substantial haemodynamic effects under conditions in which the renin-angiotensin system is activated (Muller *et al*., 1990).

In 1990, Mullins and co-workers described a hypertensive, transgenic rat strain ((mRen-2)27) which was produced by transfecting the mouse *Ren-2* gene into the genome of the Sprague-Dawley rat (Mullins *et al*., 1990). The physiological characteristics of these animals have recently been reviewed (Lee *et al*., 1996). Homozygous ((mRen-2)27) rats develop fulminant hypertension at an early age and have a relatively short life-span, but, in the heterozygotes, the hypertension is less severe and their life span is normal without treatment. There is high expression of the transgene in a number of tissues, especially the adrenal gland, but plasma and renal renin

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levels may not be elevated (Lee *et al*., 1996). The precise mechanisms underlying the hypertension are unclear. However, several groups of workers have shown that blood pressure in hypertensive, transgenic ((mRen-2)27) rats is markedly reduced by ACE inhibitors or angiotensin (AT_1)-receptor antagonists (e.g., Mullins *et al*., 1990; Bader *et al*., 1992; Hirth-Dietrich *et al*., 1994; Moriguchi *et al*., 1994). We have shown that the antihypertensive action of the latter is associated with significant increases in renal and mesenteric flows, and vasodilatation in renal, mesenteric and hindquarters vascular beds (Gardiner *et al*., 1995), but the detailed haemodynamic effects of ACE inhibition in hypertensive transgenic ((mRen-2)27 rats are unknown. We have found that the non-selective endothelin antagonist, SB 209670, causes a fall in blood pressure, together with increases in renal, mesenteric and hindquarters flows and vascular conductances in hypertensive, transgenic ((mRen-2)27) rats (Gardiner *et al*., 1995). In the same model, we found that the cardiovascular effects of SB 209670 were additive with those of losartan (AT_1-receptor antagonist), consistent with independent, but interactive, roles for angiotensin II and endothelin in the maintenance of cardiovascular status in the transgenic hypertensive rats (Gardiner *et al*., 1995). Since the conversion of big endothelin-1 to endothelin-1 is mediated by a family of metalloprotease enzymes related to NEP (see Webb, 1997) and we have shown that MDL 100,240 is able to suppress the pressor effects of big endothelin-1 (Gardiner *et al*., 1997), it is feasible that this property would enhance its cardiovascular effects in transgenic hypertensive rats, relative to an ACE inhibitor. Therefore, we compared the effects of MDL 100,240 and enalaprilat in these animals.

In other experimental models it has been claimed that bradykinin may contribute to the effects of ACE inhibition (e.g., Benetos *et al*., 1986; Carbonell *et al*., 1988; Danckwardt *et al*., 1990; Bao *et al*., 1992; Panzenbeck *et al*., 1995; Chen *et al*., 1996). Since MDL 100,240 inhibits NEP, as well as ACE, and both enzymes are involved in the degradation of bradykinin (see above), it is feasible that the latter would contribute more to the haemodynamic effects of MDL 100,240 than to those of an ACE inhibitor. So, another objective of this study was to assess the influence of the bradykinin, B_2-receptor antagonist, Hoe 140 (Wirth *et al*., 1991), on regional and on cardiac haemodynamics, 24 h after the onset of infusion of MDL 100,240 or enalaprilat.

Thus, collectively, the hypotheses to be tested in this study were that: (i) in hypertensive, transgenic ((mRen-2)27) rats, combined inhibition of ACE and NEP (with MDL 100,240) would cause greater haemodynamic effects than ACE inhibition alone (with enalaprilat), and, (ii) a vascular action of bradykinin (due to inhibition of its degradation) would contribute more to the antihypertensive effects of MDL 100,240 than to those of enalaprilat.

Methods

Experiments were carried out on male, heterozygous, hypertensive, transgenic ((mRen-2)27) rats (350–450 g), bred in the Biomedical Services Unit in Nottingham. (We are very grateful to Dr J J Mullins (Genome Research Centre, Edinburgh) for giving us the breeding stock). All surgery was carried out under sodium methohexitone anaesthesia (Brietal, Lilly; 40–60 mg kg^{-1}, i.p., supplemented as required).

Regional haemodynamics

Seven to 14 days before the study, animals had miniature, pulsed Doppler probes implanted around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor hindquarters flow) (Gardiner *et al*., 1995). Following surgery, animals were given ampicillin (Penbritin, Beecham 7 mg kg^{-1}, i.m.) and returned to individual home cages with free access to tap water and food. Twenty-four hours before experiments were begun, intravenous catheters (right jugular vein; 1 for MDL 100,240 or enalaprilat, and 1 for Hoe 140) and 1 intra-arterial catheter (distal abdominal aorta via ventral caudal artery) were implanted. Catheters were kept patent by continuous infusion of heparin-treated (15 u ml^{-1}, 0.4 ml h^{-1}) saline through a swivel system.

Cardiac haemodynamics

Five to 7 days before the study, an electromagnetic flow probe (Skalar, Delft) was implanted around the ascending aorta via a transthoracic approach (Gardiner *et al*., 1990; 1995). At least 4 days later, animals were briefly anaesthetized (sodium methohexitone 40 mg kg^{-1}, i.p.) and had an intra-arterial catheter implanted in the distal abdominal aorta (via the ventral caudal artery), and catheters implanted in the right jugular vein, 1 for administration of enalaprilat or MDL 100,240, 1 for Hoe 140, and 1 fashioned and positioned for recording central venous pressure (Gardiner *et al*., 1990). Animals were allowed to recover for at least 24 h before experiments were begun.

Experimental protocol

After a 30 min baseline recording (beginning at 07 h 00 min) a continuous infusion of MDL 100,240 or enalaprilat (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1}) was started and continued for 32 h. After 24 h, a bolus injection of Hoe 140 (1 mg kg^{-1}, Bao *et al*., 1991) was given. The dose of MDL 100,240 was based on our previous experiments (Gardiner *et al*., 1997) showing it abolished the effects of angiotensin I (250 pmol kg^{-1}) while causing marked enhancement of the action of bradykinin (3 nmol kg^{-1}). The dose of enalaprilat was supramaximal for functional ACE inhibition (Muller *et al*., 1990). At the end of the protocol (i.e., after infusion of MDL 100,240 or enalaprilat for 32 h and 8 h after injection of Hoe 140), we confirmed that the cardiovascular effects of angiotensin I (250 pmol kg^{-1}) and bradykinin (3 nmol kg^{-1}) were abolished.

Data analysis

We made recordings of all variables for 15 min across each 1 h period from 1 to 8 h and 24 to 32 h after the beginning of the experiment. For regional haemodynamics, recordings were made of phasic and mean intra-arterial blood pressures, instantaneous heart rate, and phasic and mean Doppler shift signals from the renal, mesenteric and hindquarters probes.

Cardiac haemodynamic data (mean thoracic aortic flow (i.e., cardiac output), peak thoracic aortic flow, maximum rate of rise of aortic flow (dF/dt_{max}), instantaneous heart rate, mean arterial pressure, central venous pressure, stroke volume and total peripheral conductance) were digitized by a custom-built microprocessor and stored on disk for off-line analysis (Gardiner *et al*., 1990). All variables, except heart rate, mean arterial and central venous pressures were factored by body weight (i.e., cardiac index = mean thoracic aortic flow 100 g^{-1}; stroke index = stroke volume 100 g^{-1}).

Within-group statistical analysis was carried out by use of Friedman's test, and between-group analysis was done with the Mann-Whitney U test applied to individual time points or areas under or over curves (AUC or AOC, respectively).

Drugs

MDL 100,240 ([4S-[4α, 7α (R*), 12β]]-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) (M.Wt., 480.59) was obtained from Marion Merrell Dow, enalaprilat (M.Wt., 348.5) was a gift from Merck Sharp & Dohme, and Hoe 140 ([D-Arg^0, Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) was a gift from Hoechst. In order to match the infusates, both MDL 100,240 and enalaprilat were dissolved in 1% $NaHCO_3$, 1% enthanol in isotonic saline (150 mmol l^{-1} NaCl) (French *et al*., 1994); Hoe 140 was dissolved in isotonic saline. Bolus doses of

MDL 100,240 or enalaprilat were given in 0.2 ml and the infusion rate was 0.4 ml h^{-1}.

Results

Regional haemodynamics

There were no significant differences between resting cardiovascular variables before the administration of MDL 100,240 or enalaprilat (Table 1).

During the first 8 h of infusion of MDL 100,240 or enalaprilat, there were significant falls in mean arterial pressure (MAP), but the effect of MDL 100,240 on blood pressure was significantly greater than that of enalaprilat (Figure 1). The tachycardic effect of MDL 100,240 was also more sustained than that of enalaprilat (Figure 1). Between 8 and 24 h after the onset of infusion there was a significant recovery of MAP in the presence of enalaprilat, but not in the presence of MDL 100,240 (Figure 1); there was no tachycardia at this stage in either group (Figure 1).

During the first 8 h of infusion, enalaprilat caused a significantly greater increase in renal flow than MDL 100,240, but the increases in renal vascular conductance were not significantly different (Figure 1). Although between 8 and 24 h the renal haemodynamic effects of MDL 100,240 and those of enalaprilat tended to wane, there was still a significant and similar renal vasodilatation 24 h after the onset of MDL 100,240 or enalaprilat (Figure 1).

The patterns of change in mesenteric flow were somewhat variable during the first 8 h infusion of MDL 100,240 or enalaprilat, but there were progressive increases in vascular conductance in both groups (Figure 1). By 24 h after the onset of infusion, MDL 100,240 and enalaprilat had caused significant increases in mesenteric flow and vascular conductance. However, the overall increase in mesenteric vascular conductance was greater with MDL 100,240 than with enalaprilat (Figure 1).

Over the first 8 h infusion of MDL 100,240, hindquarters flow fell; although no such reduction was seen with enalaprilat, there was no significant difference between the changes in the two groups and both showed similar and significant increases in hindquarters vascular conductance (Figure 1). Between 8 and 24 h of infusion, there were clear increases in hindquarters flow associated with vasodilatation in the presence of MDL 100,240 or enalaprilat (Figure 1), but the overall increase in hindquarters vascular conductance was greater with MDL 100,240 than with enalaprilat (Figure 1).

Effects of Hoe 140

There were no significant changes in cardiovascular variables over the 8 h following injection of Hoe 140 in those animals being infused with enalaprilat (Figure 2). In the presence of MDL 100,240, there was a slight, delayed and transient rise in MAP associated with transient reductions in renal vascular conductance, and mesenteric flow and vascular conductance (Figure 2). There were earlier reductions in hindquarters flow and vascular conductance (Figure 2). However, the integrated changes in cardiovascular variables over the 8 h following injection of Hoe 140 were not different in the animals being infused with MDL 100,240 compared to those receiving enalaprilat.

Thirty-two hours after the onset of MDL 100,240 or enalaprilat and 8 h after injection of Hoe 140, injection of angiotensin I (250 pmol kg^{-1}) or bradykinin (3 nmol kg^{-1}) had no consistent haemodynamic effects (data not shown).

Table 1 Resting regional haemodynamic variables in conscious, transgenic, hypertensive rats before the administration of MDL 100,240 or enalaprilat

	MDL 100,240	*Enalaprilat*
Heart rate (beats min^{-1})	363±13	343±11
Mean arterial blood pressure (mmHg)	176±5	173±6
Renal Doppler shift (kHz)	5.7±0.4	5.8±0.5
Mesenteric Doppler shift (kHz)	6.4±0.6	6.0±0.4
Hindquarters Doppler shift (kHz)	4.5±0.4	4.4±0.3
Renal vascular conductance ([kHz $mmHg^{-1}$]10^3)	32±3	34±4
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	37±3	35±3
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	26±3	26±2

Values are mean±s.e.mean ($n=8$).

Cardiac haemodynamics

There were no significant differences between resting cardiovascular variables before the administration of MDL 100,240 or enalaprilat (Table 2).

As observed with the animals instrumented for the assessment of regional haemodynamics (see above), the effects of

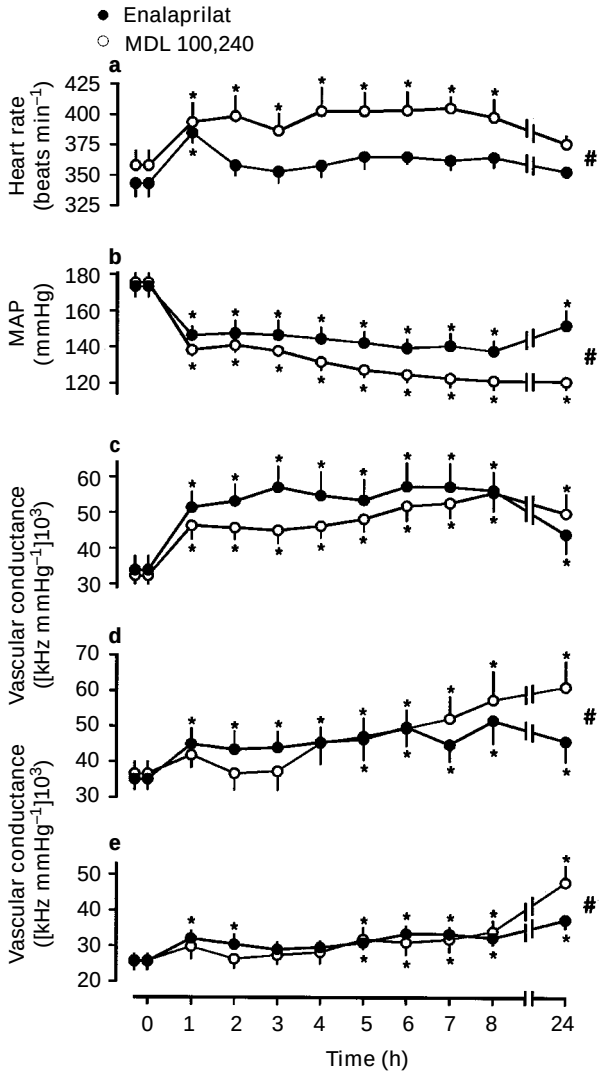

Figure 1 Regional cardiovascular variables ((a) heart rate, (b) mean arterial pressure (MAP), (c) renal, (d) mesenteric and (e) hindquarters vascular conductance) in conscious, transgenic, hypertensive rats at rest and during 24 h infusion of MDL 100,240 (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$) or enalaprilat (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$). Values are mean, and vertical lines s.e.mean; $^*P<0.05$ versus baseline. Differences between groups are given in the text.

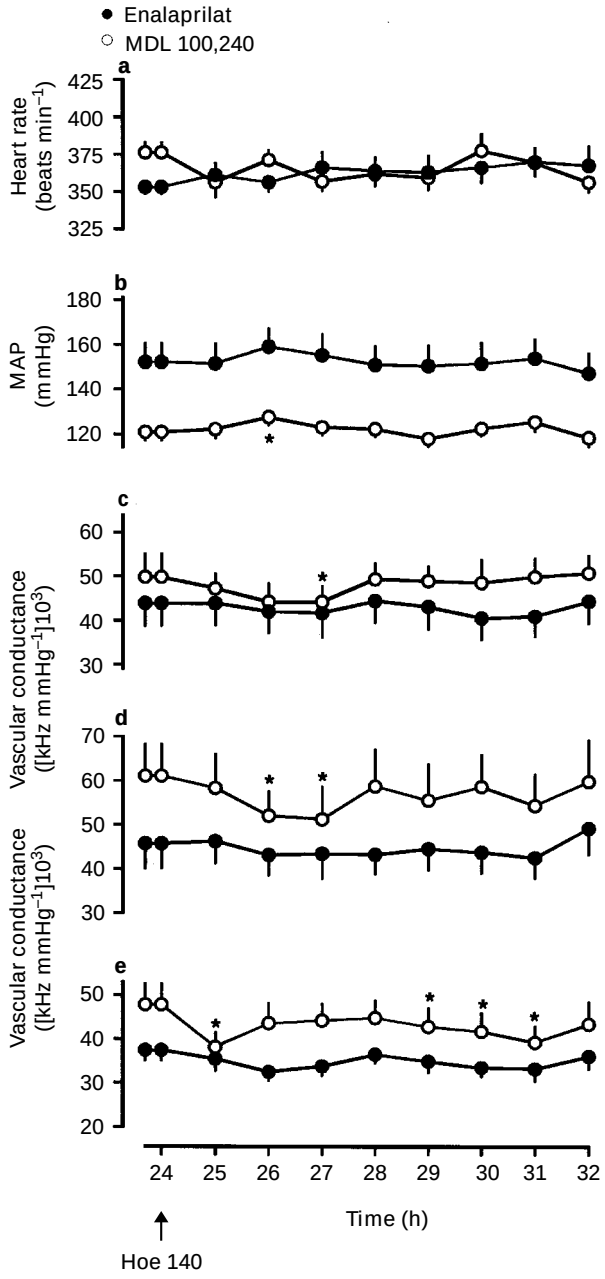

Figure 2 Regional cardiovascular variables ((a) heart rate, (b) MAP, (c) renal, (d) mesenteric and (e) hindquarters vascular conductance) in conscious, transgenic, hypertensive rats between 24 h and 32 h after the onset of infusion of MDL 100,240 (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$) or enalaprilat (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$). At the 24 h time point a bolus injection of Hoe 140 (1 mg kg^{-1}) was given. Values are mean and vertical lines s.e.mean; $^*P<0.05$ versus pre-Hoe 140 value.

MDL 100,240 on blood pressure and heart rate were greater than those of enalaprilat, with a recovery in MAP between 8 and 24 h after onset of infusion of enalaprilat, but not with MDL 100,240 (Figure 3).

Over the first 8 h infusion of both compounds, there were reductions in cardiac index, but this effect was greater with MDL 100,240 than with enalaprilat (Figure 3). By 24 h after the onset of infusion, cardiac index was not reduced in the presence of MDL 100,240, and was increased slightly with enalaprilat, but there was no significant difference between the two groups (Figure 3).

During the first hour of infusion of enalaprilat, total peripheral conductance increased and remained so thereafter (Figure 3). Although the increase in total peripheral conduc-

Table 2 Resting cardiac haemodynamic variables in conscious, transgenic, hypertensive rats before the administration of MDL 100,240 or enalaprilat

	MDL 100,240	*Enalaprilat*
Heart rate (beats min^{-1})	403±16	393±9
Mean arterial blood pressure (mmHg)	166±8	167±6
Cardiac index (ml min^{-1} 100 g^{-1})	31.5±2.2	31.3±0.9
Total peripheral conductance (μl min^{-1} mmHg^{-1} 100 g^{-1})	192±14	189±11
Stroke index (μl min^{-1} 100 g^{-1})	80±7	80±3
Peak aortic flow (ml min^{-1} 100 g^{-1})	119±8	119±4
dF/dt$_{max}$ (1 min^{-2} 100 g^{-1})	441±42	475±24
Central venous pressure (cmH_2O)	4.3±0.6	4.7±0.4

Values are mean±s.e.mean ($n=8$).

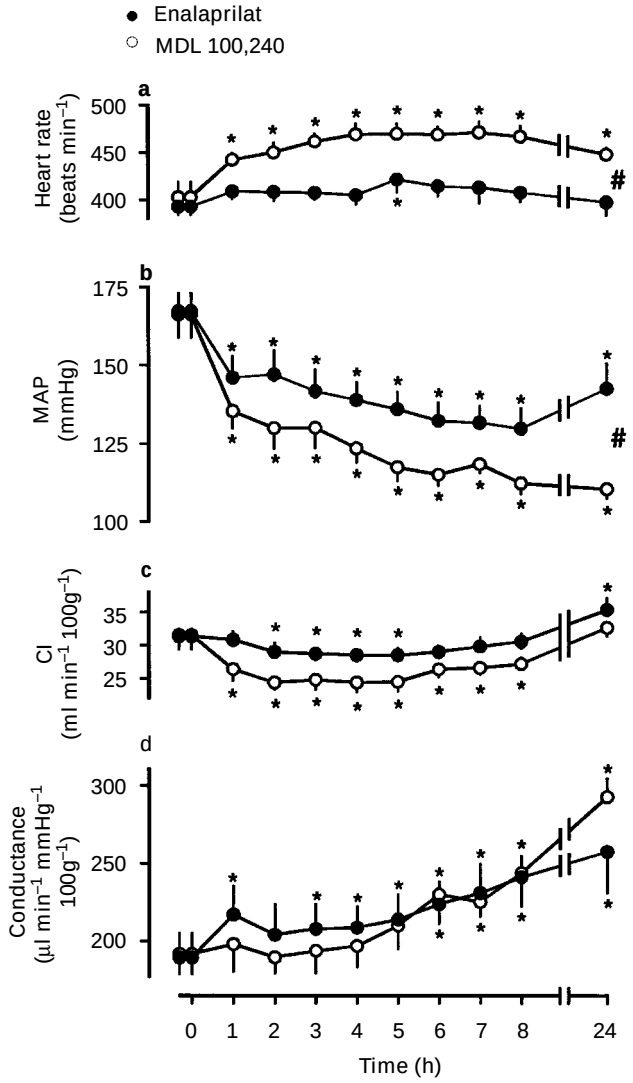

Figure 3 Central haemodynamic variables ((a) heart rate, (b) MAP, (c) cardiac index (CI) and (d) total peripheral conductance) in conscious, transgenic, hypertensive rats at rest and during 24 h infusion of MDL 100,240 (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$) or enalaprilat (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$). Values are mean, and vertical lines s.e.mean; $^*P<0.05$ versus baseline. Differences between groups are given in the text.

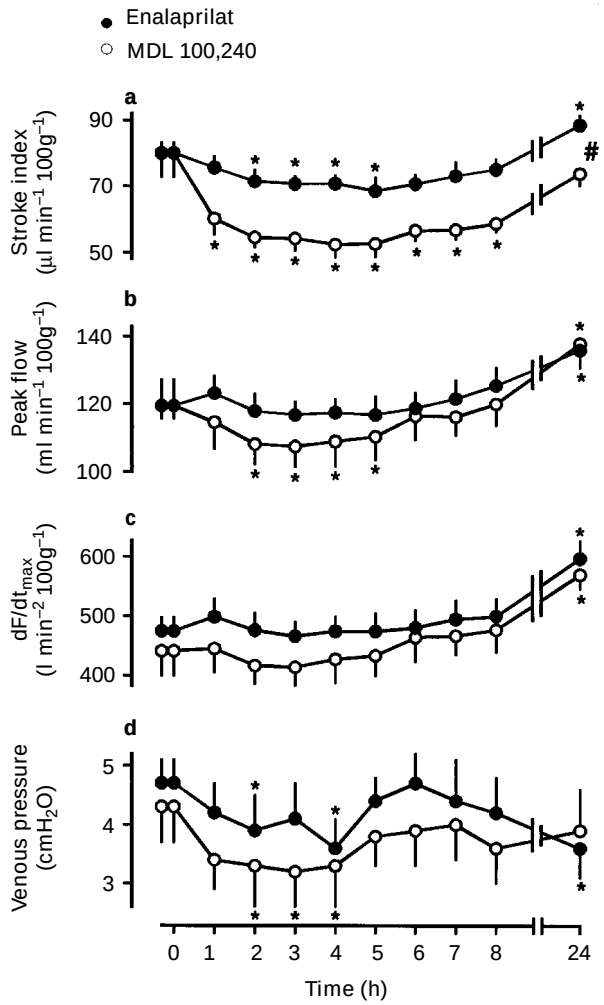

Figure 4 Central haemodynamic variables ((a) stroke index, (b) peak flow, (c) dF/dt_{max} and (d) central venous pressure) in conscious, transgenic, hypertensive rats at rest and during 24 h infusion of MDL 100,240 (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$) or enalaprilat (3 mg kg^{-1} h^{-1} infusion; $n=8$). Values are mean, and vertical lines s.e.mean; $^*P<0.05$ versus baseline. Differences between groups are given in the text.

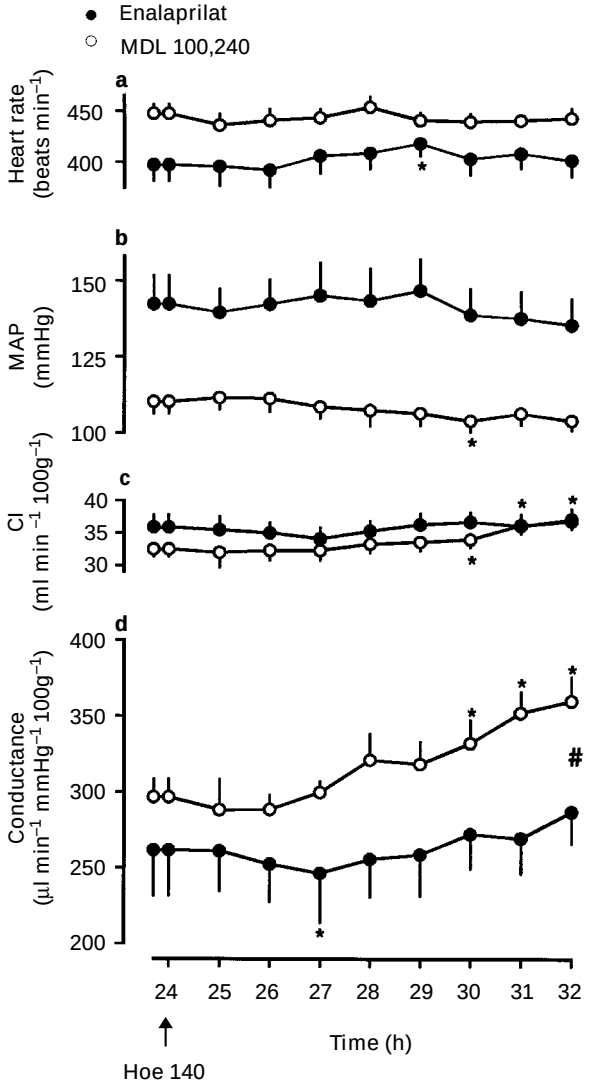

Figure 5 Central haemodynamic variables ((a) heart rate, (b) MAP, (c) cardiac index (CI) and (d) total peripheral conductance) in conscious, transgenic, hypertensive rats between 24 h and 32 h after the onset of infusion of MDL 100,240 (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$) or enalaprilat (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$). At the 24 h time point a bolus injection of Hoe 140 (1 mg kg^{-1}) was given. Values are mean and vertical lines s.e.mean; $^*P<0.05$ versus pre-Hoe 140 value.

tance developed more slowly during infusion of MDL 100,240, between 8 and 24 h it showed a marked increase such that, 24 h after the onset of infusion, the difference between the total peripheral conductance in the two groups was on the borderline of significance ($P = 0.052$) (Figure 3).

Over the first 8 h of infusion, there were reductions in stroke index in both groups, but this effect was greater and more sustained in the animals receiving MDL 100,240 (Figure 4). However, 24 h after the onset of infusion of MDL 100,240 stroke index was no longer reduced, although it was less than in the group receiving enalaprilat, in which there was a modest increase (Figure 4).

Peak aortic flow fell during the first 8 h of infusion of MDL 100,240, but did not change with enalaprilat. However, there was no significant difference between the two groups, and 24 h after the onset of infusion, both showed a slight elevation in peak aortic flow (Figure 4). A similar overall pattern of change was seen with dF/dt_{max} (Figure 4).

In both groups, there were reductions in central venous pressure during the first 8 h of infusion, but thereafter the slight changes were variable (Figure 4).

Effects of Hoe 140

During the 8 h infusion of enalaprilat, following bolus injections of Hoe 140, there were slight changes in some cardiac haemodynamic variables, but these did not appear to be related to the administration of Hoe 140 (Figures 5 and 6).

Generally, a similar picture was seen in the animals infused with MDL 100,240 for 8 h, after injection of Hoe 140 (Figures 5 and 6). However, in this group there was a slowly developing increase in total peripheral conductance which was significantly greater than that seen in the animals infused with enalaprilat after Hoe 140 (Figure 5). Thus, by the end of the experiment, total peripheral conductance was significantly higher in the group receiving MDL 100,240 compared to that given enalaprilat (Figure 5).

Discussion

The results of the present study clearly show that MDL 100,240 has a more marked and sustained antihypertensive effect than enalaprilat in conscious, hypertensive, transgenic ((mRen-2)27)rats. Since both drugs were dissolved in the same vehicle and given by primed, continuous i.v. infusion throughout the study, it is unlikely that differences in pharmacokinetics explain the results obtained. In addition, it is

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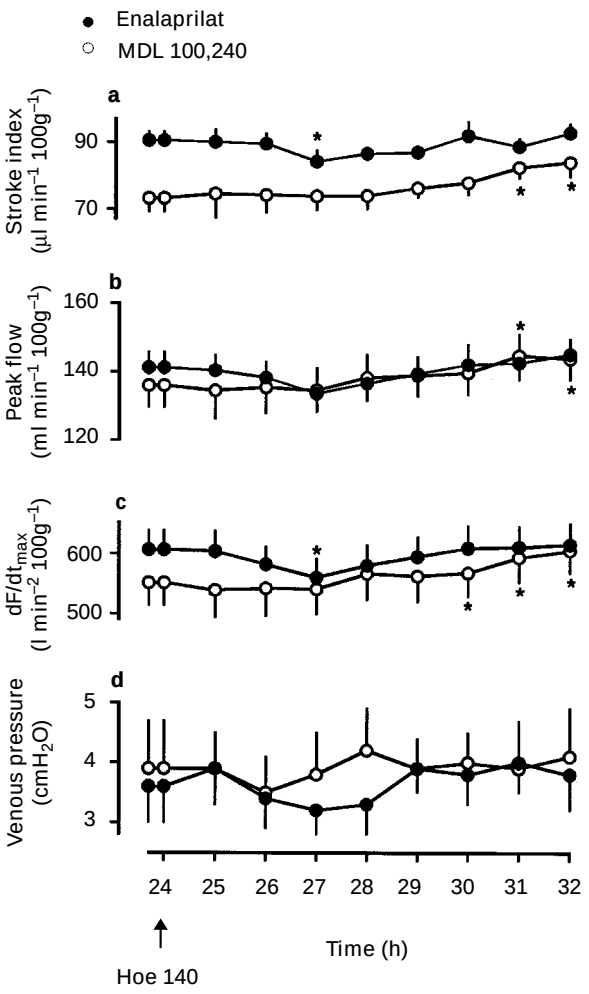

Figure 6 Central haemodynamic variables ((a) stroke index, (b) peak flow, (c) dF/dt_{max} and (d) central venous pressure) in conscious, transgenic, hypertensive rats between 24 h and 32 h after the onset of infusion of MDL 100,240 (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$) or enalaprilat (3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion; $n=8$). At the 24 h time point a bolus injection of Hoe 140 (1 mg kg^{-1}) was given. Values are mean and vertical lines s.e.mean; $^*P<0.05$ versus pre-Hoe 140 value.

unlikely that a lesser inhibition of ACE by enalaprilat than by MDL 100,240 underlies their differential effects on blood pressure, because the dose of enalaprilat used is supramaximal for functional ACE inhibition (Muller *et al*., 1990). Moreover, at the end of the experiment there were no consistent effects of exogenous AI in either group of animals, indicating substantial ACE inhibition.

Apart from the difference in the initial blood pressure lowering effects of the drugs, there was a significant recovery in MAP between 8–24 h after the onset of infusion of enalaprilat that was not seen with MDL 100,240. Whatever the explanation of this difference it was not progressive, since from 24–32 h MAP was relatively steady in the presence of enalaprilat, despite the administration of Hoe 140 (see below). The profile of change in MAP during the first 8 h of infusion of enalaprilat was similar to that seen following administration of the AT_1-receptor antagonist, losartan (Gardiner *et al*., 1995), indicating that the effect was due to suppression of the vasoconstrictor actions of angiotensin II. Thus, increased levels of angiotensin I, and/or angiotensin(1–7), and/or prostacyclin (Luque *et al*., 1996) during infusion of enalaprilat are unlikely to have been involved in its antihypertensive effect, or the regression thereof.

The greater effect of MDL 100,240 on MAP was accompanied by an enhanced tachycardia relative to enalaprilat, consistent with a component being due to baroreflex mechanisms. The MDL 100,240-induced tachycardia was associated with a significantly greater fall in cardiac and stroke index than seen with enalaprilat, and over the first 8 h of infusion this accounted for the greater hypotensive effect of MDL 100,240 on MAP, since the increases in renal, mesenteric and hindquarters vascular conductances, and of total peripheral conductance were similar with both drugs. The greater fall in cardiac index with MDL 100,240 is consistent with an inhibition of the degradation of atrial natriuretic peptide (ANP), or the other natriuretic peptides (BNP, CNP; Norman *et al*., 1991; Seymour *et al*., 1992; Kenny *et al*., 1993). Several groups have shown the blood pressure lowering effects of ANP to be due to a reduction in cardiac output by a variety of mechanisms (see Lappe, 1988) However, the difference in cardiac index did not persist and after 24 h of infusion the more marked antihypertensive effect of MDL 100,240 was associated with greater vasodilatation, particularly in the mesenteric and hindquarters vascular beds. It is feasible that this was due to local effects of ANP, since it has been shown that an antagonist of natriuretic peptide A and B receptors causes coronary vasoconstriction, but no change in renal haemodynamics (Stevens *et al*., 1994; Supaporn *et al*., 1996). However, an ability of MDL 100,240 to inhibit NEP and thereby influence the metabolism of angiotensin I, endothelin, big endothelin, substance P, calcitonin gene-related peptide (CGRP), vasopressin etc., cannot be excluded. With regard to big endothelin–1 and the ability of MDL 100,240 to suppress its cardiovascular effects (Gardiner *et al*., 1997), it is notable that, although the blood pressure lowering effects of MDL 100,240 were similar to those of a combination of losartan and the non-selective endothelin antagonist, SB 209670, in transgenic rats (Gardiner *et al*., 1995), the latter combination caused more marked regional vasodilatations. Hence, a putative ability of MDL 100,240 to suppress processing of big endothelin-1 may contribute less to its antihypertensive action than an influence on plasma volume through ANP, at least during the first 8 h of infusion. Although it is feasible that the early effects of MDL 100,240 on MAP were mediated through a negative inotropic effect of ANP (Supaporn *et al*., 1996), and/or venodilatation (Trippodo *et al*., 1995a), the changes in peak aortic flow, dF/dt_{max} and central venous pressure were similar in the presence of enalaprilat and MDL 100,240, indicating that any changes in preload or contractility were not different.

Twenty-four hours after the onset of infusion of enalaprilat or MDL 100,240, bolus i.v. injection of Hoe 140 had only slight and variable effects on cardiac and regional haemodynamics. Hence, it appears that differences in inhibition of bradykinin degradation did not account for the differences in the haemodynamic effects of MDL 100,240 and enalaprilat. Furthermore, it appears that bradykinin was not involved in the haemodynamic effects of either drug, with the possible exception of a small component of the hindquarters vasodilator effect of MDL 100,240 (see Figure 2). Considering the disparities in the literature on the involvement of bradykinin in the cardiovascular effects of ACE and/or NEP inhibitors (e.g., Panzenbeck *et al*., 1995; Vera *et al*., 1995; Trippodo *et al*., 1995a; Pham *et al*., 1996; Chen *et al*., 1996), it is likely conclusions depend on the experimental model considered.

During the last 3 h of the 8 h infusion of MDL 100,240, after the administration of Hoe 140, there was a slight, but significant further increase in total peripheral conductance. This was temporarily dissociated from the injection of Hoe 140 and, hence, probably reflected a progressive vasodilator action of MDL 100,240. Although the increase in total peripheral conductance was not accompanied by significant increases in renal, mesenteric or hindquarters vascular conductance, there was a tendency towards additional vasodilatation in the renal and mesenteric beds. Hence, the rise in total peripheral conductance was probably due to cumulative, small effects in

several vascular beds including those not monitored directly in the present study. Additional experiments would be required to determine if infusion of MDL 100,240 for periods longer than 32 h would exert increasing cardiovascular effects. Nonetheless, the present findings indicate that MDL 100,240 has more marked and persistent hypotensive effects than enalaprilat in hypertensive transgenic rats and this difference is not due to any persistent reduction in cardiac function, or to any apparent involvement of bradykinin.

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