Inhibition of Brain Angiotensin-Converting Enzyme by Peripheral Administration of Trandolapril Versus Lisinopril in Wistar Rats

Junhui Tan, Jun Ming Wang, and Frans H.H. Leenen

Background: Peripheral administration of blockers of the renin–angiotensin system (RAS) may affect the RAS in the brain to a variable degree. In the present study, we determined inhibition of angiotensin-converting enzyme (ACE) in the brain after peripheral administration of a lipophilic (trandolapril) versus hydrophilic (lisinopril) ACE inhibitor.

Methods: Trandolapril (0.2, 1, and 5 mg/kg/day, subcutaneously) was compared with lisinopril (2, 10, and 50 mg/kg/day, subcutaneously), each for 6 days. At 4 and 24 h after the last dose, $^{125}$I-351A binding on brain ACE was measured.

Results: Trandolapril and lisinopril caused similar inhibition of ligand binding outside the blood–brain barrier (BBB). However, inside the BBB, trandolapril was more effective at low and medium doses (for lisinopril, 28% to 51% inhibition at a dose of 2 mg, 63% to 72% at 10 mg, and 84% to 86% at 50 mg; and for trandolapril, 62% to 68% inhibition at a dose of 0.2 mg, 84% to 87% at 1 mg, and 88% to 93% at 5 mg). In contrast, in the brain structures caudate putamen and globus pallidus, lisinopril inhibited ligand binding better than trandolapril (for lisinopril 30% to 44% at a dose of 2 mg and 71% to 74% at 10 mg, versus for trandolapril 21% to 27% at 0.2 mg and 51% to 63% at 1 mg). At 24 h after the last dose, inhibition by trandolapril persisted more than inhibition by lisinopril both outside and inside the BBB.

Conclusions: These results suggest that peripheral administration of even hydrophilic ACE inhibitors can result in marked inhibition of brain ACE inside the BBB but that different brain structures show variable inhibition.

Key Words: Angiotensin-converting inhibitors, brain angiotensin converting enzyme, central blockades, lisinopril, trandolapril.
al6 have directly compared the inhibition in brain nuclei inside versus outside the BBB caused by a lipophilic versus hydrophilic ACE inhibitor in steady state, that is, during long-term treatment. The latter is essential because in spontaneously hypertensive rats, for example, a single oral dose of spirapril did not inhibit brain ACE, but treatment for 8 weeks caused a marked decrease.11 However, Jouquey et al6 studied trandolapril at four dose levels but enalapril at only one dose level and did not include peripheral organs for comparison. At this dose, enalapril decreased ACE activity only in brain areas not protected by the BBB.

The aim of the present study was therefore to assess the effects of chronic subcutaneous administration of two ACE inhibitors, the lipophilic trandolapril and the hydrophilic lisinopril, both over a large dose range, on brain ACE binding inside and outside the BBB relative to inhibition of ACE in peripheral organs, using in vitro autoradiography.12

Materials and Methods
Animals
Male Wistar rats (200 to 250 g; Charles River, Montreal, PQ, Canada) were housed at 24°C on a 12-h light/dark cycle, fed with regular rat chow, and allowed tap water ad libitum for at least 5 days before entering the study. All experimental procedures were approved and carried out in accordance with the guidelines of the University of Ottawa Animal Care Committee for the use and care of laboratory animals.

Experimental Groups
The rats were injected subcutaneously once daily with either ACE inhibitor at a low, intermediate, or high dose or their vehicles for 6 days using the following treatments: trandolapril (0.2, 1, and 5 mg/kg/day); lisinopril (2, 10, and 50 mg/kg/day); or vehicles (methanol water for trandolapril and 0.9% saline for lisinopril). In each group, six rats were killed 4 h after the last dose and another six rats 24 h after last dose (the latter only for the intermediate and high doses). For lisinopril, previous studies8,9 used 10 mg/kg, which resulted in marked inhibition of ACE in plasma and kidneys at 4 h but no longer at 24 h after dosing. Five-fold lower and higher doses were used for the low and high dose, respectively. A similar approach was used for trandolapril dosing based on the study by Jouquey et al6 which showed marked inhibition of ACE activity at trandolapril 1 mg/kg/day via the drinking water.

Autoradiography
The derivate of lisinopril, 351A (kindly donated by Dr. Sun, University of Tennessee Health Science Center, Memphis, TN) was iodinated by the chloramine T method.13 Tissues were rapidly sampled as described previously.14 Cryostat sections (20 μm) were incubated with 0.3 μCi/mL (30 pmol/L) of 125I-351A in 10 mmol/L phosphate buffer (pH 7.4) containing 150 mmol/L NaCl, 0.2% bovine serum albumin (BSA) at room temperature for 1 h according to Mendelsohn et al.15 After washes in ice-cold buffer without BSA, the slides were dried and then exposed to Kodak Biomax MR film (Eastman Kodak Co., Rochester, NY). In each cassette, a set of methylacrylate iodine-125 (125I) standards (Washington State University Peptide Radioiodination Service Center, Pullman, WA) was included. The 125I-351A binding density was quantified by densitometry and converted to femtomoles per gram of tissue (wet weight) by comparing with the calibrated relative optical density of 125I-standards. Non-specific binding was detected in the presence of 100 mmol/L EDTA, which completely abolished the 125I-351A binding signal.

The brain nuclei in the cryostat sections were defined according to Paxinos and Watson.15 For nuclei inside the BBB, the PVN, median preoptic nucleus (MnPO), median eminence (Me), globus pallidus (GP), and caudate putamen (Cpu) were chosen to represent nuclei with high densities of ACE involved in cardiovascular regulation (PVN, MnPO, Me) or as “control” nuclei with high ACE.16 For nuclei outside the BBB, the OVLT and SFO were chosen as nuclei involved in cardiovascular regulation. As peripheral tissues, the heart and kidneys were included.

Statistical Analysis
Data are presented as means ± SE. Differences between groups were compared by two-way analysis of variance. The level of statistical significance was set at P < .05. In all instances, the two different vehicle groups showed the same values and results were therefore combined. For relative changes caused by the two ACE inhibitors, the mean value of the control group was used to calculate percent changes.

Results
The location of 125I-351A binding in brain areas studied is presented in Fig. 1. Tables 1 and 2 show the absolute binding densities and Figs. 2 and 3 show the percent inhibition caused by the two ACE inhibitors. Differences in vehicle (data not shown) and time (Tables 1 and 2) after the last injection of the vehicle did not influence the binding densities. Both trandolapril and lisinopril administered subcutaneously for 6 days caused dose-dependent inhibition of 125I-351A binding in the brain nuclei studied (Tables 1 and 2, Fig. 2). At 4 h after the last dose, in the PVN, MnPO, Me, brain nuclei inside the BBB, at the two lower dose levels of trandolapril was more potent than lisinopril in inhibiting 125I-351A binding. At the low dose (0.2 mg/kg/day for trandolapril and 2 mg/kg/day for lisinopril), the inhibition for trandolapril was 54% to 56% but only 24% to 33% for lisinopril. At the medium dose (1 mg/kg/day for trandolapril and 10 mg/kg/day for lisin-
opril), the inhibition for trandolapril was 84% to 87% but only 71% to 72% for lisinopril. At the high dose (5 mg/kg/day for trandolapril and 50 mg/kg/day for lisinopril), the inhibition was similar for trandolapril (87% to 93%) and lisinopril (84% to 86%) (Fig. 2). On the other hand, in the SFO and OVLT, brain areas outside the BBB, the inhibition of binding was almost the same for trandolapril and lisinopril at each dose level (Table 1).

At 24 h after the last dose, the inhibition had diminished but significantly less so with trandolapril. Outside the BBB (ie, in the SFO and OVLT), at the medium dose, 36% to 46% inhibition remained for lisinopril and 52% to 64% for trandolapril. At the high dose, 55% to 65% inhibition remained for lisinopril and more than 75% inhibition still for trandolapril (Table 1, Fig. 2).

In the kidney, trandolapril was more effective than lisinopril for inhibition of the $^{125}$I-351A binding at all three dose levels 4 h after dosing but was particularly more effective after 24 h when most of the lisinopril-induced inhibition had disappeared; but this was not the case for trandolapril (Table 2, Fig. 3). In contrast, in the heart, no significant differences were found between the two ACE inhibitors at the 4-h time-point; but after 24 h, use of trandolapril resulted in more persistent inhibition (Table 2, Fig. 3).

**Discussion**

Both lisinopril and trandolapril caused significant inhibition of $^{125}$I-351A binding on ACE in the CNS and peripheral tissues but the relative potency was markedly varied. At lower doses, trandolapril caused better inhibition in some brain structures inside the BBB (ie, the PVN and MnPO) and in the kidneys, similar inhibition in nuclei outside the BBB (the OVLT and SFO) and the heart but less inhibition in two brain areas inside the BBB (the Cpu and GP). The difference in the extent of inhibition by these two ACE blockers may be due largely but not solely to the lipophilicity of the two inhibitors.

In contrast to the above pattern, the extent of the inhibition of $^{125}$I-351A binding on ACE in the brain regions Cpu and GP was the opposite (Table 2); that is, administration of lisinopril was associated with a greater inhibition of $^{125}$I-351A binding in the Cpu and GP than trandolapril (4 h after last dose, ~70% at the medium dose and ~85% at the high dose for lisinopril and 50% to 60% at the medium dose and ~70% at the high dose for trandolapril).

Drugs distribution into the brain requires penetration through the BBB, which depends on a number of factors, such as drug size, lipophilicity and concentration. The BBB consists of a continuous layer of endothelial cells joined by tight junctions. For drugs that are transported by
Table 1. Angiotensin-converting enzyme densities in brain areas involved in cardiovascular regulation in groups of rats treated for 6 days with either vehicle, lisinopril or trandolapril, at 3 dose levels

<table>
<thead>
<tr>
<th>Brain areas outside BBB</th>
<th>Control</th>
<th>Low dose</th>
<th>Medium dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tran</td>
<td>Lisi</td>
<td>Tran</td>
<td>Lisi</td>
</tr>
<tr>
<td>OVLT</td>
<td>2335 ± 34</td>
<td>939 ± 18</td>
<td>1227 ± 79*</td>
<td>312 ± 11</td>
</tr>
<tr>
<td></td>
<td>7199 ± 48</td>
<td>2891 ± 46</td>
<td>3030 ± 118</td>
<td>2755 ± 54</td>
</tr>
<tr>
<td>SFO</td>
<td>4 h after last dose</td>
<td>79* 312/11006</td>
<td>18 1227/11006</td>
<td>1489 ± 29</td>
</tr>
<tr>
<td></td>
<td>22 336/11006</td>
<td>7 389/11006</td>
<td>1609 ± 31</td>
<td></td>
</tr>
<tr>
<td>Brain areas inside BBB</td>
<td>Control</td>
<td>Low dose</td>
<td>Medium dose</td>
<td>High dose</td>
</tr>
<tr>
<td></td>
<td>Tran</td>
<td>Lisi</td>
<td>Tran</td>
<td>Lisi</td>
</tr>
<tr>
<td>PVN</td>
<td>1391 ± 28</td>
<td>565 ± 12</td>
<td>849 ± 41*</td>
<td>135 ± 7</td>
</tr>
<tr>
<td></td>
<td>821 ± 20</td>
<td>361 ± 20</td>
<td>603 ± 29*</td>
<td>139 ± 11</td>
</tr>
<tr>
<td></td>
<td>1038 ± 24</td>
<td>561 ± 16</td>
<td>803 ± 53*</td>
<td>130 ± 9</td>
</tr>
<tr>
<td>MnPO</td>
<td>24 h after last dose</td>
<td>95 ± 7</td>
<td>187 ± 39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>92 ± 9</td>
<td>127 ± 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me</td>
<td>138 ± 11</td>
<td>110 ± 7</td>
<td>138 ± 11</td>
<td></td>
</tr>
<tr>
<td>24 h after last dose</td>
<td>250 ± 11</td>
<td>629 ± 8*</td>
<td>151 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>243 ± 12</td>
<td>610 ± 20*</td>
<td>134 ± 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>183 ± 11</td>
<td>700 ± 26*</td>
<td>205 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19 ± 20</td>
<td></td>
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</tbody>
</table>

ACE = angiotensin-converting enzyme; GP = globus pallidus; Lisi = lisinopril; Me = median eminence; MnPO = median preoptic nucleus; OVLT = organum vasculosum laminae terminalis; PVN = paraventricular nucleus; SFO = subfornical organ; Tran = trandolapril.

Results are expressed as means ± SE of bound $^{125}$I-351A (fmol/g wet tissue), n = 12 for control groups and n = 6 for ACE inhibitor groups.

All ACE densities in the ACE inhibitor–treated groups are significantly lower than the densities in the control group.

* P < .05, lisinopril v trandolapril.
Table 2. Angiotensin-converting enzyme densities in the brain areas globus pallidus and caudate putamen, and the peripheral tissues of heart and kidney in groups of rats treated for 6 days with vehicle, lisinopril, or trandolapril at three dose levels

<table>
<thead>
<tr>
<th>Brain areas</th>
<th>Control</th>
<th>Low dose</th>
<th>Medium dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP</td>
<td>162</td>
<td><strong>123</strong></td>
<td><strong>145</strong></td>
<td><strong>167</strong></td>
</tr>
<tr>
<td>Cpu</td>
<td>134</td>
<td><strong>105</strong></td>
<td><strong>128</strong></td>
<td><strong>150</strong></td>
</tr>
<tr>
<td>Heart (LV)</td>
<td>112</td>
<td><strong>83</strong></td>
<td><strong>106</strong></td>
<td><strong>129</strong></td>
</tr>
<tr>
<td>Kidney (PCT)</td>
<td>98</td>
<td><strong>79</strong></td>
<td><strong>92</strong></td>
<td><strong>115</strong></td>
</tr>
</tbody>
</table>

ACE = angiotensin-converting enzyme; Cpu = caudate putamen; GP = globus pallidus; Lisi = lisinopril; Tran = trandolapril.

Results are expressed as mean ± SE of bound 125I-351A (fmol/g wet tissue), *n = 12 for control group and n = 6 for ACE inhibitor groups.

In conclusion, our data provide the first direct comparison of the effects of chronic peripheral administration over a large dose range of a lipophilic versus a hydrophilic ACE inhibitor.

passive diffusion, there is a good correlation between lipophilicity and permeability rate across the BBB. The ACE inhibitors are all drugs of low molecular weight (200 to 500 daltons), and they are most likely to penetrate the BBB by passive diffusion. Therefore, the lipophilicity of the drug may play a very important role in brain diffusion after peripheral administration of these drugs. Indeed, previous studies have shown that trandolapril caused marked inhibition of ACE activity in brain regions such as SON and PVN but that the hydrophilic inhibitor enalapril did not. After a single dose, lisinopril (10 mg/kg orally) inhibited ACE binding only in brain regions with deficient BBB (ie, outside the BBB). In contrast, the current data show that at low or regular doses the lipophilic trandolapril causes better inhibition in some areas (ie, the PVN, MnPO, and Me) but less in other areas (the Cpu and GP) inside the BBB than the hydrophilic lisinopril, whereas at higher doses lisinopril also causes marked inhibition in PVN and MnPO. In the present study, after 6 days of treatment with lisinopril, even at the low dose of 2 mg/kg/day subcutaneously, caused a significant inhibition of ACE binding inside BBB. This finding is in contrast to those of Sakaguchi et al, whose results derived from a single dose of 10 mg/kg orally. Therefore, it appears that with chronic dosing, the hydrophilic ACE blocker lisinopril can also have significant effects inside the brain BBB.

In contrast to the expected results on ACE binding in the PVN and MnPO, the binding data on Cpu and GP showed an opposite pattern (that is, greater inhibition by lisinopril than by trandolapril). A possible explanation might be that the ACE in the Cpu and GP is different from the ACE in PVN and MnPO. Chai et al reported a close correspondence between the distribution of ACE and angiotensin II (Ang II) in the brain nuclei (PVN, SON, MnPO, SFO, and OVLT) but a striking discrepancy in the basal ganglia (including the Cpu and GP), which contain very little Ang II and Ang II receptors but are very rich in ACE. These data also suggest that the ACE present in Cpu and GP may be different from the ACE in other brain nuclei.

The heart and kidneys also showed a different pattern of responses to the two ACE inhibitors. Across the dose range, trandolapril and lisinopril caused fairly similar inhibition in the heart, but in the kidney trandolapril was clearly more effective, with a twofold difference in remaining free ACE across the dose range 4 h after dosing. In addition, despite a similar plasma half-life, trandolapril use resulted in more persistent inhibition than did lisinopril, in both the periphery and CNS. At 24 h after the last dose, the remaining inhibition of ACE binding by trandolapril was much higher than that by lisinopril. In most areas, and particularly the kidneys but again not in the brain areas Cpu and GP, this phenomenon may depend on differences in tissue retention and binding affinity of the inhibitor.
ACE inhibitor on ACE binding in ACE containing nuclei inside versus outside the BBB. These results indicate that 1) trandolapril causes more ready inhibition of ACE binding in cardiovascular regulatory centers inside the BBB than does lisinopril, while producing less inhibition in the basal ganglia; and 2) the inhibition of ACE binding caused by trandolapril is more persistent than that caused by lisinopril in most, but not all, brain areas as well as in heart and particularly the kidneys.

There are several clinical implications to our findings. Tissue RAS and tissue ACE are involved in the pathogenesis of a wide spectrum of cardiovascular diseases. Differences in blockade of tissue RAS may therefore have potentially important therapeutic implications. It appears that for equivalent blockade in some areas at one time point (e.g., in the heart or SFO at 4 h after dosing), lipophilic blockers cause better blockade in other areas (e.g., the PVN) as well as causing greater and more persistent blockade in the kidneys, for example. Whether these differences are sufficient to translate into different therapeutic benefits requires further studies relating outcome parameters to degree of inhibition of tissue ACE.

**FIG. 2** Dose-dependent inhibition of $^{125}$I-351A binding in the PVN and MnPO 4 and 24 h after last dose of trandolapril and lisinopril administered for 6 days. Results are expressed as percent inhibition relative to control groups, and as mean ± SEM $(n = 6/dose)$. 0% = no inhibition; 100% = full inhibition; other abbreviations as in Fig. 1. *$P < .05$ trandolapril v lisinopril.

**FIG. 3** Inhibition of $^{125}$I-351A ACE binding in heart and kidney at 4 and 24 h after last dose of trandolapril and lisinopril, administered for 6 days. Results are expressed as percent inhibition relative to control groups, and as mean ± SEM $(n = 6/dose)$. 0% = no inhibition; 100% = full inhibition; other abbreviations as in Fig. 1. *$P < .05$, trandolapril v lisinopril.
References


