Intravenous Losartan Inhibits the Increase in Plasma Luteinizing Hormone and Water Intake Produced by Intraventricular Angiotensin II (43707)

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Abstract. **In the presence of sex hormones, intraventricular injection of angiotensin II in female rats increases luteinizing hormone (LH) secretion, and this response is blocked by intraventricular losartan. There is evidence that in doses of 3 mg/kg or more systemically administered losartan blocks brain as well as peripheral AT_1 angiotensin II receptors. Therefore, we tested the effect of intravenous losartan, 1 and 10 mg/kg, on the LH response to intraventricular angiotensin II in ovariectomized rats treated with estrogen and progesterone. The larger dose of losartan abolished the LH response. It also produced a marked reduction in the drinking response to intraventricular angiotensin II. The data provide additional evidence that in larger doses, peripherally administered losartan can penetrate the brain, and support the hypothesis that in female rats, the brain renin-angiotensin system plays an excitatory role in the regulation of LH secretion.** [P.S.E.B.M. 1994, Vol 205]

When administered peripherally in doses of 3 mg/kg, the AT_1 angiotensin II (Ang II) receptor–blocking drug, losartan, reduces the electrical responses of paraventricular neurons inside the blood-brain barrier to microinjection of Ang II (1), and 10 mg/kg reduces Ang II binding to brain tissue (2). This suggests that in these doses losartan penetrates the brain in amounts sufficient to block AT_1 receptors. Therefore, we investigated the effect of peripherally administered losartan on the increase in plasma luteinizing hormone (LH) caused by intraventricular (ivt) Ang II in ovariectomized rats treated with estrogen and progesterone. This increase is known to be blocked by ivt losartan (3). We also investigated whether systemically administered losartan affected the drinking response to ivt Ang II, following up previous reports on this point (4–6).

Materials and Methods

Animals. Female Sprague-Dawley rats weighing 225–250 g were purchased from Charles River Breeding Laboratories, Wilmington, MA. They were housed singly in the university vivarium in hanging metal cages with food and water available *ad libitum*. The temperature of the animal room was maintained at 21°–23°C, with lights on at 0:500 and off at 19:00 hr each day.

Surgery. Approximately one week after their arrival in the vivarium, the animals were anesthetized with methohexital sodium (Brevital, 50 mg/kg, supplemented as needed) and bilaterally ovariectomized. At the same time, a stainless steel cannula (22 gauge, 23 mm in length, Plastic Products, Roanoke, VA) was implanted stereotaxically in the third ventricle. The cannula was held in place with dental cement and a 30-gauge obturator was inserted to prevent obstruction.

To determine the accuracy of the cerebral ventricular cannula placement, Ang II (50 ng in 2 μl of artificial cerebrospinal fluid [aCSF]) was injected into the

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cannula. Any water intake in the 10 min period after the injection was taken as evidence of correct positioning of the cannula. Testing was performed not less than 72 hr before the experiment, in the animals' home cages. Rats that did not drink in this test were excluded from further study.

A Silastic catheter was implanted in the jugular vein 48 hr prior to the experiment, also under Brevital anesthesia. The distal end of the catheter was threaded subcutaneously to exit between the scapulas. The catheter was flushed with 500 μl of 5% heparin in saline (heparin 1000 U/ml) to maintain patency.

Experimental Protocol. Animals were injected subcutaneously with estradiol benzoate (50 μg in 500 μl corn oil; Sigma, St. Louis, MO) and progesterone (25 mg in 500 μl sesame oil; Schein Pharmaceutical Inc., Port Washington, NY) at 48 hr before the experiment.

On the day of the experiment, animals were assigned to one of three treatment groups: (i) saline intravenously (iv), Ang II ivt (12 rats); (ii) Losartan 1 mg/kg iv, Ang II ivt (15 rats); (iii) Losartan 10 mg/kg iv, Ang II ivt (14 rats). In addition, 1 animal received saline iv and aCSF ivt.

Animals were brought to the laboratory at 07:00 hr on the day of the experiment and the jugular cannulas were flushed with heparin. The jugular cannula was connected to polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ) filled with 5% heparinized saline, and the tubing was passed through the top of the cage. Animals were left unrestrained, with free access to food and water, for at least 90 min. Water was available from a drinking tube, a modified serological pipette with a spout from a rat drinking bottle.

500 μl blood samples were withdrawn from the jugular catheters. Blood was first drawn until it filled the PE tubing, then collected in a heparinized 1-ml syringe. The tubing and catheter were next flushed with 5 μl of 5% heparinized saline. Blood was temporarily stored on ice until the plasma could be separated and frozen at −20°C for subsequent radioimmunoassay of LH.

Immediately after a control blood sample was drawn, animals were given iv injections through the jugular cannula of saline, 1 mg/kg losartan, or 10 mg/kg losartan. Sixty minutes later, another blood sample was collected. After this sample, Ang II (50 ng in 2 μl of aCSF) was injected ivt with minimal handling of the animals. Additional blood samples were drawn 10 and 20 min after Ang II injection. Water intake was measured during the 30-min period after Ang II injection, using the drinking tubes. The animals were then sacrificed.

Measurement of LH. Plasma LH concentration was measured by a radioimmunoassay described elsewhere (3). The standard was changed once during the course of the experiments, and all subsequent values were corrected using the correction factor determined by comparing values for the same plasma pool measured with the old and the new standard. LH values which differed by more than two standard deviations from the mean of all values were discarded in calculating the final mean; 2 of 164 specimens were eliminated in this fashion.

Statistics. Changes in LH and plasma due to the different treatments were analyzed by one-way ANOVAs in each treatment group where time was the factor (7). Differences between individual time points were assessed by the Fisher PLSD test. The effect of saline or losartan on Ang II-induced water intake was tested by one-way ANOVA where treatment was the factor. Post hoc comparisons were done using the Fisher PLSD test.

Results

In the rat that received saline iv and aCSF ivt, there was no change in plasma LH at 60, 70, and 80 min. In rats given saline, ivt Ang II produced the expected significant increase in plasma LH with a peak 10 min after injection (Fig. 1). Neither dose of losartan changed plasma LH significantly by itself. One mg/kg did not produce a significant change in the LH peak produced by Ang II, but losartan, 10 mg/kg, abolished the LH surge.

Water intake was 5.6 ± 0.4 ml in 30 min in animals that received ivt Ang II compared to zero in the animal receiving ivt aCSF. Intravenous losartan, 1 mg/kg, reduced water intake to 4.8 ± 0.5 ml, a decrease that was not statistically significant. However, iv losartan, 10 mg/kg, reduced water intake to 2.0 ± 0.4 ml. This reduction was statistically significant compared to control ($P < 0.0001$).

Discussion

Some years ago, Steele *et al.* (8, 9) reported that ivt and not iv Ang II increased LH secretion when given to regularly cycling female rats during proestrus. They also found that ivt Ang II increased LH secretion in ovariectomized rats treated with estrogen and progesterone by increasing gonadotropin releasing hormone (GnRH) secretion. The LH response was blocked by ivt losartan (3). These findings support the hypothesis that in the presence of gonadal steroids in female rats, centrally produced Ang II facilitates GnRH secretion and hence LH secretion. In other experiments, ivt administration of the Ang II receptor–blocking drug, saralasin, and the angiotensin-converting enzyme inhibitor, enalaprilat, to normally cycling rats prevented the LH surge and ovulation, suggesting that central production and release of Ang II is necessary for the occurrence of estrous cycles. Peripherally administered saralasin and enalaprilat did

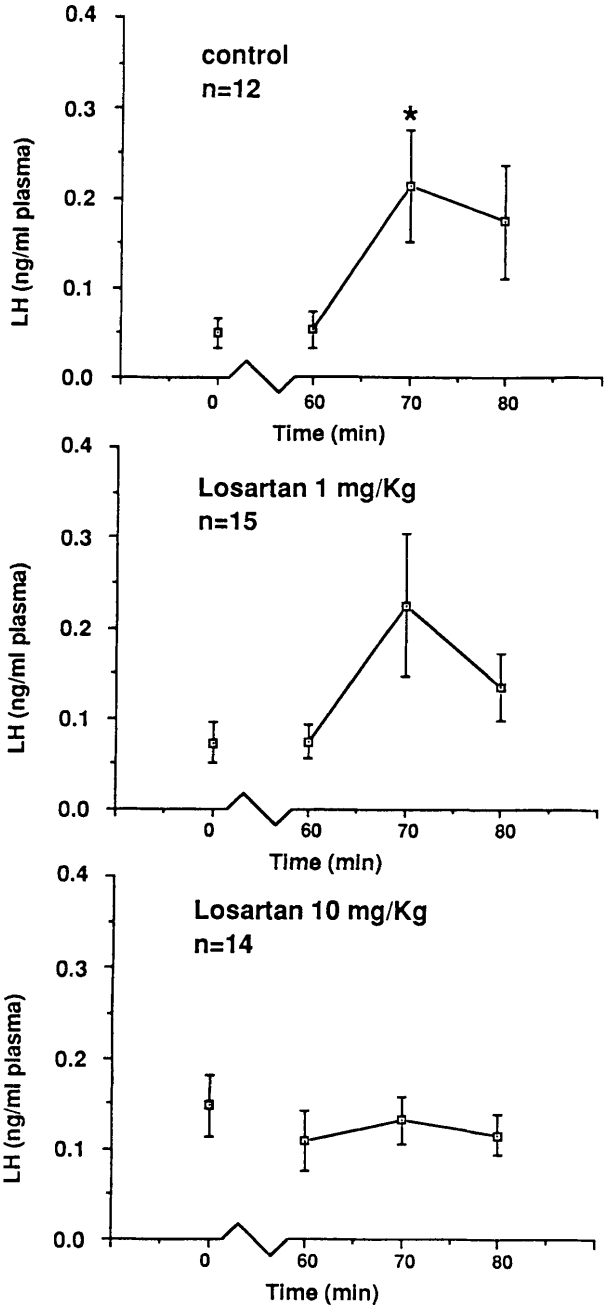

Figure 1. Effect of iv losartan on the increase in plasma LH produced by ivt Ang II. *$P < 0.05$ vs 0 and 60 min values. Losartan or saline was administered at 0 time and Ang II at 60 min.

not affect LH secretion or disrupt estrous cycles, but this could be due to failure of these compounds to cross the blood-brain barrier in amounts sufficient to disrupt the brain renin-angiotensin system (8, 10, 11). Evidence cited in the introduction to this paper (1, 2) indicates that when administered iv in doses of 3 mg/kg or more, losartan blocks central as well as peripheral AT_1 receptors. Therefore, if the hypothesis that centrally generated Ang II acts via AT_1 receptors to inhibit GnRH secretion is correct, losartan should inhibit Ang II-induced LH secretion. It did in the current experiments. One milligram per kilogram losartan administered iv did not cause a significant decline in the LH response to Ang II, but 10 mg/kg abolished it.

Our observations on water intake are similar to our observations on LH secretion; 1 mg/kg of losartan had no significant effect on the drinking response of ivt Ang II, but 10 mg/kg produced a marked reduction. Bui *et al.* (6) reported that 3 mg/kg of losartan by mouth for three days failed to alter the drinking or the pressor response produced by ivt Ang II. However, Fregly and Rowland (5) reported that in acute experiments, systemic administration of 10 mg/kg of losartan inhibited the drinking response to ivt Ang II, and Barbella *et al.* (4) reported that the same dose of losartan inhibited the drinking response to Ang II produced in the brain by ivt injection of renin. Presumably, Bui *et al.* failed to block drinking because oral administration of 3 mg/kg does not produce a great enough increase in circulating losartan to block the AT_1 receptors that mediate thirst.

It should be noted that the results of the drinking experiments, unlike the LH experiments, do not necessarily indicate that the ivt Ang II is acting on receptors inside the blood-brain barrier. ivt Ang II enters the circumventricular organs (12), the small brain areas which are outside the blood-brain barrier. These organs do not contain Ang II receptors which trigger LH secretion (9), but they do contain receptors which initiate thirst (13). Therefore, the dipsogenic response to ivt Ang II could be due to an action on these organs.

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