AT\textsubscript{1} receptor blockers prevent sympathetic hyperactivity and hypertension by chronic ouabain and hypertonic saline

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Zhang, Jing, and Frans H. H. Leenen. AT\textsubscript{1} receptor blockers prevent sympathetic hyperactivity and hypertension by chronic ouabain and hypertonic saline. Am J Physiol Heart Circ Physiol 280: H1318–H1323, 2001.—Sympathetic hyperactivity and hypertension caused by chronic treatment with ouabain or sodium-rich artificial cerebrospinal fluid (aCSF) can be prevented by central administration of an angiotensin type 1 (AT\textsubscript{1}) receptor blocker. In the present study, we assessed whether, in Wistar rats, chronic peripheral treatment with the AT\textsubscript{1} receptor blockers losartan and embusartan can exert sufficient central effects to prevent these central effects of ouabain and sodium. Losartan or embusartan (both at 100 mg·kg\textsuperscript{-1}·day\textsuperscript{-1}) were given subcutaneously once daily. Ouabain (50 μg/day) was infused subcutaneously, and sodium-rich aCSF (1.2 M Na\textsuperscript{+}, 5 μl/h) was infused intracerebroventricularly, both by osmotic minipump for 13–14 days. The mean arterial pressure (MAP) at rest and in response to air stress and intracerebroventricular injection of guanabenz (75 μg/7.5 μl), ANG II (30 ng/3 μl), and ouabain (0.5 μg/2 μl) were then measured. In control rats, chronic treatment with ouabain subcutaneously and hypertonic saline intracerebroventricularly both increased baseline MAP by 20–25 mmHg and enhanced twofold the pressor responses to air stress and depressor responses to the α\textsubscript{2}-adrenoceptor agonist guanabenz. Simultaneous treatment with losartan or embusartan fully prevented hypertension, maintained normal responses to air stress and guanabenz, and attenuated pressor responses to acute intracerebroventricular injection of ANG II and ouabain. We concluded that peripheral administration of losartan as well as embusartan can cause sufficient central effects to prevent the sympathetic hyperactivity and hypertension induced by chronic peripheral ouabain and central sodium.

AT\textsubscript{1} receptor blockers cross the blood-brain barrier, as demonstrated by autoradiographic as well as functional studies. Single doses of losartan at 1, 3, or 10 mg/kg iv inhibit ANG II receptor binding in a dose-related manner in rat brain areas containing predominantly AT\textsubscript{1} receptors both inside and outside the blood-brain barrier (25). Embusartan is a rather hydrophilic AT\textsubscript{1} receptor blocker. In whole body autoradiographic studies in rats, single doses of [\textsuperscript{14}C]embusartan at 5 mg/kg iv or 10 mg/kg orally resulted in relatively high radioactivity concentrations in the liver and other peripheral tissues, but there was no penetration of the radioactivity across the blood-brain barrier (J.-P. Stasch, Bayer AG, personal communication). The absence of [\textsuperscript{14}C]-labeled compound across the blood-brain barrier suggests that embusartan may exert less central effects than losartan.

Functional studies so far addressed the possible central effects of peripherally administered AT\textsubscript{1} receptor blockers by assessing central responses to injections of exogenous ANG II. In rats, acute or chronic treatment with losartan or irbesartan by gavage, subcutaneously, or intravenously can significantly attenuate pressor responses to central injections of ANG II (2, 3, 11, 16). The latter may or may not represent actions of endogenous ANG II in the central nervous system during chronic activation. So far, there is no evidence to substantiate whether peripheral administration of AT\textsubscript{1} receptor blockers can also inhibit excitatory responses...
to endogenous ANG II in the central nervous system during chronic stimulation. The goal of the present study was, therefore, twofold: 1) to determine whether the hypertension induced by chronic intracerebroventricular administration of hypertonic saline or chronic subcutaneous administration of ouabain can be prevented by chronic peripheral administration of an AT₁ receptor blocker, and 2) to determine whether the extent of central blockade differs between a lipophilic (losartan) versus hydrophilic (embusartan) AT₁ receptor blocker. Central blockade was assessed by evaluating their effects on resting blood pressure (BP) and changes in mean arterial pressure (MAP) and heart rate (HR) in response to air stress and intracerebroventricular injection of the α₂-adrenoceptor agent guanabenz, ANG II, and ouabain. Air jet stress was used to estimate activity in sympathoexcitatory pathways (6), and guanabenz was used to estimate activity in sympathoinhibitory pathways (10).

METHODS

Animals and Experimental Protocols

Male Wistar rats (200–250 g, Charles River; Montreal, Canada) were housed at 24°C on a 12:12-h light-dark cycle, fed 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. Two separate experiments were performed to test the effects of chronic administration of losartan and embusartan on two models of hypertension, i.e., ouabain- and hypertonic saline-induced hypertension. In experiment 1, rats received a chronic subcutaneous infusion of either 0.9% saline or 50 μg/day ouabain via osmotic pump for 14 days. In experiment 2, rats received a chronic intracerebroventricular infusion of either artificial CSF (aCSF) or aCSF containing 1.2 M NaCl (5 μl/h) via osmotic pump, also for 14 days. Each group was subdivided into three groups, which received either losartan, embusartan, or control injections. In each experiment, six groups were therefore studied. Losartan and embusartan were administered daily by subcutaneous injection at a dose of 100 mg/kg; control rats received 0.9% saline (1 ml/kg). Injections started 2 days before placement of the minipumps and continued for 16 days, and the last injection was given at the time of vascular cannulations (see Placement of Intracerebroventricular Cannula and Implantation of Osmotic Minipump). Doses of losartan and embusartan were chosen based on initial studies showing that subcutaneous injections of losartan or embusartan at 30 or 100 mg/kg for 6 days inhibited pressor responses to acute intracerebroventricular ANG II or hypertonic saline by ~40–60% (23). The final assessments were performed after 14 days infusion of ouabain or hypertonic saline. In previous studies, we showed that chronic subcutaneous administration of ouabain or intracerebroventricular infusion of 1.2 M NaCl increases baseline MAP by 20–25 mmHg within 10–14 days (7, 8).

Placement of Intracerebroventricular Cannula and Implantation of Osmotic Minipump

Experiment 1. After 5–7 days of acclimatization, under halothane anesthesia, a 23-gauge guide cannula (14 mm long) was fixed to the skull of the rat with acrylic cement. The lower end of cannula was 0.5 mm above the left lateral ventricle (coordinates: 0.4 mm posterior and 1.2 mm lateral to bregma and 2.8 mm deep to dura) (7). This cannula later served as a guide for intracerebroventricular injections. In three groups of rats, an osmotic minipump (model 2002, Alzet) filled with ouabain dissolved in saline was implanted subcutaneously on the back. The infusion rate of ouabain was 50 μg in 12 μl/day.

Experiment 2. After 5–7 days of acclimatization, guide cannulas were implanted as in experiment 1. In addition, a L-shaped stainless steel cannula was implanted into the right lateral ventricle (3.5 mm deep from dura) and fixed on the skull. By means of polyethylene (PE) tubing (PE-50 fused to PE-60), this cannula was connected to an osmotic minipump (model 2ML2, Alzet), which was implanted subcutaneously on the back, filled with aCSF or aCSF containing 1.2 M NaCl. The infusion rate of hypertonic saline was 5 μl/h.

After the intracerebroventricular guide cannula and osmotic minipump implantation, the rats were returned to their original cage with regular food and water. They were trained to stay quietly in a small experimental cage (24 × 15 × 8 cm) in which the rat could move back and forward on three to four different occasions, each lasting 1–2 h.

Femoral Artery and Vein Cannulation

After 2 wk, in the early morning, the left femoral artery and vein were cannulated with PE-10 tubing fused to PE-50 tubing filled with heparinized saline. The catheters were tunneled subcutaneously, exteriorized at the nape, and secured to the skin. Rats were given a 4–5 h recovery period before proceeding with the experiment.

Blood Pressure and HR Measurements

The arterial catheter was connected to a transducer, and MAP and HR were recorded through an IBM-compatible computer programmed by a data acquisition program (DataqLab Pro, Data Science International; St. Paul, MN) that allowed on-line analysis of the pulsatile BP signal and storage of data. MAP and HR were sampled every 30 s at a sampling rate of 500 Hz except for air stress data, in which momentary changes in MAP and HR were used. Rats were allowed an accommodation period of 30 min before resting MAP and HR were recorded.

Specific Study Protocols

On day 0, daily subcutaneous injections of losartan (100 mg·kg⁻¹·day⁻¹), embusartan (100 mg·kg⁻¹·day⁻¹), or 0.9% saline were started for 16 days. On day 2, an intracerebroventricular guide cannula and subcutaneous osmotic minipump were implanted. On days 15–16, in the early morning, a femoral artery and vein were cannulated. In the afternoon, after the resting MAP and HR had been measured, standardized air stress was provided twice at a 10-min interval by blowing the face of the rat with a jet of air (1–1.5 psi) for 30 s from a tube located ~3 cm in front of the rat. The average of peak changes in MAP and HR in response to the two applications of stress was used for comparisons. Intracerebroventricular injections were then performed using a L-shaped 30-gauge stainless steel cannula connected to a Hamilton microsyringe via PE-10 tubing, which, when injected into the intracerebroventricular guide cannula, protruded 1 mm into the left lateral ventricle. aCSF (3 μl), ANG II (30 ng/3 μl aCSF), and guanabenz (25 μg/2.5 μl aCSF and 75 μg/7.5 μl aCSF) were injected intracerebroventricularly. The next injection was administered after responses to the pre-
Previous injection had disappeared, and then a further 10-min rest was given, with a 20-min rest period between the two guanabenz injections. Thirty minutes after the responses to guanabenz had disappeared, ouabain (0.5 mg/2 ml aCSF) was injected intracerebroventricularly.

At the end of the experiment, the rat was deeply anesthetized with an overdose of pentobarbital sodium and injected intracerebroventricularly with 5 ml of methyl blue to verify cannula placement. The brain was removed and cut through the hole of the guide cannula to confirm placement of the intracerebroventricular guide cannula.

**Data Analysis**

All data are expressed as means ± SE. Statistically significant differences between control, losartan, and embusartan groups were determined by two-way ANOVA, followed by Student-Newman-Keuls test. The level of significance was set as P < 0.05.

**RESULTS**

**Baseline Values**

After subcutaneous ouabain treatment for 2 wk, resting MAP was significantly increased compared with the control group. In rats receiving control infusions of saline, both losartan and embusartan significantly decreased MAP. In the groups that received ouabain infusions combined with losartan or embusartan, MAP was similar to that in the groups receiving losartan or embusartan alone (Fig. 1A). There were no significant differences in resting HR among the six groups of rats. The body weight gain over the 2 wk of treatment was similar in all groups (data not shown).

After intracerebroventricular infusion of Na⁺-rich aCSF for 2 wk, resting MAP had significantly increased. Losartan or embusartan alone significantly decreased BP again. In the groups receiving combined hypertonic saline and losartan or embusartan, BP was similar to that in groups receiving losartan or embusartan alone (Fig. 1B). There were no significant differences in resting HR among the groups. The body weight gain over the 2 wk of treatment was similar in all groups (data not shown).

**Responses to Air Stress**

Air stress caused rapid increases in MAP. Losartan and embusartan alone did not significantly affect these responses. In rats treated with ouabain or intracerebroventricular Na⁺-rich aCSF, peak increases in MAP by air stress were approximately twice those in control rats. These enhanced responses did not develop when...
Losartan or embusartan was administered subcutaneously (Fig. 2).

Responses to Intracerebroventricular Guanabenz

After intracerebroventricular administration of guanabenz, MAP decreased in a dose-related manner and reached a plateau within 5 min. BP decreases lasted 15–20 min for the 25-μg dose and 30–40 min for the 75-μg dose. In rats treated with ouabain or intracerebroventricular Na\(^+\)-rich aCSF, maximum decreases in MAP by guanabenz were twice those in control rats. In rats receiving ouabain or intracerebroventricular Na\(^+\)-rich aCSF concomitant with losartan or embusartan, peak decreases were similar to those in control rats (Fig. 3).

Responses to Intracerebroventricular ANG II and Ouabain

Intracerebroventricular injection of aCSF did not significantly change BP. Intracerebroventricular injection of ANG II or ouabain significantly increased MAP. In the control and ouabain groups, increases in MAP by 30 ng icv ANG II were 18–20 mmHg. Subcutaneous losartan or embusartan significantly attenuated these increases in MAP to 6–7 mmHg. In the control and ouabain groups, increases in MAP by 0.5 μg icv ouabain were 13–15 mmHg. Subcutaneous losartan or embusartan significantly attenuated these increases in MAP to 4–8 mmHg. The latter responses remained significantly larger than those induced by intracerebroventricular injection of aCSF (Fig. 4A).

In the control and intracerebroventricular Na\(^+\)-rich aCSF groups, increases in MAP by 30 ng icv ANG II were 17 mmHg. Subcutaneous losartan or embusartan attenuated the increases in MAP to 4–6 mmHg. In the control and intracerebroventricular Na\(^+\)-rich aCSF groups, increases in MAP by 0.5 μg icv ouabain were 15–17 mmHg. Subcutaneous losartan or embusartan attenuated the increases in MAP to 4–5 mmHg. However, these increases remained significantly larger than those induced by intracerebroventricular injection of aCSF (Fig. 4B).

**DISCUSSION**

The present study demonstrates that, in normotensive rats, sympathetic hyperactivity and increases in MAP by 10.220.33.1 on July 9, 2017 http://ajpheart.physiology.org/ Downloaded from
resting BP caused by subcutaneous infusion of ouabain or intracerebroventricular infusion of hypertonic saline can be fully prevented by daily subcutaneous injections of losartan or embusartan.

Consistent with other recent studies, chronic treatment with ouabain caused moderate hypertension in Wistar rats (8, 21, 22). Lesions limited to the ventral part of the anteroverentral third ventricle (AV3V) region involving the organum vasculosum laminae terminalis (OVLT) and extending into the ventral median preoptic nucleus (MnPO) fully prevent the hypertension induced by chronic subcutaneous administration of ouabain (21). Peripheral mechanisms do not appear to play a significant role in the hypertension induced by subcutaneous ouabain, because central blockade of the effects of ouabain prevents hypertension in this model (8, 21). In rats, intracerebroventricular pretreatment with the ANG II receptor blocker saralasin (20) or the AT1 receptor blocker losartan (4) blocks sympathoexcitatory and pressor responses to acute intracerebroventricular ouabain. These studies suggest that activation of brain AT1 receptors occurs in the pathways mediating the effects of acute intracerebroventricular ouabain. Chronic administration of ouabain leads to increased activity in sympathoexcitatory pathways, decreased activity in sympathoinhibitory pathways, and the development of hypertension. All these responses can also be prevented by concomitant intracerebroventricular treatment with losartan (6).

In conscious rats, acute intracerebroventricular Na+-rich aCSF causes sympathoexcitatory and pressor effects that can be prevented by intracerebroventricular pretreatment with antibody Fab fragments blocking brain “ouabain” or losartan (4). Centrally administered losartan also blocks natriuretic and pressor responses to intracerebroventricular infusion of hypertonic saline in sheep and rats (13, 18). In rats, chronic central sodium loading causes enhanced sympathoexcitation and impairment of baroreflexes and hypertension, which can be prevented by concomitant intracerebroventricular Fab fragments or losartan (7). These findings suggest that, in rats, central pathways involving the organum vasculosum laminae terminalis (OVLT), which can be prevented by concomitant intracerebroventricular injections of ANG II or ouabain (8, 21). Peripheral mechanisms do not appear to contribute to circulating AT1 receptor blockers.

In conclusion, chronic infusion with ouabain subcutaneously or intracerebroventricularly enhances sympathoexcitatory and hypertensive responses to increased brain sodium and ouabain are mediated by coupling of 125I-angiotensin II and 125I-[sarcosine1, leucine8] angiotensin II, an angiotensin antagonist, by sodium ion. Eur J Pharmacol 67: 1–10, 1980.


