Orally Active Aminopeptidase A Inhibitors Reduce Blood Pressure
A New Strategy for Treating Hypertension

Laurence Bodineau, Alain Frugière, Yannick Marc, Nicolas Inguimbert, Céline Fassot, Fabrice Balavoine, Bernard Roques, Catherine Llorens-Cortes

Abstract—Overactivity of the brain renin-angiotensin system has been implicated in the development and maintenance of hypertension. We reported previously that angiotensin II is converted to angiotensin III by aminopeptidase A in the mouse brain. We then used specific and selective aminopeptidase A inhibitors to show that angiotensin III is one of the main effector peptides of the brain renin-angiotensin system, exerting tonic stimulatory control over blood pressure in hypertensive rats. Aminopeptidase A, the enzyme generating brain angiotensin III, thus represents a potential candidate central nervous system target for the treatment of hypertension. Given this possible clinical use of aminopeptidase A inhibitors, it was, therefore, important to investigate their pharmacological activity after oral administration. We investigated RB150, a dimer of the selective aminopeptidase A inhibitor, EC33, generated by creating a disulfide bond. This chemical modification allows prodrug to cross the blood-brain barrier when administered by systemic route. Oral administration of RB150 in conscious DOCA-salt rats inhibited brain aminopeptidase A activity, resulting in values similar to those obtained with the brains of normotensive rats, demonstrating the central bioavailability of RB150. Oral RB150 treatment resulted in a marked dose-dependent reduction in blood pressure in DOCA-salt but not in normotensive rats, with an ED50 in the 1-mg/kg range, achieved in <2 hours and lasting for several hours. This treatment also significantly decreased plasma arginine-vasopressin levels and increased diuresis, which may participate to the blood pressure decrease by reducing the size of fluid compartment. Thus, RB150 may be the prototype of a new class of centrally active antihypertensive agents. (Hypertension. 2008;51:1-8.)

Key Words: aminopeptidase A inhibitors ■ blood pressure ■ brain renin-angiotensin system ■ DOCA-salt rats ■ hypertension

Hypertension is a major cardiovascular risk factor, affecting ≈20% of the adult population, of which 95% have essential hypertension. Blockers of the systemic renin-angiotensin system (RAS), angiotensin (Ang) I–converting enzyme (ACE; EC 3.4.15.11) inhibitors or AngII receptor type 1 (AT1) antagonists, have since been shown to be efficient and safe for treating hypertension.1 However, they may cause secondary effects, such as coughing, deterioration of renal function in cases of underlying renal artery stenosis, and, more rarely, angioedema.2–7 In addition, these agents are not very effective in some patients, particularly African Americans, in whom high blood pressure (BP) is associated with low renin levels and a response to salt depletion.8,9 The development of new classes of antihypertensive agents with different mechanisms of action, therefore, remains an important goal. In this way, because a brain RAS hyperactivity has been implicated in the development and maintenance of hypertension,10–13 its components could constitute interesting targets. Among the bioactive peptides of the brain RAS, AngII and AngIII display similar affinities for AT1 receptors.14,15 When injected into the brain, these peptides similarly increase BP.16,17 However, because AngII is converted in vivo into AngIII, the nature of the effector peptide of the brain RAS remains to be define. We first developed specific and selective aminopeptidase A (APA; EC 3.4.11.7) and aminopeptidase N (APN; EC 3.4.11.2) inhibitors, (3S)-3-amino-4-sulfanyl-butane-1-sulfonic acid (EC33) and (2S)-2-amino-4-methylsulfanyl butane thiol (PC18), respectively,18,19 and used these new tools to demonstrate in the murine brain that APA and APN are involved in vivo in the metabolism of brain AngII and AngIII, respectively (Figure 1).20 Then, we showed in rats that ICV injection of EC33 blocked the pressor response of exogenous AngII,21 suggesting that, in the brain, the conversion of AngII to AngIII is required to increase BP.
The specificity of action of EC33 on AngII metabolism was shown by the lack of effect of EC33 on the AngIII-induced BP increase. More interestingly, ICV injection, but not IV injection, of EC33 alone caused a dose-dependent decrease in BP in alert spontaneously hypertensive rats, a model of essential hypertension sensitive to RAS blockers, and DOCA-salt rats, a salt- and volume-dependent model of hypertension resistant to blockade of the brain RAS.21 We, therefore, designed 4,4′-dithio [bis((3S)-3-aminobutyl sulfonic acid)] (RB150),24 a systematically active prodrug of EC33 obtained by disulfide bridge-mediated dimerization. We showed previously that IV RB150 enters the brain, blocks brain RAS activity, and markedly reduces BP in conscious hypertensive DOCA-salt rats.24 If APA inhibitors are to be used as orally administered centrally active antihypertensive agents, we first needed to investigate their central bioavailability after oral administration, leading to blockade of the brain RAS.

In this study, we demonstrated that RB150 was able to enter the brain, after PO administration, and caused a sustained inhibition of APA, thereby decreasing BP in conscious hypertensive DOCA-salt rats. RB150 also decreased plasma arginine vasopressin (AVP) levels, increased diuresis, and induced a mild natriuresis, without affecting kaliuresis, thereby reducing blood volume. RB150, therefore, constitutes the first orally active inhibitor of central APA and could be the prototype of a new class of antihypertensive agents that could be clinically tested as an additional treatment for hypertension.

Materials and Methods

Drugs
RB150 and EC33 were synthesized by the team of Roques and colleagues.18,19 The drugs were dissolved in saline and adjusted to pH 7.4 for in vivo administration.24 RB150 or EC33 was dissolved in 50 mmol/L of Tris-HCl (pH 7.4) supplemented with 100 equimolar of dithiothreitol per equimolar of inhibitor for the in vitro measurement of APA activity.18,24

Animals
Experiments were performed on male normotensive Wistar Kyoto (WKY), sham, and hypertensive DOCA-salt rats (200 to 300 g, Charles River Laboratories, L’Arbresle, France). Sham and DOCA-salt rats were obtained as described previously.24 All of the animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

APA Activity Measurements
In vitro APA enzymatic activity was determined using recombinant mouse APA and 2-[l-glutamyl-β-naphthylamide (Bachem) as a synthetic substrate, as described previously (please see http://hyper.ahajournals.org).18,29 The inhibitory potencies of RB150 and EC33 were evaluated in parallel (n = 15).30 For ex vivo APA enzymatic activity measurements, rats were deprived of food 12 hours before a 300-μL oral administration of saline or RB150 (7.5 to 50.0 mg/kg). Brains were collected 3.5 or 24.0 hours later, and one half of the brain was homogenized by sonication.24 Bestatin, an inhibitor of nonspecific aminopeptidases, was added at a concentration not.
Table 1. In Vitro Pharmacological Inhibition Profiles of RB150 and EC33 Against Receptors and Enzymes Involved in BP Control

<table>
<thead>
<tr>
<th>Target</th>
<th>RB150, 1 μmol/L</th>
<th>RB150, 10 μmol/L</th>
<th>EC33, 1 μmol/L</th>
<th>EC33, 10 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1*</td>
<td>−7</td>
<td>4</td>
<td>−10</td>
<td>1</td>
</tr>
<tr>
<td>AT2†</td>
<td>−3</td>
<td>−5</td>
<td>2</td>
<td>−9</td>
</tr>
<tr>
<td>ETA§</td>
<td>−6</td>
<td>−7</td>
<td>−4</td>
<td>22</td>
</tr>
<tr>
<td>ETB§</td>
<td>−16</td>
<td>−12</td>
<td>−10</td>
<td>−9</td>
</tr>
<tr>
<td>ACE</td>
<td></td>
<td>3</td>
<td>−5</td>
<td>−17</td>
</tr>
<tr>
<td>ACE-2†</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>−10</td>
</tr>
<tr>
<td>ECE-1#</td>
<td>−4</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>NEP**</td>
<td>4</td>
<td>16</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

All of the values are mean values of a percent of inhibition of control-specific binding (n=2).

*Displacement of [125I][Sar1, Ile8]-AngII from human recombinant AT1(h) expressed in HEK-293 cells (reference compound: saralasin; K: 0.63 nmol/L).
†Displacement of [125I]IC50 42112A from human recombinant AT2(h) expressed in Hela cells (reference compound: saralasin; K: 0.14 nmol/L).
‡Displacement of [125I]endothelin-1 from human recombinant ETA(h) expressed in Chinese hamster ovary cells (reference compound: endothelin-1; K: 0.2 nmol/L).
§Displacement of [125I]endothelin-3 from human recombinant ETB(h) expressed in Chinese hamster ovary cells (reference compound: endothelin-1; K: 0.2 nmol/L).

Human recombinant ACE(h) expressed in murine cells (substrate: MCA-Ang-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys-OH; reference compound: captopril; IC50: 5.6 nmol/L).
Human recombinant ECE-1(h) expressed in NSO cells (substrate: ECE-1 fluorescent substrate; reference compound: phosphoramidon; IC50: 27 nmol/L).
**Human recombinant NEP(h) expressed in HUV-EC-C cells (substrate: DAPNPs; reference compound: thrombin; IC50: 0.55 nmol/L).

Sufficient to inhibit APA activity (1 μmol/L). Assays were performed in presence or absence of EC33 (5 μmol/L).

In Vitro Pharmacological Profile

Binding affinities and inhibition activities of RB150 (1 and 10 μmol/L) and EC33 (1 and 10 μmol/L) for AT1, AT2, and endothelin A and B, and against ACE, angiotension-converting enzyme 2 (ACE-2), endothelin-converting enzyme 1 (ECE-1), and neutral endopeptidase 24.11 (NEP; Table 1) were assessed in in vitro assays performed at Cerep Co.

Surgical Methods and BP Recording

Catheters were implanted in the femoral artery and vein. RB150 (0.1 to 30.0 mg/kg PO), EC33 (15 mg/kg IV), or saline (PO) was administered (300 μL) in conscious, unrestrained rats (please see http://hyper.ahajournals.org). After PO acute treatment with RB150 or saline, BP was monitored during 7 hours on the first day of experiment. For DOCA-salt rats given saline or RB150 (15 mg/kg), another recording of 1 hour was performed at 24 and 48 hours postadministration.

For DOCA-salt rats treated with EC33, BP was recorded for 5 hours after the IV injection. Mean BP (MBP) and heart rate (HR) were calculated with the MacLab system (Phymep).21,24

Urinary Sodium and Electrolyte Output and Fluid Consumption Measurements

Normotensive WKY and hypertensive DOCA-salt rats were individually housed in metabolic cages (Tecniplast). After acclimatization over a period of 3 days, fluid consumption, urinary excretion of water, and electrolytes were measured for each rat after saline or RB150 (15 mg/kg) PO administration. Urinary Na+ and K+ concentrations were determined using a flame photometer (model 243, Instrumentation Laboratory).

AVP Radioimmunoassay

Plasma AVP concentrations were estimated as described previously (please see http://hyper.ahajournals.org) from 0.2 mL of plasma using a specific radioimmunoassay kit (Peninsula Laboratories).

Data Analysis and Statistics

Data are presented as means±SEMs. Comparisons among groups of APA activities, baseline MBP and HR (values obtained 1 hour before PO administration), MBP and HR after RB150 and EC33 administration, plasma AVP concentration, drinking fluid, urine flow rate, and urinary electrolytes were made with paired or unpaired Student’s t test or 1-way ANOVA for repeated measures and ANOVA followed by Fisher’s posthoc least-squares differences correction for multiple comparisons. Differences were considered significant if P value was <0.05.

Results

Selective Inhibition of Recombinant Purified Mouse APA by RB150

In vitro selectivity on recombinant purified mouse APA showed that the inhibitory potency of the reduced form of RB150 (inhibition constant [K]: 1.7±0.2×10−7 mol/L) obtained in the presence of dithiothreitol was similar to that of EC33 (K: 2.4±0.1×10−7 mol/L). In the absence of dithiothreitol, RB150, with an intact disulfide bridge, had no effect on APA activity (K: >10−7 mol/L). The concentration of dithiothreitol used was also inactive against APA. Neither RB150 nor EC33 showed affinity for recombinant AT1, AT2, endothelin A, and endothelin B receptors when tested at 1 and 10 μmol/L in various in vitro binding assays. RB150 and EC33 were also found not to inhibit enzymatic activity of ACE, ACE-2, ECE-1, and NEP (Table 1).

Inhibitory Effect of Oral RB150 Administration on Brain APA Activity in Conscious Hypertensive DOCA-Salt Rats

Brain APA activity in hypertensive DOCA-salt rats was 103% and 98% higher than that in normotensive WKY and sham rats (91.5±9.9 nmol of β-naphthylamide (βNA) per milligram of protein per hour versus 45.1±4.1 and 46.2±3.7 nmol of βNA per milligram of protein per hour, respectively; Figure 2A).

PO RB150 administration (7.5 to 50 mg/kg) in DOCA-salt rats progressively inhibited brain APA activity after 3.5 hours (Figure 2A). With doses of 15, 30, and 50 mg/kg, APA activity was decreased to ≈54% of that in DOCA-salt rats given saline PO (43.6±3.8, 41.2±5.0, and 42.6±3.3 nmol of βNA per milligram of protein per hour, respectively, versus 91.5±9.9 nmol of βNA per milligram of protein per hour) and reached the control levels measured in normotensive WKY or sham rats given saline PO (Figure 2A). The brain APA activity of DOCA-salt rats, which have received RB150 (15 mg/kg), is at 93.4±10.0 nmol of βNA per milligram of protein per hour for 24 hours after the administration. The brain APA activity of normotensive WKY rats was not modified by the PO RB150 administration (15 mg/kg):
Oral RB150 Administration Decreases BP in Conscious Hypertensive DOCA-Salt Rats

The baseline MBP of hypertensive DOCA-salt rats (149.5±3.5 mm Hg; n=38) was significantly higher than that of normotensive WKY (n=5) and sham (n=4) rats (105.2±4.1 and 106.9±2.0 mm Hg, respectively). Basal HR was 343.1±7.0 bpm in hypertensive DOCA-salt rats, 371.0±15.5 bpm in normotensive WKY rats, and 330.5±10.0 bpm in sham rats. No significant difference in baseline MBP and HR was found between the different groups of hypertensive DOCA-salt rats used in this study.

The PO administration of RB150 (0.1 to 30.0 mg/kg) decreased MBP in a dose-dependent manner in conscious DOCA-salt rats, with an ED50 of 0.84 mg/kg (Figure 2B and 2C), but did not significantly decrease HR (Table 2). The minimum dose of RB150 (PO) required to normalize brain APA activity and to observe a maximal decrease (−29.0±4.3 mm Hg) in MBP in DOCA-salt rats was 15 mg/kg. The hypotensive effect began 2 hours after administration, was maximal at 5 hours, and remained significant after 7 hours. A nonsignificant hypotensive effect remained after 24 hours, but this effect had disappeared after 48 hours (Figure 2D). The oral administration of saline in conscious DOCA-salt rats, used as an internal control, did not significantly alter MBP or HR, regardless of the time elapsed between administration and recording (between 0 and 48 hours). Interestingly, 5 hours after its oral administration, RB150 (15 mg/kg) had no significant effect on MBP or HR in conscious normotensive WKY and sham rats (Table 2), whereas it decreased MBP in DOCA-salts rats to values significantly different not only from baseline (Figure 2) but also from variations in MBP measured in normotensive WKY and sham rats (Table 2). In contrast, the IV injection of EC33 (15 mg/kg) into conscious DOCA-salt rats did not modify MBP or HR (ΔMBP: −6.0±5.6 mm Hg and ΔHR: 4.0±11.7 bpm) corresponded with the maximal changes obtained 4.0±0.4 hours after injection; n=5; Table 2).

Oral RB150 Administration in Conscious Hypertensive DOCA-Salt Rats Enhances Diuresis, Slightly Affecting Natriuresis but not Kaliuresis, With No Effect on Water Intake

Oral RB150 (15 mg/kg) administration in DOCA-salt rats increased the urinary excretion of water by 50% (3.6±0.3 mL/100 g) and Na⁺ excretion by 13% (61.8±3.7 μmol/L per 100 g) over those after the oral administration of saline, for a 5-hour analysis (2.4±0.7 mL/100 g, P<0.05, and 54.8±3.8 μmol/L per 100 g, P<0.04, respectively; Figure 3A and 3C). No parallel change in kaliuresis was observed (Figure 3B). The increases in urinary water and Na⁺ excretion became significant 2 hours after oral administration of the drug (Figure 3A and 3C). In contrast, in the same experimental conditions, we observed no difference in water intake after
oral RB150 and oral saline administration in a 5-hour analysis (Figure 3D).

**Oral RB150 Administration in Conscious Normotensive WKY Rats Did Not Modify the Diuresis, Natriuresis, Kaliuresis, and Water Intake**

The oral administration of RB150 (15 mg/kg; n=5) in normotensive WKY rats did not induced any significant change in diuresis (1.0±0.1 and 0.8±0.1 mL/100 g, respectively; paired Student’s t test P=0.07), water intake (1.5±0.1 and 1.8±0.1 mL/100 g, respectively; paired Student’s t test P=0.13), natriuresis (48.6±11.4 and 47.4±13.6 µmol/L per 100 g; paired Student’s t test P=0.40) or kaliuresis (63.3±11.3 and 61.6±5.0 µmol/L per 100 g; paired Student’s t test P=0.43) for a 5-hour analysis as compared with normotensive WKY rats receiving an oral saline administration.

**Effect of an Oral Administration of RB150 on Plasma AVP Levels**

Plasma AVP levels in DOCA-salt rats (saline PO; n=5) were 59% higher than plasma AVP levels in normotensive WKY (saline PO; n=4; 25.3±0.6 and 15.9±1.3 pg of AVP per milliliter of plasma, respectively; unpaired Student’s t test P=0.01). In DOCA-salt rats receiving RB150 (15 mg/kg, PO) plasma AVP levels were 20.3±1.5 pg/mL. The difference in plasma AVP levels between normotensive WKY and DOCA-salt rats receiving saline (PO) is 9.4 pg/mL, and the difference in plasma AVP levels between normotensive WKY and DOCA-salt rats receiving RB150 (15 mg/kg, PO) is 4.4 pg/mL. This showed that RB150 treatment reduced by 54% the increase in plasma AVP levels observed in DOCA-salt rats as compared with normotensive WKY rats (unpaired Student’s t test P<0.01).

**Discussion**

This study describes an original way for the acute normalization of BP in a salt-dependent model of hypertension based on oral administration of the EC33 APA inhibitor prodrug, RB150, producing the blockade of brain APA activity. After its oral administration, this prodrug enters the brain. Brain reductases then generate 2 active molecules of the specific and selective APA inhibitor EC33, inhibiting brain APA activity. This prevents brain AngIII formation and markedly decreases BP in conscious DOCA-salt rats. Oral administration of RB150 also decreases plasma AVP levels and increases diuresis, thereby reducing blood volume and participating in the decrease in BP.

As developed in the introduction, we reported previously that brain AngIII, generated by APA from brain AngII, is one of the main effector peptides of the brain RAS, increasing systemic AVP release and exerting a tonic central control over BP in alert hypertensive rats. In such a context, if APA inhibitors are to be used as central antihypertensive agents, they must be able to cross the blood-brain barrier and inhibit brain APA activity when administered orally. The central bioavailability of thiol inhibitors of zinc metallopeptidases has been shown to be enhanced by coupling a NEP inhibitor and an APN inhibitor via a disulfide bridge to produce a prodrug. We applied a similar approach, increasing the bioavailability of EC33 by dimerization involving a disulfide bond, to generate RB150. In this symmetrical molecule, the thiol group of EC33 is involved in a disulfide bridge and, therefore, cannot interact with the zinc atom present in the active site of zinc metallopeptidases, such as APA. In vivo, the disulfide bridge of the prodrug is cleaved by brain reductases, generating 2 active molecules of EC33, which inhibit APA. The selectivity of EC33 and RB150 toward APA was shown by the lack of affinity of these compounds for other zinc metallopeptidases involved in the production or metabolism of vasoactive peptides, such as APN, ACE, ACE-2, ECE-1, and NEP, as well as by the absence of binding of these compounds to AT1 and AT2 or endothelin A and B receptors (Table 1) known to be involved in BP regulation. Consistent with these results, the ICV injection of RB150 into conscious mice has been shown to induce an intense and sustained decrease in brain APA activity and AngIII formation, with a time course similar to that for the free thiol inhibitor EC33, demonstrating the rapid cleavage of RB150 in the brain to generate EC33. In a similar way, the oral administration of increasing doses of RB150 in DOCA-salt rats resulted in a progressive inhibition of brain APA activity 3.5 hours after treatment, with a maximal reduction of 54% observed for a dose of 15 mg/kg (12 000 nmol per rat). In these conditions, brain APA activity in RB150-treated DOCA-salt rats was similar to that measured in the brain of normotensive WKY rats. However, the RB150-induced decrease in brain APA activity in DOCA-salt rats disappeared.
occurs in normotensive mice after IV injection of a high dose showed previously that a reduction of brain APA activity orally received RB150 at a dose of 15 mg/kg. However, we intestinal, hepatic, and blood brain barriers. The brain APAported from the gastrointestinal tract to the blood and then toing that the disulfide-bonded dimer is very efficiently trans-

Effects of the oral administration of RB150 on the uri-

cation.37 Such an alteration could facilitate the penetration of
blood-brain barrier observed in the case of chronic hyperten-
sion.38 DOCA-salt rats, even at the high dose of 15 mg/kg (Table 2). The RB150-induced hypotensive effect was large and long lasting, but HR was not significantly affected, suggesting that the sensitivity of the baroreflex was decreased by brain AngIII, consistent with the findings of Lin et al.38 These data are consistent with previous data showing that RB150 given IV inhibits brain APA activity, blocks the formation of brain AngIII, and markedly reduces BP for up to 24 hours. Interestingly, there was a difference of ≈2 hours in the time required to obtain a maximal decrease in BP after the oral administration of 15 mg/kg of RB150 to DOCA-salt rats and that observed after IV injection, reflecting the time required for the prodrug to reach its central target. This illustrates the high potency of oral RB150 treatment to inhibit brain RAS activity, decreasing BP several hours after a single administration, without changing HR, in an experimental salt-dependent model of hypertension. The hypotensive effect of RB150 is a central but not a peripheral effect, resulting from the blockade of brain APA activity, because its active moeity, EC33, had no effect on BP when given IV to DOCA-salt rats, even at the high dose of 15 mg/kg (Table 2).

The brain RAS controls BP via 3 different mechanisms: increasing systemic AVP release, activating sympathetic premotor neuron activity in the rostral ventrolateral medulla (a key BP regulatory center containing the neurons generating vasomotor tone), and inhibiting the baroreflex in the nucleus of the solitary tract. Consistent with the mechanism of action of the brains RAS, the oral administration of RB150 to DOCA-salt rats induced a decrease in plasma AVP levels. This suggests that the oral administration of RB150, by generating EC33 in the brain and blocking the formation of brain AngIII, decreases, similar to EC33, the activity of AVP neurons, resulting in a decrease in AVP release into the bloodstream. The increase in diuresis observed after oral RB150 administration may, therefore, be because of the decrease in plasma AVP levels. The consequence of such an increase diuresis would be a reduction in blood volume that may participate in the decrease in BP. RAS hyperactivity has been observed in the brain of DOCA-salt rats and may be partly because of the greater APA activity.
observed in the brain of this strain compared with normotensive WKY rats.\textsuperscript{23,44} This may account for the high efficiency with which APA inhibitors decreased BP in this model.

The brain RAS is involved in drinking behavior,\textsuperscript{17,44} but we observed no change in water intake after oral RB150 administration. This result is consistent with previous studies showing either that AngII is the main effector peptide\textsuperscript{45–47} or that brain AngII and AngIII are both active in the induction of water intake.\textsuperscript{48}

**Perspectives**

RB150 is the first orally active prodrug inhibiting brain but not systemic RAS activity, strongly reducing BP several hours after a single administration, without changing HR, in an experimental salt-dependent model of hypertension. APA inhibitors may, therefore, constitute a new class of central antihypertensive agents that could be clinically tested as an additional therapy for the treatment of hypertensive patients. This treatment may be particularly beneficial in hypertensive patients with low plasma renin and high plasma AVP levels, resistant to the antihypertensive medication generally used.

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**Disclosures**

None.

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**Orally Active APA Inhibitors and Hypertension**

22. Corvol P, Llorens-Cortes C. PC18, a specific aminopeptidase N inhibitor, shows either that AngII is the main effector peptide\textsuperscript{45–47} or that brain AngII and AngIII are both active in the induction of water intake.\textsuperscript{48}


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