Dissociation between the circulating renin-angiotensin system and angiotensin II receptors in central losartan-induced hypertension

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Abstract

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Losartan, an AT_1 angiotensin II (ANG II) receptor non-peptide antagonist, induces an increase in mean arterial pressure (MAP) when injected intracerebroventricularly (*icv*) into rats. The present study investigated possible effector mechanisms of the increase in MAP induced by *icv* losartan in unanesthetized rats. Male Holtzman rats (280-300 g, N = 6/group) with a cannula implanted into the anterior ventral third ventricle received an *icv* injection of losartan (90 µg/2 µl) that induced a typical peak pressor response within 5 min. In one group of animals, this response to *icv* losartan was completely reduced from 18 ± 1 to 4 ± 2 mmHg by intravenous (*iv*) injection of losartan (2.5-10 mg/kg), and in another group, it was partially reduced from 18 ± 3 to 11 ± 2 mmHg by *iv* prazosin (0.1-1.0 mg/kg), an α_1-adrenergic antagonist (P<0.05). Captopril (10 mg/kg), a converting enzyme inhibitor, injected *iv* in a third group inhibited the pressor response to *icv* losartan from 24 ± 3 to 7 ± 2 mmHg (P<0.05). Propranolol (10 mg/kg), a ß-adrenoceptor antagonist, injected *iv* in a fourth group did not alter the pressor response to *icv* losartan. Plasma renin activity and serum angiotensin-converting enzyme activity were not altered by *icv* losartan in other animals. The results suggest that the pressor effect of *icv* losartan depends on angiotensinergic and α_1-adrenoceptor activation, but not on increased circulating ANG II.

Introduction

The AT_1 angiotensin II (ANG II) receptor antagonist losartan induces an increase in arterial pressure when injected into the brain ventricular system (1-3). Activation of brain ANG II receptors is traditionally associated with an increase in arterial pressure (4,5), but there are also reports of a decrease in arterial pressure induced by exogenous ANG II (for a review, see Ref. 3). Thus, the hypertensive effect of central losartan is explained, at least in part, by its action on central depressor ANG II receptors. However, nothing is known about the efferents that mediate this effect of central losartan. Thus, in the present study we determined whether sympathetic output and the systemic renin-angiotensin system mediate the pressor effect induced by losartan injected into the third cerebral ventricle (3rdV).

Material and Methods

Animals

Male Holtzman rats weighing 260-300 g at the beginning of the experiment were individually housed in a room on a 12:12-h light/dark cycle beginning at 7:00 am. Standard Purina (Campinas, SP, Brazil) pellets and tap water were available *ad libitum* unless otherwise stated. All experiments began at 8:00 am.

Brain surgery and histology

Each animal received a stainless steel guide cannula (0.7 mm OD) stereotaxically implanted into the anterior part of the 3rdV under tribromoethanol (Aldrich Chemical Company Inc., Milwaukee, WI, USA) anesthesia (20 mg/100 g body weight). Coordinates for the anteroventral 3rdV were: 0.2 mm caudal to bregma, 6.6 mm from duramater, 1.2 mm lateral to sagittal suture, incisor bar 2.5 mm below the interaural line, and cannula at an angle of 10° from the sagittal plane. A prophylactic dose of penicillin (30,000 IU) was administered *im* before surgery. At the end of the experiments, the animals were deeply anesthetized with chloral hydrate (800 mg/100 g body weight) and perfused with 10% formalin through the left ventricle of the heart. The brain was removed, fixed in 10% formalin, frozen and sectioned for light microscopy examination.

Drugs and injection techniques

Losartan (90 µg) was injected into the 3rdV in a volume of 2 µl. Single pulse intracerebroventricular (*icv*) injections were made with an injector (0.3 mm OD) that protruded 1.0 mm beyond the tip of the guide cannula. Prazosin hydrochloride, an α_1-adrenergic antagonist (0.1 and 1.0 mg/kg), propranolol hydrochloride, a ß-adrenergic antagonist (10 mg/kg), losartan, an AT_1 ANG II receptor antagonist (1.25, 2.5, 5.0 and 10.0 mg/kg), captopril, an angiotensin-converting enzyme (ACE) blocker (10 mg/kg), or 0.9% saline was injected intravenously (*iv*) 20 min before the *icv* injection of losartan through a catheter previously implanted into the right jugular vein. Except for losartan (a gift from Dr. Ronald D. Smith, DuPont-Merck, Wilmington, DE, USA), all other drugs were purchased from Sigma (St. Louis, MO, USA). Each drug was dissolved or suspended (prazosin) in 0.9% saline.

Measurement of plasma renin and serum angiotensin-converting enzyme activities

Plasma renin activity was measured by radioimmunoassay. One hundred microliters of each plasma sample was mixed with 200 µl of 0.2 M Tris-HCl buffer, pH 7.4, 20 µl of 48 mM 8-OH-quinoleine and 10 µl of 161 mM phenylmethylsulfonyl fluoride and incubated at 37°C for 3 h. Blanks were prepared by incubating the plasma samples under the same conditions at 4°C. After incubation, the amount of ANG I generated from angiotensinogen was determined by radioimmunoassay. The linear range of the method extends from plasma renin activity values of 0.3 to 11 ng ml^{-1} h^{-1}.

Serum ACE activity was measured by coupling an indicator reaction, catalyzed by γ-glutamyltransferase, to the ACE-catalyzed hydrolysis of hippuryl-glycyl-glycine (6). ACE activity was calculated from the production of the chromophore 3-carboxy-4-nitroaniline, determined with a spectrophotometer at 410 nm, and defined as µmol hippuric acid, and therefore of glycyl-glycine (coupler), released by one liter of serum per minute (U/l). The increase in absorbance due to the formation of 3-carboxy-4-nitroaniline in the γ-glutamyltransferase reaction was linear to the concentration of glycyl-glycine and therefore to the activity of ACE. The linear range of the method extends from

ACE values less than 50 to 1300 U/l.

Arterial pressure recording

Four days after surgery, the animals were anesthetized with tribromoethanol (20 mg/100 g body weight), a catheter was fitted into the femoral artery and another one into the right jugular vein. Both catheters were tunneled subcutaneously and exteriorized at the nape of the neck. On the next day, the femoral catheter was connected to a Narco (P-1000B) pressure transducer coupled to a multichannel recorder (Narcotrace 40, Narco Bio-System, Austin, TX, USA). Direct mean arterial pressure (MAP) was recorded from the abdominal aorta in unanesthetized, unrestrained, normovolemic rats. Baseline was defined when MAP remained stable for at least 5 min of continuous recording before any treatment. Each animal was submitted to only one day of cardiovascular recording.

Statistical analysis

Peak variations in arterial pressure, which occurred up to 5 min after the *icv* injection of losartan, were submitted to analysis of variance followed by the *post hoc* Student-Newman-Keuls test. Dose zero of the antagonists corresponds to 0.9% saline. The unpaired *t*-test was used for analysis of the biochemical plasma renin and ACE activity data. Data are reported as means ± SEM and the level of significance was set at P<0.05. A minimum of six animals were used in each group. Each animal submitted to arterial pressure recordings received only two *icv* injections separated by a 6-h interval. Blood for plasma renin and ACE was collected on the same day from both treated and control animals.

Experimental protocols

Antagonists or enzyme blocker iv + losartan icv. Different doses of the antagonists were randomly assigned to each experimental day. Half of the animals received one dose of the antagonists in the first injection and half received the other dose. Then, the doses given were reversed in the second injection given at least 6 h later. The antagonists were injected *iv* immediately after the baseline recording, 20 min before *icv* injection of losartan.

Effects of icv losartan on plasma renin and ACE activity. A group of 16 animals received an *icv* injection of losartan (90 µg) or isotonic saline and were left in their home cages for 5 min before decapitation. This interval includes the peak pressor response to *icv* losartan (1,3). Trunk blood was collected from each animal into chilled tubes containing either 7.5% EDTA (100 µl/ml) for collection of plasma or gel separator (Becton Dickinson, Plymouth, UK) for collection of serum. Plasma was obtained from whole blood centrifuged at 3,000 rpm at 4°C for 15 min. Plasma and serum were then processed for determination of plasma renin and ACE activity, respectively.

Results

Effects of *icv* injection of losartan on arterial pressure

Losartan (90 µg) injected *icv* induced a peak pressor response of 20 ± 4 mmHg within 5 min compared to a 1 ± 1 mmHg response to *icv* saline (N = 14/group, P<0.05).

Effects of antagonists or the enzyme blocker injected *iv* on the pressor response to losartan injected into the 3rdV

Losartan (1.25, 2.5, 5.0 and 10.0 mg/kg) *iv* produced 80 to 100% inhibition of the peak pressor response to *icv* losartan (Figure 1). Captopril (10 mg/kg) injected *iv* induced a 70% inhibition of the pressor response to *icv* losartan (Figure 2).

Prazosin (0.1 and 1.0 mg/kg) injected *iv* produced an inhibition of 46% in the pressor

Figure 1. Effect of intravenous injection of losartan on the pressor response to icv losartan (90 µg). The number of animals is given in parentheses. *P<0.05 vs doze zero (Student-Newman-Keuls test). MAP = mean arterial pressure.
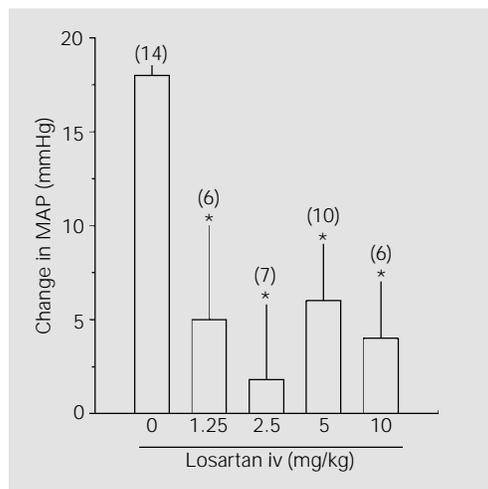

Figure 2. Effect of intravenous injection of captopril on the pressor response to icv losartan (90 µg). N = 7 for each group. *P<0.05 vs doze zero (Student-Newman-Keuls test). MAP = mean arterial pressure.

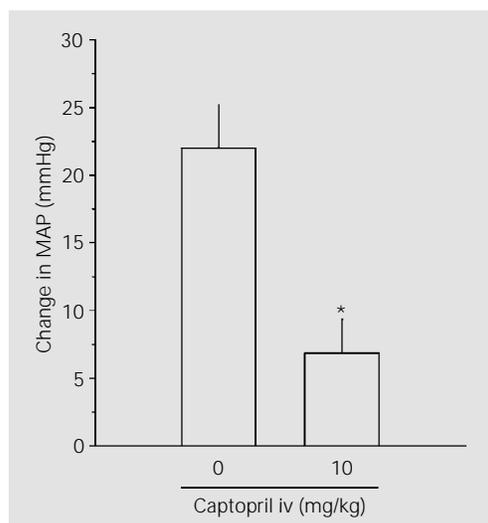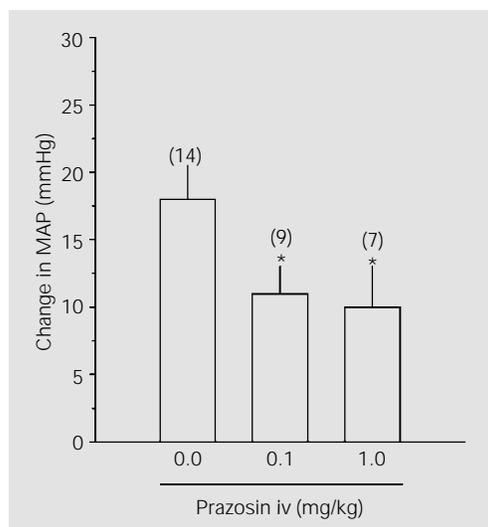
Figure 3. Effect of intravenous injection of prazosin on the pressor response to icv losartan (90 µg). The number of animals is given in parentheses. *P<0.05 vs doze zero (Student-Newman-Keuls test). MAP = mean arterial pressure.

response to *icv* losartan (Figure 3). Propranolol injected *iv* (10 mg/kg) did not alter the pressor effect of *icv* losartan (Figure 4).

Effect of *icv* losartan on plasma renin and ACE activity

Losartan injected *icv* did not alter plasma renin or ACE activity. The values for 90 µg losartan-treated (N = 8) and saline-treated (N = 8) rats were 1.8 ± 0.6 and 1.8 ± 0.7 ng ANG ml^{-1} h^{-1} and 535 ± 45 and 537 ± 82 U/l, respectively.

Effects of the antagonists injected *iv* on basal mean arterial pressure

Basal MAP (Table 1) was reduced by the lowest dose of losartan and by the two doses of prazosin injected *iv*. Captopril and propranolol injected *iv* did not alter basal MAP.

The lowest dose (1.25 mg/kg) of losartan is similar to the dose (1.0 mg/kg) which reduces the pressor effect of systemic ANG II by 50% (7). This was confirmed by injecting the lowest dose *iv* prior to 25 ng of *iv* ANG II. The pressor response to ANG II was reduced from 43 ± 6 to 22 ± 6 mmHg (P<0.05, N = 4).

The patency of the propranolol solution was confirmed by a fall in heart rate (-91 ± 15 bpm, N = 8) compared to the effect of 0.9% saline (0 ± 3 bpm, N = 14).

Discussion

Hypertension induced by *icv* injection of losartan was reduced by *iv* injection of losartan, captopril or prazosin, and was not altered by *iv* injection of propranolol. Intracerebroventricular injection of losartan altered neither plasma renin nor ACE activity.

The increase in arterial pressure induced by *icv* losartan could result from an action on central AT_1 receptors. This is consistent with the less explored hypotensive effect induced by central actions of ANG II. Localization of

receptor mechanisms and interactions with neurotransmitters related to such effect has been more systematically investigated in the hindbrain. Peptide and non-peptide ANG II antagonists, including losartan, produce sympathoactivation and an increase in arterial pressure when injected into the rostral and the caudal ventrolateral medulla (8-11). Losartan may also interact with catecholamines in the hindbrain to produce sympathoactivation (12). When injected into the 4th ventricle, but not into the anterior ventricles, losartan induces bradycardia (1,2) likely related to the modulatory effect hindbrain ANG II receptors have on baroreflex (8-12). However, better hypertensive effects are obtained with losartan injected into the 3rd than into the 4th cerebral ventricles in the rat (1,2). Contrary to what has been suggested (12), this effect does not depend on potassium associated with losartan (1). Thus, the hypotensive ANG II receptors accessible through the ventricular route should be found mainly in the forebrain, but scant information is available on how ANG II acts there to produce hypotension (13,14).

The arterial pressure increase induced by losartan injected into the 3rdV depends, at least in part, on the periventricular tissue surrounding the anteroventral 3rdV (3) which connects through hypothalamic nuclei to the sympathetic system (15,16). Thus, it is possible that the pathway that activates the α_1-adrenergic receptors mediating the pressor effect of losartan starts in forebrain structures that converge to the intermediolateral column and from there proceed through efferent signals to blood vessels. The sympathetic system also provides efferents to ß-adrenergic receptors of the juxtaglomerular cells to release renin (16) and ß-adrenergic receptors activate the intrinsic vascular renin-angiotensin system (17,18). Moreover, ß-adrenergic activation in the heart increases cardiac output (16). Nevertheless, ß-adrenergic receptors and the corresponding responses are probably not involved in the
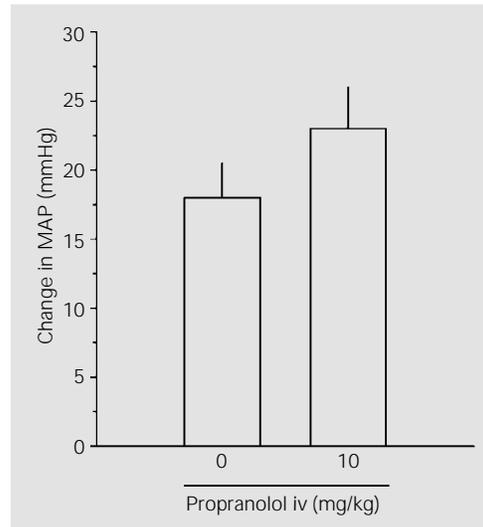

Figure 4. Effect of intravenous injection of propranolol on the pressor response to icv losartan (90 µg). N = 8 for each group. MAP = mean arterial pressure. There was no statistical effect of propranolol (Student-Newman-Keuls test).

Table 1. Peak variation in mean arterial pressure (ΔMAP) after intravenous injections of 0.9% saline, losartan, captopril, prazosin or propranolol.

Treatment	ΔMAP (mmHg)
0.9% Saline (N = 14)	0 ± 1
Losartan, 1.25 (N = 6)	-9 ± 1*
Losartan, 2.5 (N = 7)	-4 ± 3
Losartan, 5.0 (N = 10)	-6 ± 2
Losartan, 10.0 (N = 6)	-3 ± 3
Captopril, 10.0 (N = 7)	-4 ± 2
Prazosin, 0.10 (N = 9)	-21 ± 3*
Prazosin, 1.0 (N = 7)	-34 ± 7*
Propranolol, 10.0 (N = 8)	0 ± 3

*P<0.05 compared to 0.9% saline (Student-Newman-Keuls test). Drug concentrations are reported as mg/kg.

pressor responses to *icv* losartan since they were not altered by systemic propranolol.

Central losartan-induced hypertension is possibly also mediated by ANG II receptors since antagonism of the renin-angiotensin system reduced this response. The receptors are likely to be peripheral since clear opposite effects are obtained between *icv* and *iv* injections of losartan (present results and Ref. 2). This implies an elusive efferent activation to the systemic renin-angiotensin system to produce hypertension. The efferents do not depend on ß-adrenergic sympathetic

output as discussed above or on the increase of circulating ANG II since plasma renin or serum ACE activity were not altered by *icv* losartan.

The pressor response to *icv* losartan results from the activation of α_1-adrenergic and ANG II receptors. This activation probably depends on increased α_1-sympathetic output and a bypass of the circulating renin-angiotensin system.

Acknowledgments

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