Effect of Losartan, a Nonpeptide Angiotensin II Receptor Antagonist, on Drinking Behavior and Renal Actions of Centrally Administered Renin (43551)

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Abstract. **Losartan, a nonpeptide angiotensin II receptor antagonist, was used to establish the role of brain AT_1 angiotensin II receptor subtype on the natriuretic, antidiuretic, and dipsogenic actions of centrally administered renin. Intracerebroventricular administration of renin reduces urine volume and increases sodium excretion and water intake in conscious, male, hydrated rats. Losartan (3 or 10 mg/kg, sc) reduced the increased sodium excretion and totally inhibited the antidiuretic action induced by intracerebroventricular renin. When both renin and Losartan were given intracerebroventricularly, at the highest dose, there was a potent inhibition of the antidiuretic and natriuretic actions. Peripheral and central administration of the AT_1 receptor blocker significantly lengthened the onset of drinking behavior and reduced the cumulative water intake observed after intracerebroventricular injection of renin.**

Our results strongly suggest that the brain AT_1 receptor subtype mediates the physiologic actions of angiotensin II, such as drinking behavior, the increase in sodium excretion, and vasopressin release. [P.S.E.B.M. 1993, Vol 202]

The renin-angiotensin system (RAS) is an important physiologic mechanism in the regulation of blood pressure, fluid, and electrolyte homeostasis in normal and in various pathophysiologic states through its effector peptide, angiotensin II (ANG) (1, 2). The existence of a brain renin-angiotensin system is now firmly established (3–7). Exogenous administration of renin or angiotensin II into the central nervous system markedly affects body hydration. Indeed, intracerebroventricular infusion of ANG in conscious rats increased water intake and sodium excretion and decreased plasma (Na^+) and urine volume (8–10), which suggests that brain RAS is reciprocally related to renal RAS and thus functioning to defend the body against hypernatremia. The mechanism of the natriuretic response after intracerebroventricular renin or ANG is still undefined. Angiotensin II exerts these effects through activation of specific receptors on brain areas, such as circumventricular structures (11–12). Therefore, intervention of RAS by drugs directed against the ANG receptor should provide insight into the possible mechanisms involved in these actions. Recently, with the development of selective nonpeptide ANG receptor antagonists, it has become evident that in peripheral tissues, such as the adrenal gland and uterus, there appear to be two different ANG receptor subtypes: AT_1 and AT_2 (13, 14). Binding of ANG to the AT_1 receptor is selectively displaced by Losartan and to the AT_2 receptor by CGP 42112A and PD 123177 (13–16). The brain contains both receptor subtypes distributed in localized areas (17). Specifically, AT_1 receptors, located in brain areas outside the blood-brain barrier such as the subfornical organ and nucleus of the solitary tract (17–19), are related to the homeostasis of fluid and electrolyte metabolism and blood pressure regulation. In addition, AT_1 receptors have also been localized in areas inside the blood-brain barrier and related to vasopressin secretion, such as the paraventricular nucleus

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of the hypothalamus (17, 18). In the present study, we evaluated the functional role of brain AT_1 receptors in the regulation of homeostasis of fluid and electrolytes.

Materials and Methods

Adult, male, Sprague-Dawley rats (230–290 g) were housed under controlled conditions of temperature and photoperiod (lights on from 0600 to 1800 hr) and provided with free access to laboratory chow and water. A cannula (20) was implanted in the left lateral cerebroventricle, 1 mm caudal to the coronal suture and 1.5 mm lateral to the midsagittal suture, with the aid of a stereotaxic instrument and under pentobarbital anesthesia (40 mg/kg, ip). The cannula was secured to the skull with acrylic cement. A minimum of 2 days was allowed for recovery. A single intracerebroventricular injection was made with a Hamilton syringe fitted with a stop to prevent needle penetration past the cannula tip.

Experimental Protocol. Two days after ventricular cannulation, the animals were randomly distributed into the following groups: Group 1 received a subcutaneous injection of saline; Group 2, received a subcutaneous dose of Losartan (L_1, 3 mg/kg, or L_2, 10 mg/kg, 1 hr before intracerebroventricular injections); Group 3, received an intracerebroventricular injection of saline (5 μl); and Group 4, was administered Losartan intracerebroventricularly (L_1, 10 μg/5 μl, or L_2, 100 μg/5 μl). Intracerebroventricular pretreatments were performed 5 min before intracerebroventricular renin or saline. Animals were weighed and placed in metabolic cages. At 0900 hr, half of the rats receiving each pretreatment were injected intracerebroventricularly, as a bolus in 10 sec, with saline solution (5 μl) or with renin in saline solution (10 mGU/5 μl). (Renin from porcine kidney [0.78 units/mg protein, 1 unit will liberate 100 μg of ANG I from angiotensinogen/hr at pH 6.0 and 37°C] was purchased from Sigma Chemical Co., St. Louis, MO), followed by 20 ml of water/kg orally. Urine was collected at 3 and 6 hr; the bladder was emptied at 6 hr by gentle suprapubic massage. Food and water were not available during the experiment. Ventricular cannula placement was confirmed postmortem by examining the distribution of an intracerebroventricular injection of 5 μl of fast green dye, given before animal sacrifice. Data were used only if the dye was distributed in the lateral, third, and fourth ventricles. Urine samples were assayed for sodium and potassium content by flame photometry.

Measurement of the Onset of Drinking Behavior. In the same experimental groups, and before oral hydration and urine collection, the drinking test was performed. The rats were placed individually in metabolic cages. Water and food were removed. Rats were injected with intracerebroventricular renin (10 mGU/5 μl) as described above, and tap water was presented at the front of the cage. The time of onset of drinking behavior was recorded during a 60-min period.

To assess drinking behavior, rats were divided into the following experimental groups: subcutaneously pretreated with saline, or Losartan-sc (3 or 10 mg/kg, sc, 1 hr before intracerebroventricular renin); and intracerebroventricularly pretreated with saline (5 μl), or Losartan-icv (10 or 100 μg/5 μl, 5 min before intracerebroventricular renin). After treatment, all the animals were injected with intracerebroventricular renin (10 mGU/5 μl) and the cumulative volume consumed was recorded every 15 min during 1 hr.

All data are presented as mean ± SE. Statistical differences between groups were analyzed using Students t test or two-way analysis of variance and by the Newman-Keul's Student range statistics.

Results

Effect of Subcutaneous Administration of Losartan on Renal Actions Induced by Intracerebroventricular Renin. The effect of subcutaneous administration of Losartan is illustrated in Figure 1. Two-way analysis of variance and Newman-Keul's test revealed that renin (R) clearly evoked a reduction of water excretion at the 3- and 6-hr collection periods (all $P < 0.01$) ($F(R) = 3$ hr, 17.05, and 6 hr, 7.1). This effect was prevented by L_2 pretreatment (at 3 hr, $V-R=L_1-R<L_2-S=L_2-R=L_1-S=V-S$, $L_2-R<V-R$, and at 6 hr, $V-R<L_1-S=L_2-R=L_2-S=L_1-S=V-S$). There was a significant interaction between pretreatment and intracerebroventricular renin at 3 hr ($F(I) = 3.4$; $P < 0.05$). The decrease in urinary volume was associated with an enhanced natriuresis at the 3- and 6-hr collection periods ($F(R) = 3$ hr, 12.2, and 6 hr, 26.8; $P < 0.001$). Losartan pretreatment reduced the increase of sodium excretion induced by intracerebroventricular renin (3 hr, $V-S=L_1-S=L_2-S=L_2-R<L_1-R=V-R$, $L_1-S=L_1-R=L_2-R$, and 6 hr, $V-S=L_1-S=L_2-S<V-R=L_1-R$, $V-R>L_2-R$). Intracerebroventricular renin-induced kaliuresis was statistically significant and was not altered by Losartan pretreatment ($F(R) = 3$ hr, 4.8, and 6 hr, 11.3; $P < 0.005$). The urinary Na to K ratio was increased by intracerebroventricular renin injection, an effect that was totally inhibited by Losartan pretreatment (all $P < 0.005$) (3 hr, $V-S=L_1-S=L_2-S=L_1-R=L_2-R<V-R$, and 6 hr, $V-S=L_1-S=L_2-S=L_2-R<V-R$, $L_1-R<V-R$).

Effect of Centrally Administered Losartan on Diuretic and Natriuretic Actions of Intracerebroventricular Renin. The effect in conscious hydrated rats of intracerebroventricular administration of either saline or two doses of Losartan (L_1, 10 μg/5 μl, or L_2, 100 μg/5 μl, icv, 5 min before intracerebroventricular renin) with and without renin is presented in Figure 2. Intracerebroventricular renin decreased the urine volume at 3 and 6 hr. The volume reduction induced by intracer-

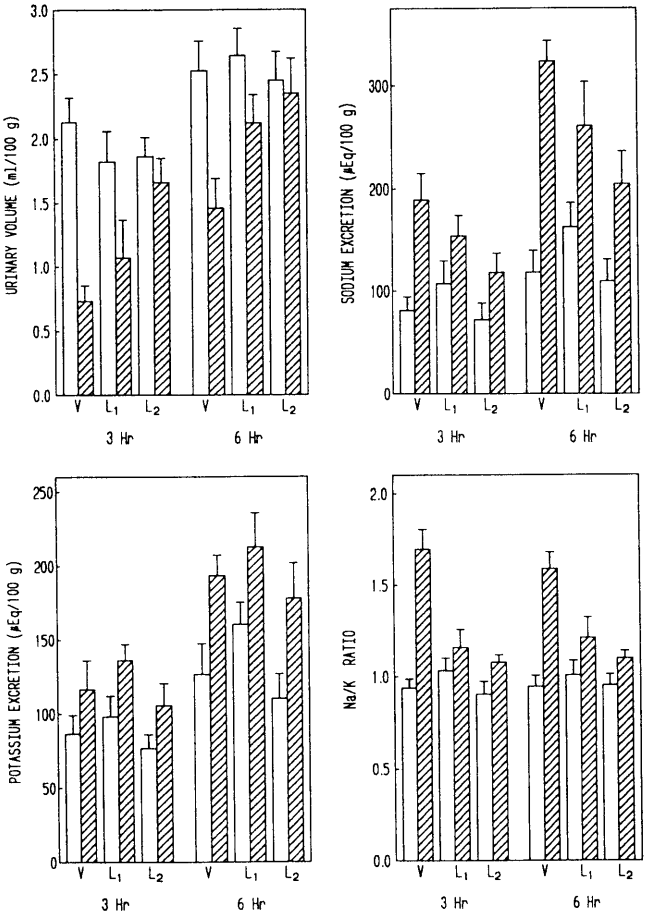

Figure 1. Urinary response to intracerebroventricular administration of renin in rats pretreated subcutaneously with Losartan (L), a nonpeptide angiotensin II receptor antagonist. Groups of rats were pretreated with vehicle (V) or two doses of Losartan (L_1, 3 mg/kg, or L_2, 10 mg/kg, sc), 1 hr before intracerebroventricular renin (10 mGU/5 μl) (dashed bars) or saline (5 μl) (open bars) followed by water orally (20 ml/kg) (n = 9–12 rats/group). The following results were significant (significant F ratios [$P < 0.01$] from two-way analysis of variance); volume-, Na^+, and Na to K ratio-renin effect at 3 and 6 hr and L effect at 3 and 6 hr ($P < 0.01$).

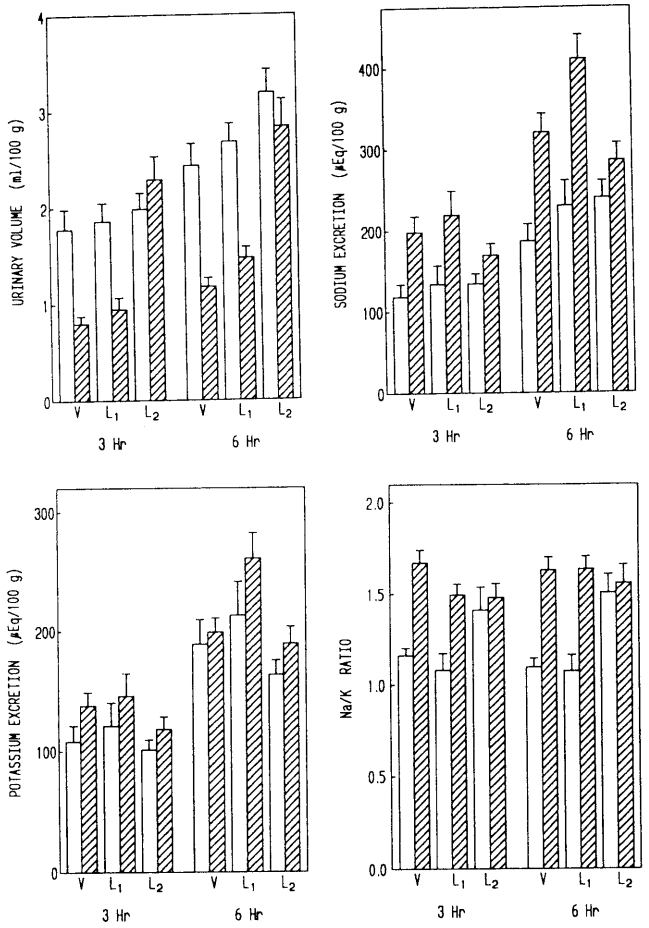

Figure 2. Urinary response to central administration of renin in rats pretreated intracerebroventricularly with Losartan. Groups of rats (12–21 rats/group) were intracerebroventricularly injected with renin (10 mGU/5 μl) (dashed bars) or saline (open bars) followed by water orally (20 ml/kg). Vehicle (V): intracerebroventricular administration of saline (5 μl); intracerebroventricular Losartan: L_1, 10 μg/5 μl, or L_2, 100 μg/5 μl. Significant F ratios ($P < 0.01$) from two-way analysis of variance were volume-, Na^+- and Na to K ratio-renin and L_2 effect at 3 and 6 hr.

ebroventricular renin was not altered by the lower dose (10 μg/5 μl) of Losartan; however, an inhibitory effect by the highest dose (100 μg/5 μl) was observed (F(3 hr) = 11.0 and F(6 hr) = 27.08; $P < 0.0001$) (V−R =L_1−R<V−S=L_1−S=L_2−R=L_2−S and V−S<L_2−S). The reduction in urinary volume induced by intracerebroventricular renin was associated with an enhancement in sodium excretion at the 3- and 6-hr collection periods (F′ ratios >20). Losartan at 100 μg/5 μl totally inhibited the natriuretic action of intracerebroventricular renin. In addition, there was a significant interaction between pretreatment and intracerebroventricular renin injection at 6 hr (F(I) = 3.8; $P < 0.05$) (3 hr, V−S=L_1−S=L_2−S=L_2−R<V−R=L_1−R, and 6 hr, V−S =L_1−S=L_2−S<L_2−R=V−R<L_1−R). Renin-induced kaliuresis was unaffected by renin or by Losartan pretreatment. Evaluation of the urinary sodium to potassium ratio revealed a significant effect of renin at both periods of collection (F′ ratios >19). This effect was blocked by Losartan (100 μg/5 μl) pretreatment (F′ ratios >3.0; $P < 0.05$). There was a significant interaction between renin and Losartan pretreatment (V−S=L_1−S=L_1−R<V−R=L_2−S=L_2−R).

Effect of Losartan on Drinking Behavior Induced by Intracerebroventricular Renin. Intracerebroventricular renin induced a significant water-drinking behavior that was already apparent at 1–2.5 min after intracerebroventricular renin. Compared with the vehicle-treated group (2.08 ± 0.266 min, N = 21). Peripheral (7–9 rats/group) (3 or 10 mg/kg, sc) or central administration (12–19 rats/group) of Losartan (10 μg/5 μl or 100 μg/5 μl, icv) significantly delayed the onset of drinking behavior (Fig. 3). In addition, both subcutaneous and central administration of Losartan significatively reduced the cumulative water drinking (Fig. 4).

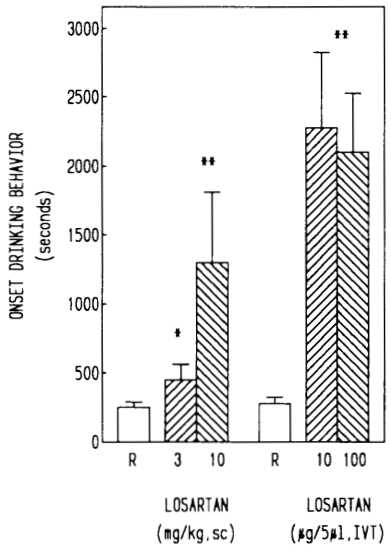

Figure 3. Effect of either peripheral (3 or 10 mg/kg, 1 hr before) or central (10 or 100 μg/5 μl, 5 min before) administration of Losartan on the onset of drinking behavior induced by intracerebroventricular renin. Open bars: intracerebroventricular renin (R; 10 mGU/5 μl); dashed bars: renin + Losartan. Shown are means ± SE for n = 7–19 rats/group; $^*P < 0.01$ and $^{**}P < 0.001$ compared with renin-treated control group.

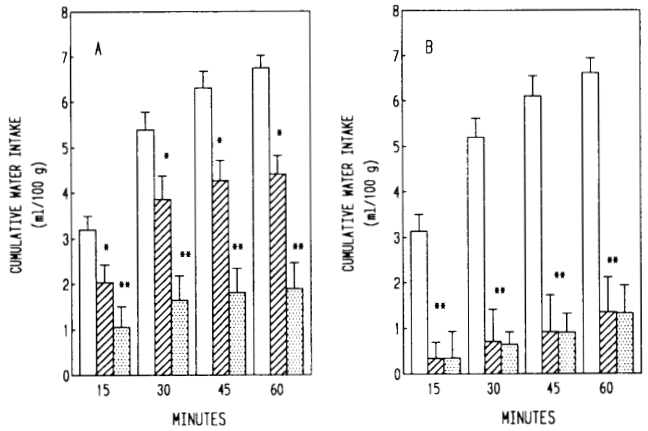

Figure 4. Cumulative water intake induced by central administration of renin in rats pretreated with (A) Losartan subcutaneously, 1 hr before intracerebroventricular renin. (open bars: intracerebroventricular renin (10 mGU/5 μl); dashed bars: 3 mg/kg; and speckled bars: 10 mg/kg); and (B) Losartan intracerebroventricularly, 5 min before intracerebroventricular renin (open bars: intracerebroventricular renin, 10 mGU/5 μl; dashed bars: 10 μg/5 μl; and speckled bars: 100 μg/5 μl). Water intake was recorded every 15 min during 1 hr. Shown are means ± SE for n = 7–19 rats/group; $^*P < 0.01$ and $^{**}P < 0.001$ compared with renin-treated control group.

Discussion

Angiotensin II is a circulating peptide that exerts some of its action through stimulation of brain angiotensin receptors accessible from the blood (1–3). Specific receptors with high affinity for ANG have been identified and localized in the central nervous system, with the highest concentration in areas such as the subfornical organ, nucleus medianus, septum, dorsal motor nucleus of the vagus (nucleus of the solitary tract), area postrema, and organum vasculosum lamina terminalis (OVLT) (11, 12). Studies with a labeled biologically active ANG antagonist revealed the ventricular surface of the OVLT as a receptor site for intracerebroventricularly applied ANG (21). Rat brain has recently been shown to contain two ANG receptor subtypes, AT_1 and AT_2 (16–19). Most brain areas mainly contain one of the ANG receptor subtypes. The selective anatomical distribution of the ANG receptor subtypes indicates that they may play specific roles. AT_1 receptors have been demonstrated in areas such as the subfornical organ and nucleus of the solitary tract, known to participate in cardiovascular and fluid regulation, and located outside the blood-brain barrier and accessible to circulating ANG (16–19). AT_1 receptors have also been localized in structures inside the blood-brain barrier such as the paraventricular nucleus (16–19), an area associated with the regulation of vasopressin release.

Stimulation of ANG receptors in the central nervous system elicits a complex but very reproducible and concerted pattern of behavioral, as well as cardiovascular and endocrine, responses. In effect, central administration of ANG or renin has been shown to induce a pressor response, drinking behavior, and release of vasopressin from the pituitary gland (9, 20, 22, 23). The possible involvement of AT_1 receptors in the pressor response has been shown (24, 25). In fact, peripheral administration of Losartan inhibits the pressor response to intravenous administration of ANG, which suggests that, at least, AT_1 receptors located in circumventricular structures accessible to blood-borne ANG, play an important role in the regulation of blood pressure. Furthermore, central, but not peripheral, administration of Losartan, inhibited the pressor response to intracerebroventricular ANG (24) which indicates that peripheral administration of Losartan does not affect brain areas that are accessible to intracerebroventricular ANG.

In addition to the known effects of intracerebroventricular ANG, it has been observed that in conscious rats, intracerebroventricular injection of angiotensin II, at doses well below the pressor threshold, induces an immediate and sustained natriuresis and vasopressin release (2, 9, 10). Our present results point to the specific role of the angiotensin AT_1 receptor subtype in this action. Indeed, in the present study, we demonstrated that peripheral administration of Losartan reduces the increase of sodium excretion and inhibits the antidiuretic action of centrally administered renin. Although it has been suggested that Losartan does not cross the blood-brain barrier after oral administration of a single dose of the drug (24), the inhibition of ANG receptor binding in rat brain after intravenously administered Losartan reported by Song *et al.* (26) and our present results indicate that, unlike peptide antagonist

or converting enzyme inhibitors, Losartan and/or its active metabolite can readily cross the blood-brain barrier and exert potent and selective inhibition of AT_1 receptors either outside or within the blood-brain barrier and strongly suggest that AT_1 receptors in circumventricular structures and/or in areas inside the blood-brain barrier may be implicated in the regulation of vasopressin release and in the natriuretic action of intracerebroventricular renin. The OVLT is a brain structure that can be reached from the cerebrospinal fluid as well as from the blood and it has been suggested that, at least in the rat, this area may be an important site of action of cerebrospinal fluid- and blood-borne ANG (21).

Administration of ANG has been demonstrated to stimulate water drinking in a variety of animal species (22, 23). Intracerebroventricularly administered renin produced instantaneous drinking behavior (<2.5 min after intracerebroventricular renin injection). Both peripherally and centrally administered Losartan significantly delayed the onset of drinking behavior and reduced cumulative water intake. As drinking response can be elicited by systemic as well as by central administration of ANG, circumventricular organs lacking a blood-brain barrier appear to be the areas in which renin stimulates drinking behavior. Our results confirm previous observations (15, 27–29) for a role of AT_1 receptors in the drinking response to ANG.

Our findings suggest a physiologic role for brain AT_1 receptors in the regulation of fluid and electrolyte metabolism, specifically with respect to the actions of angiotensin II on sodium excretion, vasopressin release, and drinking behavior.

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