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Effect of chronic ET_A-selective endothelin receptor antagonism on blood pressure in experimental and genetic hypertension in rats

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1 Chronic treatment with a combined ET_A/ET_B endothelin receptor antagonist has been shown to reduce blood pressure in experimental rat models of hypertension in which endothelin-1 gene overexpression occurs in the walls of blood vessels, particularly small, resistance-sized arteries, but not in those genetic or experimental models of hypertension in which there is no overexpression of vascular endothelin-1. Failure of some experimental models of hypertension to respond to treatment with the combined ET_A/ET_B endothelin antagonist may be due in part to blockade of vasorelaxant endothelial ET_B receptors which could in theory reduce the efficacy of endothelin antagonism.

2 In this study the orally active ET_A-selective endothelin antagonists A-127722.5 and LU 135252 were used in chronic experiments on deoxycorticosterone acetate (DOCA)-salt hypertensive rats (which overexpress vascular endothelin-1 and respond with blood pressure lowering to combined ET_A/ET_B endothelin receptor antagonism), on spontaneously hypertensive rats (SHR) (which do not overexpress vascular endothelin-1 and do not respond with blood pressure lowering to the combined ET_A/ET_B receptor antagonist), and in 1-kidney 1 clip Goldblatt (1-K 1C) hypertensive rats (which present mild overexpression of vascular endothelin-1 but do not respond with blood pressure lowering to the combined ET_A/ET_B receptor antagonist). Additionally, it has been suggested that interruption of the renin-angiotensin system may sensitize responses to endothelin antagonism. Accordingly, SHR were treated with an angiotensin converting enzyme inhibitor, cilazapril, in addition to the ET_A receptor antagonist.

3 Blood pressure of DOCA-salt hypertensive rats was lowered by a mean of 24 and of 27 mmHg ($P<0.01$) by A-127722.5 after 4 weeks of treatment, when given orally at two different doses (10 and 30 mg kg^{-1} day^{-1}), and by 18 mmHg by LU 135252 50 mg kg^{-1} day^{-1}.

4 SHR treated with A-127722.5 for 8 weeks starting at 12 weeks of age exhibited the same progressive rise in blood pressure as untreated SHR. Addition of cilazapril resulted in similar reduction of blood pressure in A-127722.5-treated and untreated SHR.

5 Treatment of 1-K 1C hypertensive rats with the dose of LU 135252 which lowered blood pressure in DOCA-salt hypertensive rats did not cause any reduction in blood pressure relative to untreated rats.

6 These results demonstrate that treatment with either dose of the selective ET_A receptor antagonists A-127722.5 or LU 135252 caused reductions in blood pressure similar to those obtained for a combined ET_A/ET_B endothelin antagonist. Blood pressure was lowered only in hypertensive rats known to overexpress vascular endothelin-1 (DOCA-salt hypertensive rats) but not in those which do not (SHR) or only have mild vascular overexpression of endothelin-1 gene (1-K 1C hypertensive rats). Reduction in activity of the renin-angiotensin system in SHR did not sensitize blood pressure to potential hypotensive effects of an ET_A-selective receptor antagonist.

Keywords: Spontaneously hypertensive rats (SHR); DOCA-salt hypertensive rats; one-kidney one clip Goldblatt hypertensive rats; angiotensin converting enzyme inhibition; renin-angiotensin system; blood vessels; endothelin-1 gene expression; A-127722.5; LU 135252

Introduction

Endothelins are 21 amino acid peptides with a variety of physiological actions which are produced ubiquitously, in different tissues. Endothelin-1, the endothelin originally discovered and produced by vascular endothelium, has been implicated in blood pressure elevation through its potent vasoconstrictor effects (Yanagisawa *et al*., 1988), and possibly via its potential growth inducing action (Bobik *et al*., 1990; Chua *et al*., 1992; Schiffers *et al*., 1994). However, the role of endothelins in hypertension remains unclear (Lüscher *et al*., 1993; Vanhoutte, 1993; Schiffrin, 1995). Enhanced expression of endothelin-1 within the endothelium (Larivière *et al*., 1993a; Day *et al*., 1995; Schiffrin *et al*., 1995a) together with a hypotensive effect of endothelin antagonists has suggested that some hypertensive rats such as deoxycorticosterone acetate (DOCA)-salt hypertensive rats (Li *et al*., 1994; Bird *et al*., 1995) and spontaneously hypertensive rats (SHR) treated with DOCA (Schiffrin *et al*., 1995b) may have a form of hypertension which is in part endothelin-1-dependent (Schiffrin, 1995). In contrast, SHR (Larivière *et al*., 1995; Schiffrin *et al*., 1995a) do not exhibit enhanced endothelin-1 expression in blood vessels, and neither acute (Bird *et al*., 1995) nor chronic treatment (Li & Schiffrin, 1995a,b) with endothelin antagonists lower blood pressure. One-kidney one clip (1-K 1C) Goldblatt hypertensive rats exhibit a mild degree of vascular overexpression of endothelin-1 (Sventek *et al*., 1996b), but treatment with bosentan does not cause any lowering of blood pressure (Li *et al*., 1996). Chronic endothelin antagonism has only been tested until now with the orally active combined ET_A/ET_B endothelin receptor antagonist, bosentan (Clozel *et al*., 1994).

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Since combined ET_A/ET_B endothelin antagonism may block both smooth muscle vasoconstrictor ET_A and ET_B endothelin receptors and endothelial vasorelaxant ET_B endothelin receptors, the possibility exists that the endothelin receptor antagonist which blocks both receptor subtypes may be less effective than a selective ET_A endothelin receptor antagonist. This could possibly account, for example, for the failure of chronically applied bosentan to lower blood pressure in SHR (Li & Schiffrin, 1995a,b), in contrast to the effect of acute intravenous infusion of an ET_A endothelin receptor antagonist (Bazil *et al*., 1992).

In this study the orally active, selective ET_A endothelin receptor antagonists A-127722.5 *trans-trans*-2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-((N,N-dibutylamino)carbonylmethyl) pyrrolidine-3-carboxylic acid (Opgenorth *et al*., 1996) and LU 135252 (+)-(S)-2-(4,6-dimethyoxy-pyrimidin-2-yloxy)-3-methoxy-3,3 diphenyl-propionic acid (Müenter *et al*., 1996; Riechers *et al*., 1996) were given for 4 weeks to DOCA-salt hypertensive rats, SHR and 1-K 1C Goldblatt hypertensive rats, in order to compare the blood pressure lowering effects of these agents with those of bosentan, that we have previously demonstrated (Li *et al*., 1994; Li & Schiffrin, 1995a,b; Schiffrin *et al*., 1995b). Additionally, because it has been suggested that inhibition of the renin-angiotensin system may potentiate the response to endothelin receptor antagonism (Richard *et al*., 1995), the angiotensin I-converting enzyme inhibitor cilazapril, which is effective in reducing blood pressure in SHR, was administered to these rats together with an endothelin receptor antagonist, in order to establish whether it would enhance the effects of the latter.

Methods

Animal experiments

The protocol was approved by the Animal Care Committee of the Clinical Research Institute of Montreal and followed the recommendations of the Canadian Council for Animal Care. Rats were exposed to a 12 h light:dark cycle, an ambient temperature of 22°C and humidity of 60%. DOCA-salt hypertension was induced by the method of Ormsbee and Ryan (1973). Male Sprague-Dawley rats (Charles River Laboratories, St-Constant, Quebec) were unilaterally nephrectomized under anaesthesia with sodium pentobarbitone. Silicone rubber impregnated with DOCA, 200 mg per rat, was implanted, and rats were offered 1% saline to drink. Starting the day of surgery rats received either vehicle or A-127722.5 (a low dose of 10 mg kg^{-1} weight day^{-1} or a high dose of 30 mg kg^{-1} day^{-1}) in their drinking water. A-127722.5 was dissolved in 95% ethanol, 60 mg ml^{-1}, and then diluted in tap water to the desired concentration. The concentration of drug in the drinking water was adjusted daily to ensure that the full dose of drug was received. The low dose of A-127722.5 when given by gavage blocks the action of pressor doses of intravenously injected endothelin-1 for more than 24 h (Opgenorth *et al*., 1996). Peak pressor responses to 0.3 nmol kg^{-1} endothelin-1 administered intravenously into xylazine/ketamine anaesthetized rats were inhibited by 24% in the rats which had received the antagonist A-127722.5 at the low dose and by 35% in the rats which had received A-127722.5 at the high dose for 4 days in a previous study in our laboratory (Sventek *et al*., 1997). SHR were bought from Taconic Farms (Germantown, N.Y.), and were received aged 10 weeks. SHR were offered either vehicle or A-127722.5 (10 mg kg^{-1} weight day^{-1} in their drinking water), starting at 12 weeks of age. After 8 weeks of treatment with A-127722.5 and while they continued on this drug at the same dose and route of administration, SHR started receiving for an additional 2 weeks cilazapril in the drinking water (10 mg kg^{-1} weight day^{-1}, a dose which effectively lowers blood pressure in SHR (Li & Schiffrin, 1996)).

To study the effects of LU 135252, a second group of DOCA-salt hypertensive rats was investigated in parallel with the DOCA-salt hypertensive rats treated with A-127722.5. These DOCA-salt hypertensive rats received the ET_A-selective endothelin receptor antagonist LU 135252 (50 mg kg^{-1} day^{-1}) in the drinking water. This dose given by gavage reduced by 50% the area under the curve of the blood pressure response to 0.8 nmol kg^{-1} endothelin-1 administered intravenously 4 h later in urethane-anesthetized rats (Müenter *et al*., 1996). LU 135252 was dissolved in 1 mol l^{-1} NaOH (pH 11.5), 100 mg in 0.25 ml, followed by addition of 9 ml of water and adjustment of pH to 7.5 with 0.1 mol l^{-1} HCl, and finally addition of tap water to the desired concentration. This dose of LU 135252 offered in the drinking water to normal rats was shown to antagonize effectively intravenous endothelin-1 in preliminary experiments in our laboratory (see below). A final group of Sprague-Dawley rats was used to study effects of endothelin antagonism on 1-kidney 1 clip Goldblatt hypertension. Renal hypertension was induced in rats weighing 150 g, at age 5–6 weeks, as previously described (Li *et al*., 1996; Sventek *et al*., 1996b) by applying under pentobarbitone anesthesia (40 mg kg^{-1}, intraperitoneally) a silver clip with a 0.2 mm lumen to the left renal artery and performing a right nephrectomy. Control normotensive rats were unilaterally nephrectomized as well. These rats were treated with the antagonist LU 135252 (50 mg kg^{-1} day^{-1}) in the drinking water as described above, after unilateral nephrectomy and renal artery clipping.

Systolic blood pressure was measured indirectly in all rats after warming and under light restraint in a plexiglass cage, by use of the tail-cuff method with a model PCPB photoelectric pulse sensor, and recorded on a Grass Model 7 polygraph fitted with a 7P8 preamplifier (all from Grass Medical Instruments, Quincy, MA). The average of three pressure readings was recorded. We previously demonstrated that these systolic blood pressure measurements are very close to intravascular systolic blood pressures of hypertensive N^G-nitro-L-arginine methyl ester (L-NAME)-treated rats implanted with telemetric transmitters (TA11PA-C40) and a catheter placed into the distal portion of the descending aorta for recording of arterial pressure (Sventek *et al*., 1996a).

Effectiveness of endothelin blockade by A-127722.5 and LU 135252

The efficacy of both A-127722.5 and LU 135252 to block endothelin-1 pressor responses were compared in preliminary experiments. Both agents were prepared and administered to normal Sprague-Dawley rats as described above. After 4 days of drug administration of either A-127722.5 (10 and 30 mg kg^{-1} day^{-1}) or LU 135252 (50 mg kg^{-1} day^{-1}) in drinking water, rats were anaesthetized with ketamine/xylazine 90/12 mg kg^{-1}, i.p., and then heparin-treated normal saline-filled PE-50 polyethylene catheters (Intramedic, Clay Adams, Parsippany, NJ) were introduced into a carotid artery and jugular vein. Blood pressure was monitored with a Gould P23ID pressure transducer on a Grass polygraph. Bolus intravenous injections of 50 ng angiotensin II and cumulative doses of 2 ng–2 μg endothelin-1 in 0.1 ml normal saline were successively administered and compared to responses in parallel control rats which had not received either endothelin antagonist.

Measurement of plasma endothelin and plasma renin activity

Blood was obtained from the neck during the first few seconds after decapitation, in tubes containing potassium ethylenediaminotetraacetate, for measurement of plasma endothelin-1 and plasma renin activity. Immunoreactive endothelin-1 was extracted from plasma by passage through C18 Sep-Pak cartridges (Waters Assoc., Milford, MA), and measured by radioimmunoassay as previously described (Larivière *et al*.,

1993b; Schiffrin *et al*., 1995a). The antibody against endothelin-1 was from Peninsula (Palo Alto, CA). The minimum detectable concentration of endothelin was 0.4 pmol l^{-1}, and recovery of 5 pmol l^{-1} of endothelin-1 added to plasma was 75%. The cross-reactivity of the antibody was 10% with big endothelin and 7% with endothelin-3. Plasma renin activity was measured by radioimmunoassay of angiotensin I generated during a 2 h incubation in the presence of 8-hydroxyquinoline and sodium edetate as angiotensinase inhibitors, at pH 6.5 and at 37°C as previously described (Larivière *et al*., 1993b; Schiffrin *et al*., 1995a).

Analysis of data

Values are given as means ± s.e.mean. Statistical differences were evaluated by ANOVA followed by a Newman-Keuls *post-hoc* test. Results were considered significantly different when $P<0.05$.

Results

The effectiveness of endothelin blockade by A-127722.5 and LU 135252 was evaluated in preliminary experiments in Sprague-Dawley rats treated with the same doses and route of administration for 4 days as that used in hypertensive rats. Whereas pressor responses to 50 ng angiotensin II given intravenously were unaffected by treatment with either endothelin antagonist (not shown), administration of the low or high dose of A-127722.5 or of the dose used of LU 135252 resulted in reduced peak pressor responses and a displacement to the right of the dose-response curve to intravenous endothelin-1 (Figure 1). These results demonstrated the efficacy of the blockade of endothelin-1 pressor effects at the doses of both endothelin antagonists given in the drinking water.

DOCA-salt hypertensive rats treated with either the low or high dose of A-127722.5 presented a similar reduced rise in systolic blood pressure relative to untreated rats at 4 weeks of treatment (Figure 2 and Table 1). Body weight of DOCA-salt hypertensive rats was lower than that of normotensive controls, as found previously, and was not significantly affected by treatment. Plasma renin activity was suppressed in DOCA-salt hypertensive rats, as expected, and was unaffected by treatment with A-127722.5. Plasma immunoreactive endothelin concentration was significantly higher in DOCA-salt hypertensive rats than in normotensive controls and was unchanged in A-127722.5-treated rats.

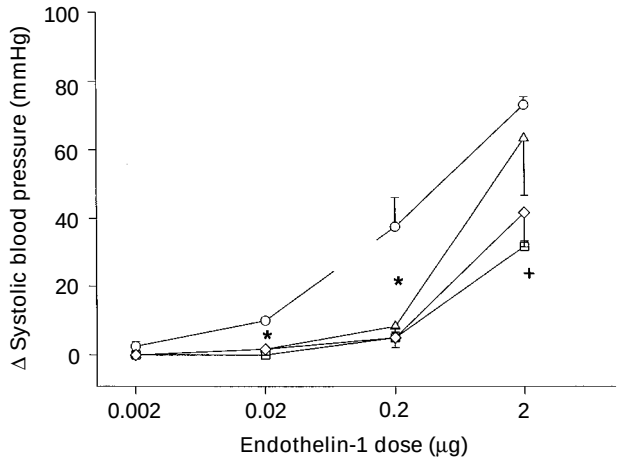

Figure 1 Dose-response curve of pressor responses to endothelin-1 administered intravenously to Sprague-Dawley rats anaesthetized with ketamine/xylazine (90/12 mg kg^{-1} i.p.) which had received for the preceding four days in their drinking water the ET_A-selective endothelin receptor antagonists A-127722.5 at a low (10 mg kg^{-1} day^{-1} (△)) or high (30 mg kg^{-1} day^{-1} (□)) dose or LU 135252 (◇). Control rats (○) were identically treated but did not receive the endothelin antagonists. Results are mean and vertical lines show s.e.mean; $n=3$ rats per group. *$P<0.01$ all groups vs control, $^{+}P<0.01$ rats treated with A-127722.5 at the high dose or LU 135252 vs control.

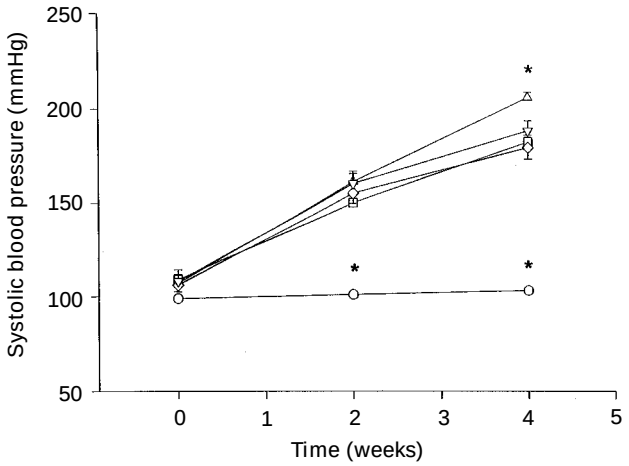

Figure 2 Time-course of systolic blood pressure in DOCA-salt hypertensive rats without (△) or with administration of the ET_A-selective endothelin receptor antagonist A-127722.5 at a low (10 mg kg^{-1} day^{-1} (□)) or high (30 mg kg^{-1} day^{-1} (◇)) dose or the ET_A-selective endothelin receptor antagonist LU 135252 (▽). Unilaterally nephrectomized rats (○) served as normotensive controls. $n=8-11$ rats per group. *$P<0.01$ vs other groups. Time indicates time from unilateral nephrectomy, implantation of DOCA, administration of 1% saline and of A-127722.5 or LU 135252. s.e.mean in some groups is hidden by the symbols.

Table 1 Blood pressure, body weight, plasma renin activity and plasma endothelin in DOCA-salt rats treated with the ET_A-selective endothelin receptor antagonists A–127722.5 and LU 135252 for 4 weeks

Parameter	*Unilaterally nephrectomized*	*DOCA-salt hypertensive*	*DOCA-salt + A-127722.5 (low dose)*	*DOCA-salt + A-127722.5 (high dose)*	*DOCA-salt + LU 135252*
Number	8	9	9	11	8
Systolic BP (mmHg)	103 ± 2**	206 ± 4**	182 ± 3	179 ± 4	188 ± 5
Body weight (g)	359 ± 6**	248 ± 6	268 ± 13	261 ± 9	273 ± 9
Plasma renin activity (ng A1 ml^{-1} h^{-1})	1.28 ± 0.29**	0.27 ± 0.09	0.04 ± 0.02	0.10 ± 0.04	0.08 ± 0.06
Plasma immunoreactive endothelin (pmol l^{-1})	2.94 ± 0.16*	4.52 ± 0.41	4.18 ± 0.50	4.64 ± 0.35	4.36 ± 0.38

Data are mean ± s.e.mean. DOCA-salt = DOCA-salt hypertensive rats; BP = blood pressure. *$P<0.05$; **$P<0.01$ vs other groups.

SHR were treated with A-127722.5 for 8 weeks starting at 12 weeks of age. In contrast to DOCA-salt hypertensive rats, SHR treated with the low dose of A-127722.5 had a similar systolic blood pressure to the untreated SHR (Figure 3 and Table 2). Blood pressure appeared to rise slower in SHR treated with A-127722.5 and this difference from untreated SHR achieved transiently statistical significance after 4 weeks of treatment, disappearing after that. When A-127722.5 treated SHR received the angiotensin I-converting enzyme inhibitor cilazapril, blood pressure decreased significantly. There was no difference in the extent of the blood pressure reduction between the A-127722.5-treated and untreated SHR also receiving cilazapril. Plasma endothelin immunoreactivity did not show any significant changes in rats treated with the endothelin antagonist and/or the angiotensin converting enzyme inhibitor.

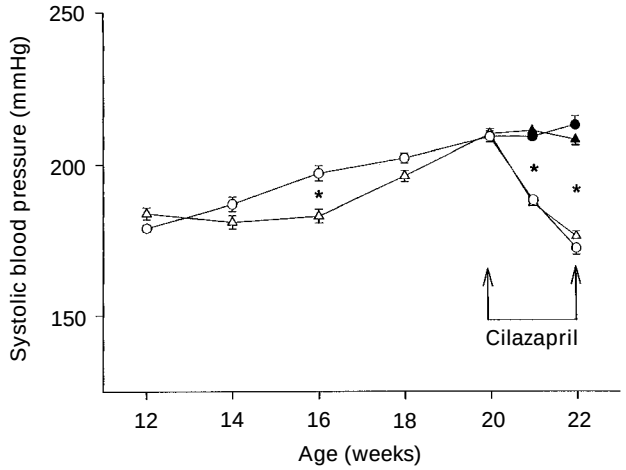

Figure 3 Time-course of systolic blood pressure in spontaneously hypertensive rats (SHR) without (○, ●) or with (△, ▲) administration of the low dose of the ET_A-selective endothelin receptor antagonist A-127722.5. Where indicated, the angiotensin I-converting enzyme inhibitor cilazapril was also administered (10 mg kg^{-1} day^{-1}) in the drinking water (open symbols). Rats which did not receive cilazapril are shown by the solid symbols. $n=8$ rats per group during the period of cilazapril treatment and 16 before that. *$P<0.05$. Age indicates age of rats. s.e. mean in some groups is hidden by the symbols.

DOCA-salt hypertensive rats treated with LU 135252 presented a blunting of the rise of systolic blood pressure relative to untreated rats at 4 weeks of treatment (Figure 2 and Table 1). LU 135252 treatment did not significantly affect the body weight, the suppressed plasma renin activity or the raised plasma endothelin immunoreactivity in DOCA-salt hypertensive rats. One-kidney 1 clip Goldblatt hypertensive rats treated with LU 135252 had a similar systolic blood pressure to untreated 1-K 1C hypertensive rats (Figure 4 and Table 3). Body weight was lower in 1-kidney 1 clip Goldblatt hypertensive rats, and neigher it nor plasma renin activity were affected by LU 135252 treatment. In contrast, immunoreactive endothelin in plasma was slightly elevated in 1-kidney 1 clip hypertensive rats and rose slightly more in the LU 135252-treated 1-K 1C rats.

Discussion

This study demonstrates that hypertensive rats which have in previous studies responded with a reduction in blood pressure to chronic treatment with the combined ET_A/ET_B endothelin receptor antagonist bosentan (DOCA-salt hypertensive rats) (Li *et al*., 1994) responded with similar reductions in blood pressure to the ET_A-selective endothelin receptor antagonists A-127722.5 and LU 135252. Similarly, models which did not respond to bosentan (SHR, (Li & Schiffrin, 1995a,b) or one-kidney one clip Goldblatt hypertensive rats (Li *et al*., 1996)) failed to respond to A-127722.5 or to LU 135252. The low dose of A-127722.5 used in the case of SHR appears to exert maximal effects, since DOCA-salt hypertensive rats responded with equal lowering of blood pressure to both doses. The blunting of the blood pressure rise in DOCA-salt hypertensive rats was small, of approximately 20 mmHg, as has been previously observed after chronic administration of bosentan (Li *et al*., 1994). Similar results to those found with A-127722.5 were obtained with LU 135252: the dose which lowered blood pressure by approximately 20 mmHg in

Table 2 Blood pressure, body weight and plasma endothelin in SHR treated with the ET_A-selective endothelin receptor antagonist A-127722.5 for 8 weeks with or without the angiotensin converting enzyme inhibitor cilazapril for the last 2 weeks

Parameter	*SHR*	*SHR + A-127722.5*	*SHR + cilazapril*	*SHR + A-127722.5 + cilazapril*
Number	8	8	8	8
Systolic BP (mmHg)	213±2.9	208±2	172±2*	176±2*
Body weight (g)	392±4	393±6	380±4	376±5
Plasma immunoreactive endothelin (pmol l^{-1})	2.51±0.26	2.81±0.29	2.21±0.16	2.22±0.17

Data are mean±s.e.mean. BP = blood pressure. *$P<0.01$ vs SHR not receiving cilazapril.

Table 3 Blood pressure, body weight, plasma renin activity and plasma endothelin in one-kidney one clip Goldblatt hypertensive rats treated with the ET_A-selective endothelin receptor antagonist LU 135252 for 4 weeks

Parameter	*Unilaterally nephrectomized*	*Untreated 1-K 1C*	*1-K 1C + LU 135252*
Number	9	8	8
Systolic BP (mmHg)	103±1.7**	206±4	205±3
Body weight (g)	346±6**	306±6	299±8
Plasma renin activity (ng A1 ml^{-1} h^{-1})	1.82±0.39	1.67±0.36	1.88±0.29
Plasma immunoreactive endothelin ($pmol^{-1}$)	2.90±0.39	3.37±0.30	4.61±0.48*

Data are mean±s.e.mean. 1-K 1C = one-kidney one Goldblatt hypertensive rats; BP = blood pressure; BW = body weight; AI = angiotensin I. *$P<0.05$ **$P<0.01$ vs other groups.

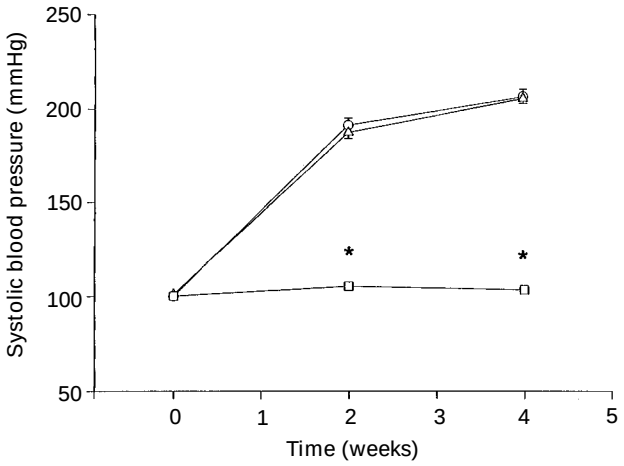

Figure 4 Time-course of systolic blood pressure in DOCA-salt hypertensive rats without (○) or with (△) administration of the ET_A-selective endothelin receptor antagonist LU 135252 (50 mg kg^{-1} day^{-1}). Unilaterally nephrectomized rats (□) served as normotensive controls. $n=8-9$ rats per group. $^*P<0.05$ vs other groups. Time indicates time from unilateral nephrectomy, application of 2 mm silver clip to the left renal artery and administration of LU 135252. s.e.mean in some groups is hidden by symbols.

DOCA-salt hypertensive rats (responsive to bosentan) was ineffective in one-kidney one clip Goldblatt rats (which do not demonstrate a hypotensive response to bosentan). Thus the ET_A-selective endothelin receptor antagonists appeared to have similar effects on blood pressure as the combined ET_A/ET_B endothelin receptor antagonist bosentan, and both discriminated between apparently endothelin-1-dependent hypertension such as DOCA-salt hypertension (Larivière *et al*., 1993a,b; Day *et al*., 1995; Schiffrin *et al*., 1995a), and endothelin-1-independent hypertension, such as SHR and one-kidney one clip Goldblatt hypertension (Larivière *et al*., 1993b; 1995; Schiffrin *et al*., 1995a; Sventek *et al*., 1996b). This suggests that potential blockade of endothelial ET_B endothelin receptors by the combined ET_A/ET_B receptor antagonist bosentan does not influence blood pressure responses to this drug. However, it should be noted that blood pressure appeared to rise at a slower rate in the SHR treated with A-127722.5 and this difference (approximately 14 mmHg) was statistically significant after 4 weeks of treatment (Figure 2). Whether this was due to the presence of a transient pathophysiologically significant endothelin-dependent component in SHR is unclear.

Only after 4 weeks of treatment was the blood pressure lower in the model which did respond to endothelin antagonism: the DOCA-salt hypertensive rat. As already mentioned, a blood pressure lowering effect of endothelin antagonists may occur only in hypertensive rat models over expressing endothelin-1 in the endothelium of small arteries (Schiffrin, 1995). In DOCA-salt hypertensive rats endothelin-1 mRNA was found to be increased in blood vessels only after 3 weeks of treatment (Schiffrin *et al*., 1996). It was, therefore, not unexpected that endothelin antagonism resulted in lower blood pressure only after several weeks of treatment. It is likely that this delay in the triggering of endothelin-1 gene activation is the result of endothelial damage or other factors which activate expression when blood pressure surpasses a certain threshold. Thus, endothelin antagonism succeeds in having a blood pressure lowering effect only in this later period.

In this study possible interactions of the endothelin system and the renin-angiotensin system were also examined. L-NAME-induced hypertension in rats has been shown to respond acutely to endothelin antagonism with bosentan when rats have been treated previously with an angiotensin I-converting enzyme inhibitor, apparently unmasking an endothelin-dependent vasopressor action (Richard *et al*., 1995). Angiotensin II may stimulate endothelin-1 expression by the endothelium (Imai *et al*., 1992), and this may occur particularly in SHR (Dohi *et al*., 1992). However, in the present study there was no evidence of unmasking of an endothelin-dependent vasopressor tone after interruption of the renin-angiotensin system. The reason for this may be the chronic nature of these experiments compared to the acute study in anaesthetized rats treated with angiotensin converting enzyme inhibitors performed by Richard *et al*. (1995). In the latter there may have been an increase in plasma endothelin, possibly from the pituitary (Kaufmann *et al*., 1991), in part as a result of anaesthesia. Under these circumstances an endothelin-dependent pressor tone may indeed contribute to the maintenance of blood pressure after interruption of the renin-angiotensin system.

In this study, as in previous work (Larivière *et al*., 1993b), DOCA-salt hypertensive rats were shown to exhibit increased plasma immunoreactive endothelin levels and these were unaffected by treatment with the endothelin antagonists. Bosentan has been shown to raise plasma endothelin immunoreactivity, perhaps as a result of occupation of endothelial ET_B receptors (Löffler *et al*., 1993). Selective ET_A receptor antagonists would, therefore, not be expected to cause an elevation in endothelin immunoreactive concentrations in plasma, and indeed in DOCA-salt hypertensive rats neither A-127722.5 nor LU 135252 had this effect. In 1-K 1C hypertensive rats a slight elevation of endothelin immunoreactivity in plasma was found, in agreement with the mild endothelin-1 gene expression previously demonstrated in blood vessels of these rats in other studies (Sventek *et al*., 1996b). LU 135252 induced slight but significantly higher plasma immunoreactive levels of endothelin in these hypertensive rats, but the mechanism for this is unclear.

The effect of A-127722.5 on blood pressure of DOCA-salt hypertensive rats appeared to be slightly greater than that of LU 135252, even though the blockade of exogenous endothelin-1 by LU 135252 appeared to exceed that induced by A-127722.5 in the pilot experiments destined to demonstrate the endothelin blocking ability of the two compounds when administered in the drinking water. Both antagonists have similar affinity for the ET_A receptor *in vitro* (Opgenorth *et al*., 1996; Riechers *et al*., 1996). Since the effect of the antagonists are exerted at the receptor level, and we do not know the concentrations of endogenous endothelin (probably mostly endothelin-1 (Day *et al*., 1995)) occurring in DOCA-salt hypertensive rats in comparison to those when exogenous endothelin-1 is infused intravenously, or the precise pharmacokinetics of penetration of the antagonists, it is difficult to compare the efficacy of the antagonism of exogenous endothelin-1 in a normotensive rat with that of endogenous endothelin in a hypertensive rat. Essentially those pilot experiments attempted to show that with the mode of administration used, there was persistent endothelin antagonism which extended over many hours of the day even though rats drink mostly at night, when they are most active.

In summary, these results demonstrate that treatment with either of two ET_A-selective endothelin receptor antagonists (A-127722.5 or LU 135252) resulted in a small reduction in blood pressure (approximately 20 mmHg), similar to that obtained with a combined ET_A/ET_B endothelin receptor antagonist. Blood pressure was lowered only in hypertensive rats which have been previously shown to overexpress vascular endothelin-1 (DOCA-salt hypertensive rats) but not those which do not overexpress endothelin-1 in blood vessels (SHR) or do so in a limited fashion (one-kidney one clip Goldblatt hypertensive rats). This suggests that blockade of ET_B endothelin receptors does not interfere with the hypotensive effects of combined ET_A/ET_B endothelin receptor antagonists like bosentan. Interruption of the renin-angiotensin system did not affect the blood pressure reduction induced by chronic endothelin antagonism with an ET_A-selective receptor antagonist.

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