Additive Effects of Enalapril and Losartan in (mREN-2)27 Transgenic Rats

Christine Richer, Patrick Bruneval, Joël Ménard, Jean-François Giudicelli

Abstract—Blockade of angiotensin II AT$_1$ receptors combined with angiotensin I–converting enzyme inhibition might amplify the potency of the renin-angiotensin system blockade. We studied whether chronic and simultaneous administration of enalapril and losartan would result in additive or synergistic effects in the (mREN-2)27 transgenic rat (TGR), the investigated targets being blood pressure, cardiac hypertrophy, renin-angiotensin system blockade achieved, and plasma active renin concentration. In addition, the origin (renal or extrarenal, rat or mouse) of the induced renin release was determined. Adult TGRs were treated orally and daily for 5 to 7 weeks with 1 mg/kg (E1) or 3 mg/kg (E3) enalapril or 1 mg/kg (L1) or 3 mg/kg (L3) losartan, or their combinations (E1L1 and E3L3). At the end of the treatment period, enalapril and losartan exerted dose-dependent and, when combined, additive effects in terms of blood pressure fall and cardiac hypertrophy limitation, and synergistic effects in terms of plasma active renin stimulation and blockade of exogenous angiotensin I pressor effects, with E3L3 > E3 > L3, E1L1 > E1 > L1, and E1L1 = E3 > L3. This indicates that in the TGR, (1) the greater the renin-angiotensin system blockade achieved, the greater are the reduction in blood pressure, the limitation of cardiac hypertrophy, and the reactive rise in plasma renin concentration elicited, and (2) the enalapril-losartan combinations are more potent at achieving these goals than any of their constituents individually. In contrast, there was no interaction between the two drugs regarding aldosteronuria reduction. Measurement of plasma renin concentration and renal renin at pH 6.5 and 8.5, ie, the optimal pH values for rat and mouse renin activities, respectively, indicates that in TGRs the counterregulatory process for renin release elicited by enalapril, losartan, or their combination involves primarily rat renin of renal origin, a finding supported further by the observed increase in the rat renal renin hybridization index. (Hypertension. 1998;31:692-698.)

Key Words: enalapril ■ losartan ■ renin-angiotensin system ■ rats, TGR(mREN-2)27

Both Ang II AT$_1$, receptor antagonists and ACEIs aim at the inhibition of the same pressor system, but because of different sites of action they may still have additive or synergistic effects. There are a number of theoretical reasons for this potential mutual reinforcement. First, ACEIs are unable to strongly inhibit plasma Ang II levels, 1,2 This phenomenon, which is linked (1) to the reactive rise in plasma active renin and Ang I secondary to the interruption of the Ang II feedback on renin release and (2) to the existence, at the level of the heart and blood vessels, of other Ang II–forming enzymatic pathways not sensitive to ACEIs, 3,4 can be neutralized by AT$_1$ receptor blockade. Second, the reactive rise in plasma Ang II induced by AT$_1$ receptor blockade can be strongly inhibited by Ang I–converting enzyme inhibition. 5 Therefore, addition of an AT$_1$, receptor antagonist to an ACEI might help to achieve a more efficient RAS blockade than that obtained after each drug used individually. Finally, the ancillary properties of ACEIs, eg, interruption of kinin metabolism and interaction with nitric oxide, 6 and of AT$_1$, receptor antagonists, eg, interaction with nitric oxide 6 and prostaglandins, 7 could lead, when these two groups of drugs are combined, to additive effects. Indeed, additive effects on blood pressure and renin release have been described recently after combined acute ACE inhibition (captopril or enalapril) and AT$_1$, antagonism (losartan) in sodium-depleted normotensive volunteers 8,9 and in SHRs. 9 In addition, the reactive losartan–induced plasma Ang II peak was completely suppressed by their combination. 5

In this context, the first goal of this study was to reinvestigate the issue of additive or synergistic effects between ACEIs and AT$_1$, receptor antagonists in the hypertensive TGR(mREN-2)27 rats because, despite the controversy about its plasma renin levels, 10-13 this model is a renin-dependent one, as shown by its greater sensitivity to losartan and enalapril as compared with SHRs. 14-15 TGRs were preferred to two-kidney one-clip Goldblatt rats to avoid the possible confounding effects of renal ischemia and the heterogeneity in renin values observed, at least in our experience, 16 between individual animals. The test drugs were enalapril and losartan administered chronically either alone or in combination, and the investigated targets...
were blood pressure and cardiac hypertrophy. We also included the assessment of the achieved blockade of Ang I pressor effects which, despite its unique ability to investigate simultaneously the residual function of ACE and Ang II AT1 receptors in humans, has not yet been investigated after combined blockade of the RAS both experimentally and clinically.

PRC, an index of the status of the negative feedback of Ang II on renin release, is another target for investigating potential additive or synergistic effects between ACEI and AT1 receptor antagonists. However, if the interruption of this feedback at the level of the juxtaglomerular cells explains the escape from ACEIs, it is to be expected that the phenomenon will be functionally less important in the TGR than in the other models, because the extrarenal production of mouse renin, especially in the adrenals, is not sensitive to the Ang II feedback. In this context, the second goal of this study was to investigate further the RAS blockade-induced reactive renin release in TGRs, to assess the origin (mouse or rat) of this released renin, and to determine the effects of the combined administration of ACEIs and AT1 receptor antagonists.

Methods
All experiments were performed in accordance with the rules for animal health care of the French Ministry of Agriculture.

Animals and Treatments
Fifty-six heterozygous male TGR(mREN2)27 rats (Mollegaard Breeding and Research Center), kept in a temperature-controlled environment (20°C to 22°C) under 12-hour light/dark cycles and having free access to food and tap water, were used. At the age of 6 weeks, they were weight-matched and divided into seven groups. One group served as untreated controls (C, n=8), whereas the six other groups (n=8 each) were treated, starting from 8 weeks of age and for a period of 5 to 7 weeks, with 1 mg/kg (EI) or 3 mg/kg (E3) enalapril, 1 mg/kg (L1) or 3 mg/kg (L3) losartan, or their combinations (1 mg/kg enalapril plus 1 mg/kg losartan [E1L1] or 3 mg/kg enalapril plus 3 mg/kg losartan [E3L3]) dissolved in their drinking fluid. BW and drinking volume were determined weekly, and the concentrations of drugs in the drinking fluid were adjusted accordingly.

SBP and HR were determined weekly by the tail-cuff method using a photoelectric pulse detector (PC model 139, IITC) according to the method of Bunag and Butterfield.[20]

Hemodynamic Assessment of RAS Blockade
All surviving animals were anesthetized between the ages of 13 and 15 weeks with sodium pentobarbitone (50 mg/kg IP), pithed, bivalvotimized, intubated, and ventilated with room air (Harvard Respirator, model 680). Catheters were placed in a femoral vein and in a carotid artery for infusion of drugs and for measurement of arterial blood pressure via a pressure transducer (Statham P100EZ, Gould Instruments), respectively. Miniaturized pulsed Doppler probes were implanted around the upper abdominal aorta and the left renal artery, respectively, and connected to a pulsed Doppler flow meter (Directional Pulsed Doppler, model 545C, University of Iowa) as previously described.[21]

CO and RBF signals were collected on a PC (Dynamit Compag) with an on-line data-acquisition system (PRX Software, Notoxoc System) and continuously displayed. TPR and RVR were estimated as the MAP to corresponding mean flow ratios and expressed in arbitrary units.

After instrumentation, the animals were given atropine sulfate (1 mg/kg IV), and after a 15-minute stabilization period baseline values of all investigated parameters were measured. Next, systemic and regional vascular responses to increasing bolus doses of Ang I (10, 30, 100, 300, and 1000 ng/kg) were recorded. Ang I dose–response curves for MAP, HR, CO, RBF, TPR, and RVR were constructed in the seven groups of animals. In addition, for MAP, TPR, and RVR, AUCs versus log-dose of Ang I were calculated in each rat according to the trapezoidal rule and averaged within each group.

At the end of the experiments, the rats were killed and their hearts (left ventricles) and kidneys were removed and weighed. The right kidney was frozen at −80°C for determination of R.R. content. The left kidney was rapidly frozen in liquid nitrogen for evaluation of rat RR mRNA by in situ hybridization.

Assessment of the Renin-Angiotensin System Components in the Plasma, Urine, and Kidney
Urinary aldosterone was measured on the 16-hour urine of the rats, collected after adaptation in metabolic cages during the 11th week of age. Aldosteronuria was determined by radioimmunoassay (Coat-a-Count Aldosterone Kit).

At 12 weeks of age, ie, after a 4-week treatment period, blood was obtained from the jugular vein under pentobarbitone anesthesia (50 mg/kg IP) and collected into heparinized tubes. Plasma was then stored at −80°C until the time of plasma angiotensinogen, PRC, and total renin concentration and PRA assessments.

Plasma angiotensinogen was measured by incubating plasma to exhaustion with an excess of pure mouse submaxillary gland renin.22 PRC, total renin concentration, and R.R. content were measured as previously described22–25 by the in vitro production of Ang I at pH 6.5, 7.4, and 8.5 in the presence of an excess of angiotensinogen provided by bunaphrectomized rat plasma. At pH 6.5, Ang I generation is dependent mainly on rat renin, whereas at pH 8.5, it is dependent mainly on mouse renin.21

PRA was measured after a 1-hour incubation of experimental plasma, at 37°C at pH 7.4, in the absence of exogenous angiotensinogen.24 Both renin species contribute to Ang I generation at this pH, but the kinetics of the enzymatic reaction is faster for mouse than for rat renin in the presence of rat angiotensinogen. Protein measurements of the tissue extracts were performed according to the method described by Bradford.[25]

In Situ RRRHII
A 1.2-kb rat renin cDNA26 was labeled with 32P-dCTP (Amersham) with a random primer kit (Amersham) yielding a specific activity of 2×106 cpm/μg. In situ hybridization was performed on renal tissue

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**Selected Abbreviations and Acronyms**

- ACE = angiotensin-converting enzyme
- ACEI = ACE inhibitor
- Ang I = angiotensin I
- Ang II = angiotensin II
- AT1, AT2 = angiotensin type 1, type 2 receptor
- AUC = area under the curve
- BW = body weight
- CO = cardiac output
- HR = heart rate
- LVW/BW = ratio of left ventricular weight to body weight
- MAP = mean arterial pressure
- PRA = plasma renin activity
- PRC = plasma renin concentration
- RAS = renin-angiotensin system
- RBF = renal blood flow
- RR = renal renin
- RRRHI = rat renal renin hybridization index
- RVR = renal vascular resistance
- SBP = systolic blood pressure
- SHR = spontaneously hypertensive rat
- TGR = transgenic rat
- TPR = total peripheral resistance
TABLE 1. Mean Values of SBP, LVW/BW, Plasma Active Renin Concentration, and Urinary Aldosterone Calculated in Control or Treated TGRs at the End of the Treatment Period

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP, mm Hg</th>
<th>LVW/BW, mg/g</th>
<th>PRC 6.5, ng Ang I · mL⁻¹ · h⁻¹</th>
<th>Aldo, ng/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>274.0±4.0</td>
<td>3.694±0.148</td>
<td>42.6±8.7</td>
<td>45.9±11.2</td>
</tr>
<tr>
<td>E1</td>
<td>200.0±8.5*</td>
<td>2.393±0.057†</td>
<td>23.6±7.4†</td>
<td>23.6±3.8*</td>
</tr>
<tr>
<td>L1</td>
<td>221.7±9.8*</td>
<td>2.780±0.084‡</td>
<td>20.0±5.1‡</td>
<td>16.5±1.5*</td>
</tr>
<tr>
<td>E1L1</td>
<td>177.0±8.6*</td>
<td>2.242±0.096*</td>
<td>145.8±47.‡</td>
<td>18.0±2.8*</td>
</tr>
<tr>
<td>E3</td>
<td>176.4±5.0*</td>
<td>2.319±0.041†</td>
<td>271.6±69.1†</td>
<td>22.5±5.3*</td>
</tr>
<tr>
<td>L3</td>
<td>192.1±5.1*</td>
<td>2.480±0.057‡</td>
<td>31.9±12.9†</td>
<td>12.5±2.3*</td>
</tr>
<tr>
<td>E3L3</td>
<td>169.4±4.3*</td>
<td>2.195±0.028*</td>
<td>679.2±143.6†</td>
<td>22.9±4.6*</td>
</tr>
</tbody>
</table>

PRC 6.5 indicates plasma active renin concentration measured at pH 6.5; Aldo, urinary aldosterone.

Values are mean±SEM.

*P<.05 vs C; †P<.05 vs E1L1; ‡P<.05 vs E3L3.

Results

Treatment Effects on Blood Pressure and Heart Weight

In 6-week-old TGRs, BW and SBP values were 194±5 g and 205±3 mm Hg, respectively. During the treatment period, a number of animals died, either because of their disease or because of anesthesia performed for blood sampling or before pithing, so that at the end of the study, there were 4, 5, 6, 5, 6, 6, and 7 surviving animals in the C, E1, L1, E1L1, E3, L3, and E3L3 groups, respectively.

Table 1 indicates the SBP values measured in the seven groups of conscious animals at the end of the treatment period. It shows that all six treatments significantly lowered SBP compared with the control group. Enalapril at both doses (E1, –74 mm Hg, P<.001 versus C; E3, –98 mm Hg, P<.001 versus C) significantly decreased SBP, but E3 was not statistically more potent than E1 in this respect. Losartan also exerted a significant blood pressure–lowering effect (L1, –52 mm Hg, P<.001 versus C; L3, –82 mm Hg, P<.001 versus C) that was dose-dependent (L3, P<.01 versus L1). The E1L1 combination (–97 mm Hg, P<.001 versus C) was more potent than L1 alone (P<.001) but not significantly more potent than E1 alone, and the E3L3 combination (–105 mm Hg, P<.001 versus C) was more potent than L3 alone (P<.01) but not significantly more potent than E3 alone at reducing SBP (Fig 1), which indicates additive effects of the two drugs on this parameter. Finally, there was no statistical difference between E3L3 and E1L1 effects on SBP.

No significant difference in BW or HR evolutions were observed among the seven experimental groups during the entire treatment period (data not shown). Table 1 also shows that the six treatments significantly reduced LVW/BW compared with C. Enalapril at both doses (E1, –35%, P<.001 versus C; E3, –37%, P<.001 versus C) opposed development...
TABLE 2. Systemic and Regional Hemodynamics Measured in Control or Treated Pithed TGRs at the End of the Entire Treatment Period

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
<th>CO, AU</th>
<th>RBF, AU</th>
<th>TPR, AU</th>
<th>RVR, AU</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>4</td>
<td>54.8 ± 0.6</td>
<td>307.3 ± 13.5</td>
<td>3.6 ± 0.2</td>
<td>1.9 ± 0.3</td>
<td>15.2 ± 0.8</td>
<td>30.8 ± 4.6</td>
</tr>
<tr>
<td>E1</td>
<td>5</td>
<td>49.4 ± 2.2</td>
<td>308.4 ± 10.6</td>
<td>4.3 ± 0.6</td>
<td>2.3 ± 0.3</td>
<td>12.3 ± 1.6</td>
<td>22.8 ± 3.6</td>
</tr>
<tr>
<td>L1</td>
<td>6</td>
<td>50.9 ± 1.6</td>
<td>309.2 ± 10.6</td>
<td>4.2 ± 0.5</td>
<td>2.3 ± 0.3</td>
<td>12.8 ± 1.4</td>
<td>23.0 ± 2.6</td>
</tr>
<tr>
<td>E1L1</td>
<td>5</td>
<td>55.7 ± 2.7</td>
<td>307.8 ± 11.6</td>
<td>4.2 ± 0.3</td>
<td>2.1 ± 0.1</td>
<td>13.6 ± 1.1</td>
<td>26.3 ± 1.0</td>
</tr>
<tr>
<td>E3</td>
<td>6</td>
<td>49.6 ± 1.9</td>
<td>308.3 ± 11.1</td>
<td>4.2 ± 0.4</td>
<td>2.3 ± 0.2</td>
<td>121.9 ± 0.9</td>
<td>224.2 ± 2.3</td>
</tr>
<tr>
<td>L3</td>
<td>6</td>
<td>52.4 ± 2.4</td>
<td>310.7 ± 14.2</td>
<td>5.2 ± 0.6</td>
<td>2.1 ± 0.2</td>
<td>106.0 ± 0.9</td>
<td>28.6 ± 3.1</td>
</tr>
<tr>
<td>E3L3</td>
<td>7</td>
<td>41.4 ± 1.7†‡¶</td>
<td>308.9 ± 4.3</td>
<td>4.3 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>10.0 ± 0.7†</td>
<td>241 ± 3.2</td>
</tr>
</tbody>
</table>

AU indicates arbitrary units. Values are mean ± SEM.

*P < 0.01 vs C; † P < 0.05 vs E1L1; ‡ P < 0.01 vs E3; ¶ P < 0.01 vs L3.

Treatment-Induced RAS Blockade: Biochemical Aspects

In 11-week-old TGRs, ie, after 3 weeks of treatment, the 24-hour water intake (53 ± 3 mL) and diuresis (31 ± 2 mL) values measured in control animals were not affected by any of the six treatments. In contrast, aldosteronuria was significantly reduced (−48% to −72%) by the six treatments (Table 1), and there was no significant difference among them.

Table 3 indicates the values of the different components of the RAS measured at pH 7.4 in the plasma of the control and treated TGRs. Whereas E1 and L1 did not affect PRC and PRA, their combination strongly increased these parameters (+311% and +70%, respectively); this effect, however, did not reach statistical significance. E3 significantly increased PRC (+430%) and PRA (+149%). E3L3 induced an even greater increase in PRC (+1119%), which indicates synergistic effects of the two drugs on this parameter, but not in PRA (+122%). E3L3 also induced a strong and significant (−66%) reduction in plasma angiotensinogen. Total renin concentration did not change with the different treatments and was in all groups much greater than usually observed in normal rats.

Fig 3 illustrates the ratios of renin concentration measured at pH 6.5 (optimum pH for rat renin activity) to renin concentration measured at pH 8.5 (optimum pH for mouse renin activity) in the plasma (PRC, 6.5/8.5) and in the kidney (RR, 6.5/8.5) in the seven experimental groups. The PRC 6.5/8.5 of left ventricular hypertrophy, but E3 was not statistically more potent than E1 in this respect. Losartan also exerted a significant limitation of left ventricular hypertrophy (L1, −25%, P < 0.01 versus C; L3, −33%, P < 0.001 versus C) that was dose-dependent (L3, P < 0.01 versus L1). The E1L1 combination (−39%, P < 0.001 versus C) was more potent than L1 alone (P < 0.001) but not significantly more potent than E1 alone, and the E3L3 combination (−41%, P < 0.01 versus C) was more potent than L3 alone (P < 0.01) and E3 alone (P < 0.05) at opposing left ventricular hypertrophy (Fig 1), which again indicates some additive effects of the two drugs. Finally, there was no statistical difference between E3L3 and E1L1 effects on cardiac hypertrophy.

Treatment-Induced RAS Blockade: Hemodynamic Aspects

Table 2 compares the mean values of MAP, HR, CO, RBF, TPR, and RVR determined in the pithed TGRs at the end of the treatment period (5 to 7 weeks). All treatments tended to decrease TPR, the effect being significant only with L3 and E3L3; with RVR, the effect was not significant. E3L3 also decreased MAP (−25%), and this effect was significantly greater than those observed with E3 (P < 0.01), L3 (P < 0.001), and E1L1 (P < 0.05). CO tended to be slightly increased by all treatments, but RBF and HR were not modified.

In control TGRs, Ang I induced dose-dependent increases in MAP, TPR, and RVR. These responses were reduced or even abolished by the different treatments (Fig 2). Thus, pressor responses were dose-dependently decreased by enalapril (E1, −40% versus C; E3, −60%, P < 0.05 versus C) and losartan (L1, −20% versus C; L3, −29% versus C). The E1L1 combination (−57%, P < 0.05 versus C) was more potent than E1 alone or L1 alone (P < 0.05) and as potent as E3 at limiting Ang I pressor responses, whereas the E3L3 combination (−85%, P < 0.05 versus C) was more potent than E3 alone (P < 0.01) and L3 alone (P < 0.01) (Fig 2), which indicates synergistic effects of the two drugs versus Ang I–induced increases in MAP. Similar results were obtained for TPR. (E1, −54% versus C; E3, −64% versus C; L1, −37% versus C; L3, −79% versus C; E1L1, −69% versus C; E3L3, −90% versus C) and, to a larger extent, for RVR. (E1, −72% versus C; E3, −74% versus C; L1, −29% versus C; L3, −65% versus C; E1L1, −82% versus C; E3L3, −98% versus C) (Fig 2).
value was 0.45 ± 0.08 in C and remained <1 in the E1, L1, and L3 groups. It was increased by E1L1 (+344%, P = NS), E3 (+400%, P < .01), and E3L3 (+513%, P < .001). Regarding the RR 6.5/8.5 ratio, its value was 1.97 ± 0.45 in C and was strongly increased by E1 (+108%, P < .05), E1L1 (+125%, P < .05), E3 (+184%, P < .001), L3 (+95%, NS), and E3L3 (+132%, P < .05). Kidney structure was normal in all treated rats, whereas 4 of the 5 control TGRs surviving at 12 weeks had mild to severe nephroangiosclerotic lesions (arteriolar wall thickening, fibrinoid necrosis, interstitial fibrosis and inflammation, and glomerulosclerosis). Rat renin hybridization signal formation, and glomerulosclerosis). Rat renin hybridization signal formation, and glomerulosclerosis). Rat renin hybridization signal formation, and glomerulosclerosis).

Discussion

It appears from this study that in the transgenic (mREN2)27 rat, a renin-dependent model of hypertension, chronic Ang I—converting enzyme inhibition and Ang II AT1 receptor blockade, when combined, exert additive effects in terms of blood pressure fall and cardiac hypertrophy limitation and synergistic effects in terms of level of RAS blockade achieved and reactive renin release. Our study also supports the view that this reactive renin secretion is mainly of renal, ie, of rat origin.

At the end of the treatment period, the decreasing rank order of potency of these six treatments at reducing SBP in the conscious animal was found to be as follows: E3L3 > E3 = E1L1 > L3 > E1 > L1. As a result, E3L3 proved to be more potent than E3 or L3 alone, E1L1 more potent than E1 or L1 alone, and E1L1 as potent as E3, clearly indicating that the two drugs developed at least additive effects on this parameter when administered together (Fig 1). Regarding the LVW/BW ratio, our data indicate that enalapril and losartan also tended to exert additive effects at limiting cardiac hypertrophy development, again with E3L3 > E3 > L3, E1L1 > E1 > L1, and E1L1 > E3 > L3, ie, almost the same profile as that observed for SBP reduction.

One important finding in this study is that the effects of enalapril, losartan, and their combinations on blood pressure and cardiac hypertrophy clearly parallel those of the two drugs on the level of RAS blockade achieved, especially in the kidney. Thus, whereas both enalapril and losartan dose-dependently opposed the pressor, systemic, and renal vasoconstrictor effects of Ang I in the pithed TGR—enalapril being at a given dose usually more potent than losartan—their combination resulted in a dramatic and dose-dependent potentiation of Ang I inhibition. This first demonstration that ACEIs and AT1 receptor antagonists exert additive and possibly synergistic inhibitory effects versus the vascular responses to Ang I is evidenced by the fact that (1) the E1L1 combination was not only more potent than E1 and L1 but also more potent than L3 and equipotent to E3 and (2) the E3L3 combination resulted in a strong reduction of the pressor, systemic vasoconstrictor, and above all renal vasoconstrictor responses, thereby indicating an almost complete blockade of the RAS at the kidney level. In addition, the order of potency of the six treatments for Ang I blockade was very similar to those observed for the antihypertensive and cardiac antihypertrophic effects. All of these data thus demonstrate that (1) the greater the level of RAS blockade achieved, the greater are the reduction in blood pressure and the limitation of cardiac hypertrophy elicited and (2) the enalapril-losartan combinations are more potent at achieving these goals than any of their constituents taken individually.

In this study, enalapril and losartan also exerted synergistic effects on active PRC. Thus, whereas E1 and L1 had no effect

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**TABLE 3. Plasma Angiotensinogen, Total Renin Concentration, Active Renin Concentration, and Renin Activity Values Measured at pH 7.4 in 12-Week-Old Control or TGRs after a 4-Week Treatment Period**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Aogen, ng Ang I/mL</th>
<th>Total Renin Concentration, ng Ang I · mL⁻¹ · h⁻¹</th>
<th>PRC, ng Ang I · mL⁻¹ · h⁻¹</th>
<th>PRA, ng Ang I · mL⁻¹ · h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5</td>
<td>567 ± 52</td>
<td>1024 ± 188</td>
<td>33.6 ± 8.0</td>
<td>10.5 ± 1.6</td>
</tr>
<tr>
<td>E1</td>
<td>5</td>
<td>621 ± 49</td>
<td>710 ± 190</td>
<td>27.0 ± 9.4</td>
<td>9.4 ± 0.7</td>
</tr>
<tr>
<td>L1</td>
<td>6</td>
<td>579 ± 29</td>
<td>660 ± 153</td>
<td>20.4 ± 4.2</td>
<td>6.9 ± 1.1</td>
</tr>
<tr>
<td>E1L1</td>
<td>5</td>
<td>435 ± 58</td>
<td>630 ± 72</td>
<td>138.2 ± 45.9</td>
<td>17.8 ± 2.7</td>
</tr>
<tr>
<td>E3</td>
<td>7</td>
<td>785 ± 73</td>
<td>624 ± 67</td>
<td>178.2 ± 32.8</td>
<td>26.1 ± 4.3</td>
</tr>
<tr>
<td>L3</td>
<td>7</td>
<td>536 ± 28</td>
<td>827 ± 164</td>
<td>44.4 ± 9.7</td>
<td>9.8 ± 1.4</td>
</tr>
<tr>
<td>E3L3</td>
<td>8</td>
<td>195 ± 35†</td>
<td>1196 ± 180</td>
<td>409.5 ± 100.8</td>
<td>23.3 ± 4.5</td>
</tr>
</tbody>
</table>

Aogen indicates plasma angiotensinogen. Values are mean ± SEM.

*P < .01; †P < .001 vs C.
on this parameter, their combination (E1L1) strongly increased it (+311%). Also, whereas E3 (+430%) and, to a lesser extent, L3 (+32%) raised PRC, their combination (E3L3) further increased it (+1119%). These synergistic effects of enalapril and losartan on renin release are not surprising, because the enalapril-induced fall in plasma Ang II and losartan-elicited AT1 receptor blockade both contribute to it. As a result of the strong E3L3 combination–induced increase in PRC, consumption of substrate was greatly increased, as shown by the low angiotensinogen value in this group, so that PRA was only slightly increased.22 In contrast to what we observed for blood pressure reduction, cardiac hypertrophy limitation, RAS blockade, and renin release, we were unable in this study to detect any interaction between enalapril and losartan on aldosterone urinary excretion, because the latter was equally decreased in the six treatment groups. All of these biochemical data are thus in good agreement with those previously reported after acute administration in healthy volunteers.5,8

The second issue that we investigated in this study was the origin of the renin synthesized and released in TGRs during the treatment by enalapril, losartan, and their combinations. Recently, Tokita et al19 demonstrated that perindopril, an ACEI, administered to TGRs for 6 consecutive days suppressed plasma Ang II and markedly increased PRC and kidney renin. Moreover, they showed that a mouse REN-2 renin antibody (1) suppressed PRC only in control and not in perindopril–treated TGRs and (2) suppressed adrenal renin but had almost no effect on kidney renin in both groups of animals, thereby demonstrating that in TGRs, basal circulating renin is mainly mouse renin of adrenal origin, whereas RAS blockade–induced renin release is mainly rat renin of renal origin. In this study, we confirmed these findings with enalapril and extended them to the AT1 receptor antagonist losartan and to the enalapril–losartan combination. By measuring Ang I generation at two different pH values (6.5 and 8.5, i.e., the optimum pH values for the rat and mouse renin activities, respectively), we were able to calculate the ratio of Ang I generated at pH 6.5 and pH 8.5. This method, although less selective than the use of specific antibodies for independently quantifying mouse and rat active renin, allowed us to determine indirectly the relative contributions of the renin of both species to the activity of the plasma RAS. In control TGRs, the PRC 6.5/8.5 value was well below 1, indicating that circulating Ang I depends mainly on the enzymatic activity of mouse renin of extrarenal (adrenal) origin, and the RR 6.5/8.5 ratio was well above 1, indicating that RR activity was mainly attributable to rat renin. In the E1-, L1-, and L3-treated TGRs, PRC 6.5/8.5 remained unchanged, although some shift from mouse to rat renin in the kidney was already detectable, as indicated by the observed rises in RR 6.5/8.5. When kidney renin stimulation became more marked in the E1L1-, E3-, and E3L3–treated groups, both RR 6.5/8.5 ratio and RRRHI increased in parallel. The resulting strong increases in the activity of plasma renin were predominantly of rat origin, as shown by PRC values 6.5/8.5 well above 1. These data thus confirm that in TGRs, mouse renin is more active in the plasma than rat renin and that the increment in circulating renin induced by RAS blockade is rat renin of renal origin. The data also indicate that this phenomenon is identical whether RAS blockade is produced by ACEIs or AT1 receptor antagonists and that quantitatively, it is more marked with the former than with the latter when administered at identical doses.

In conclusion, in a renin–dependent model of hypertension, inhibition of the RAS by either ACE inhibition or AT1 receptor blockade was found to be dose–dependent. Furthermore, simultaneous inhibition of the RAS at multiple sites by combined administration of an ACEI and an AT1 receptor antagonist induced additive effects in terms of blood pressure reduction and left ventricular hypertrophy limitation and synergistic effects in terms of active renin release and level of RAS blockade achieved. As a result, a combination of low doses of each blocker was more potent than high doses of each of them administered individually.

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References

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