

Effect of Central Losartan on DOCA-Salt Hypertension Rats

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ABSTRACT

Background: The purpose of this study was to investigate whether brain AT₁ receptor stimulation contributes as a hypertensive mechanism to deoxycorticosterone acetate (DOCA)-salt hypertension. **Methods:** 1) Acute injection: Losartan (1 mg/4 μ L) or artificial cerebrospinal fluid (aCSF) was injected into the lateral cerebral ventricle (icv) of conscious control uninephrectomized Wistar rats or rats with DOCA-salt at 2 or 4 weeks, and mean arterial pressure (MAP) and heart rates (HR) were recorded. 2) Chronic injection: Using osmotic minipump, losartan (1 mg/kg/d) or aCSF was injected to a sham group or three DOCA-salt rat groups [icv-aCSF, icv-losartan, sc-losartan (subcutaneous) groups] for 4 weeks, after which the MAP and HR were recorded in addition to the weights of the left (LV) and right ventricles (RV) and kidneys. **Results:** 1) Acute injection: In rats treated with DOCA-salt, resting MAP significantly increased compared to the control group [144 ± 6 mmHg (2 weeks), 170 ± 5 mmHg (4 weeks) vs $115-120$ mmHg (controls)]. MAP decreased significantly (2 weeks, 4 weeks) at 4, 8, 24 hours after icv injection of losartan to the level of the control group. 2) Chronic injection: The general trend showed that MAP decreased more in the icv-losartan group than in the icv-aCSF group (127 ± 15.2 mmHg vs 141.1 ± 5.5 mmHg, $p=0.0578$). In all DOCA-salt groups, no differences in RV weight were found. In the icv-aCSF and sclosartan groups, the kidney weight increased compared to the control group, but there was no difference in LV and kidney weight between the icv-losartan group and the control group. **Conclusions:** Normalization of MAP after acute or chronic icv administration of the AT₁ receptor antagonist suggests that the stimulation of the brain AT₁ receptor plays a significant role in the development and maintenance of hypertension in the DOCA-salt hypertensive rat model. Losartan icv injection appeared to have a protective effect on the heart and kidney. (Korean Circulation J 2004;34(1):84-91)

KEY WORDS: Losartan; Angiotensin II; Receptor, angiotensin, type 1; Brain; DOCA-salt hypertension.

Introduction

In deoxycorticosterone acetate (DOCA)-salt hypertensive rats the development and maintenance of hypertension is considered to be independent of the renin-angiotensin system because plasma renin in this model

is low,¹⁾ and peripheral administration of angiotensin converting enzyme (ACE) inhibitors²⁾ or angiotensin (Ang) II receptor antagonists³⁾ have no effect on blood pressure (BP).

However, there is some evidence of a role of the brain renin-angiotensin system in the pathogenesis of DOCA-salt hypertension in rats. DOCA-salt-treated rats show higher Ang II receptor density in brain areas involved in cardiovascular regulation, such as the nucleus of the solitary tract, area postrema, median preoptic nucleus, subfornical organ, and solitary vagal area,⁴⁻⁶⁾ and have elevated levels of renin and Ang II in the hypothalamus and brain stem nuclei.^{7,8)}

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Acute intracerebroventricular (icv) administration of captopril decreases the blood pressure of DOCA-salt hypertensive rats.⁹⁾ Chronic icv administration of captopril attenuates the development of hypertension in DOCA-salt hypertensive rats.¹⁰⁾ However, the interpretation of these findings is complicated because ACE is involved in the metabolism of various peptides that may participate in regulating BP, including bradykinin, enkephalin, and substance P.¹¹⁾

Three studies assessed the effects of icv Ang II receptor antagonists on BP in DOCA-salt hypertensive rats. Acute icv administration of salarasin did not lower BP during 30 min of follow-up.¹²⁾ In contrast, injection of losartan icv into the rostral part of the third ventricle did lower BP for more than 1 h in DOCA-salt hypertensive rats at 4 weeks.¹³⁾ Moreover, continuous icv infusion of CV-11974 (active metabolite of candesartan) for 7 days lowered BP from the fourth day after infusion.¹⁴⁾ However, because hemodynamic measurements in these studies were done under anesthesia in DOCA-salt hypertensive rats at 4 or 6 weeks, the role of brain Ang II in the development of hypertension in the DOCA-salt hypertensive rat model is not yet known.

The aim of the present study was to determine the acute and chronic central effects of the type 1 Ang II (AT₁) receptor antagonist losartan in the development and maintenance of hypertension in conscious DOCA-

salt hypertensive rats.

Methods

Animals

Male Wistar rats aged 5 weeks, weighing 140–170 g, were purchased from Charles River, Montreal, Canada and housed two per cage at 24°C on a 12 hour light/dark cycle. They were allowed normal rat chow and tap water ad libitum for at least 5 days prior to 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.

DOCA-salt hypertension

After 5–7 days of acclimatization, under halothane anesthesia, all rats underwent left nephrectomy. After surgery, they were randomly assigned to either the control or DOCA-salt group. Twenty-four hours after surgery, the rats in the DOCA-salt group received the first injection of DOCA (25 mg/kg s.c. in 0.1 mL sesame oil/100 g body weight) and 1% NaCl as drinking water. The rats in the control group (Control) received sesame oil only and distilled water as drinking water. Treatments continued at 3 x/week for 2 or 4 weeks in two separate experiments. In acute injection, for the 2-week treatment ex-

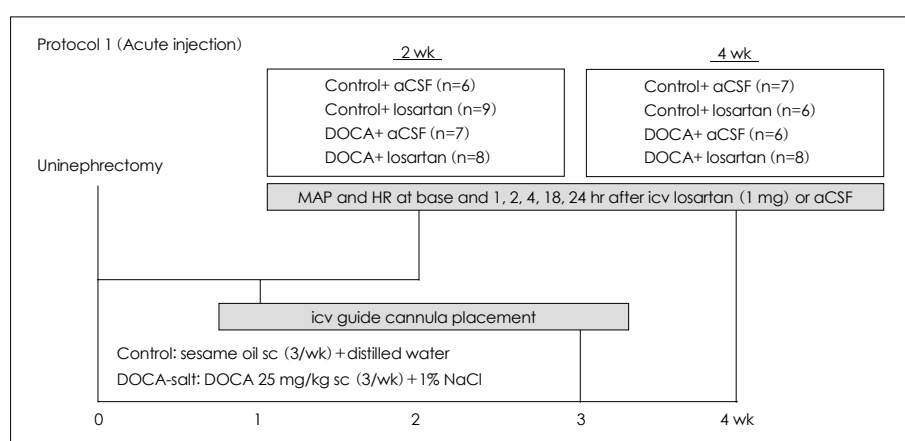


Figure 1. Protocol 1 (acute injection). To evaluate the effect of losartan in the development and maintenance of DOCA-salt hypertensive rats, the study group was divided into 2-week and 4-week groups. DOCA: deoxycorticosterone acetate, aCSF: artificial cerebral space fluid, MAP: mean arterial pressure, HR: heart rate.

periment, the following groups were studied: Control+icv aCSF (n=6), Control+icv losartan (n=9), DOCA+icv aCSF (n=7), and DOCA+icv losartan (n=8). For the 4-week treatment, the groups studied were Control+icv aCSF (n=7), Control+icv losartan (n=6), DOCA+icv aCSF (n=6), and DOCA+icv losartan (n=8) (Figure 1). In chronic injection (4 week), Control (n=8), DOCA+icv aCSF (n=8), DOCA+sc losartan (n=7), and DOCA+icv losartan (n=7) were studied (Figure 2).

Intracerebroventricular cannulation

Intracerebroventricular cannulation was performed at least 1 week before arterial cannulation. A guide cannula (23 gauge, stainless steel tubing) was implanted just above the left lateral cerebroventricle and fixed on the skull of the rat. The cannula was located 0.5-mm posterior and 1.4-mm lateral to the bregma, and its lower end was about 0.3 mm above the ventricle as previously described.¹⁵⁾

Direct blood pressure measurement and icv injection

About 2 or 4 weeks after nephrectomy, in the early morning under halothane anesthesia, the right carotid artery was cannulated with PE-50 polyethylene tubing

filled with heparinized saline. After recovering from the anesthesia for 4–5 hrs, the intra-arterial catheter was connected to a pressure transducer to record mean arterial pressure (MAP) and heart rate (HR). The output signals of the transducer were amplified and fed to an IBM-compatible computer with a data acquisition program (Dataquest Labpro; Data Science International, St. Paul, MN) that allowed on-line analysis of the pulsatile blood pressure signal and storage of data.

For icv injection, a 26-gauge stainless cannula was inserted into the guide cannula so that its tip protruded 0.8–1.0 mm into the lateral ventricle. In acute injection, a 20- μ L volume Hamilton microsyringe was used for icv injections. Injections consisted of a volume of 4- μ L delivered manually over a period of 2 min (losartan 1 mg dissolved in 1 mL aCSF, or 1 mL aCSF). Each rat received only a single injection. In chronic injection, an osmotic minipump was implanted subcutaneously and connected to the icv cannula to allow continuous injection (1 mg/kg/day for 4 weeks) in the DOCA+icv losartan group. In the DOCA+sc losartan group, the osmotic minipump was not connected to the icv cannula; instead, losartan was injected subcutaneously (1 mg/kg/day for 4 weeks) (Figure 2).

The resting BP and HR were taken at baseline, and at 1, 2, 4, 18, and 24 hours after acute icv injection. The accuracy of the icv cannulation was checked at autopsy with an icv injection of methylene blue. In the chronic injection study, after 4 weeks of injection, the resting BP and HR were recorded and the weights of the right and left ventricles and kidneys were measured.

All data are expressed as means \pm SEM. One-way ANOVA was used to analyze MAP and HR responses to icv losartan, followed by a Newman-Keuls' to compare individual readings to the baseline. Values of $p < 0.05$ were considered statistically significant.

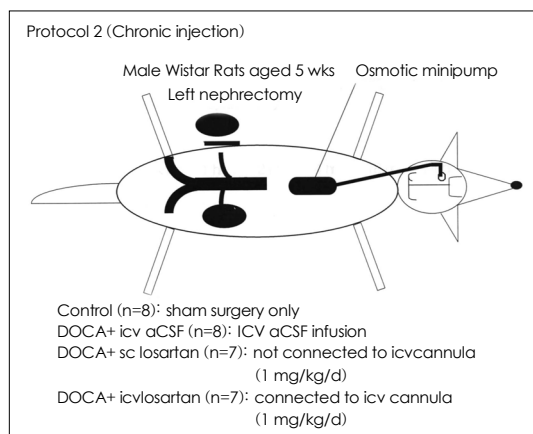


Figure 2. Protocol 2 (chronic injection). After 4 weeks of injection, the resting BP and HR and the weights of the kidneys and the right and left ventricles of each group were recorded. DOCA: deoxycorticosterone acetate, aCSF: artificial cerebral space fluid, ICV: intracerebroventricle, BP: blood pressure, HR: heart rate.

Results

Acute injection

After 2 and 4 weeks of treatment, the average resting

mean arterial pressure (MAP) of the 2 DOCA-salt hypertensive groups increased significantly compared to that of the 2 control groups (144 ± 6 mmHg versus 118 ± 5 mmHg and 170 ± 5 mmHg versus 115 ± 3 mmHg, $p < 0.01$ for both). No significant difference in resting basal HR was detected between the two groups. Body weight in DOCA-salt hypertensive rats was similar to that in the

Table 1. Resting mean arterial pressure, heart rate, and body weight in rats 2 or 4 weeks on DOCA-salt in acute injection study

	MAP (mmHg)	HR (bpm)	BW (gm)
2 week			
Control			
aCSF (n=6)	110 ± 5	387 ± 13	301 ± 12
Losartan (n=9)	122 ± 6	415 ± 7	302 ± 10
DOCA-salt			
aCSF (n=7)	$142 \pm 8^*$	392 ± 16	303 ± 8
Losartan (n=8)	$146 \pm 9^*$	418 ± 18	284 ± 8
4 week			
Control			
aCSF (n=7)	115 ± 3	383 ± 8	342 ± 9
Losartan (n=7)	115 ± 3	390 ± 16	357 ± 9
DOCA-salt			
aCSF (n=6)	$163 \pm 8^\dagger$	403 ± 4	$301 \pm 12^*$
Losartan (n=8)	$174 \pm 7^\dagger$	405 ± 20	$289 \pm 6^*$

Values are expressed as mean \pm SEM. *: $p < 0.05$ and † : $p < 0.01$ when compared to the control group treated with icv aCSF or losartan. BW: body weight, MAP: mean arterial pressure, HR: heart rate, DOCA: deoxycorticosterone acetate, bpm: beats per minute

controls after 2 weeks, but was significantly less after 4 weeks (Table 1).

In rats with DOCA-salt for 2 weeks, MAP did not show a significant change at 1 and 2 hours after icv injection of losartan, but started declining at 4 hours, and significant decreases in MAP were found at 18 and 24 hours after icv losartan. In hypertensive rats with DOCA-salt for 4 weeks, MAP decreased significantly at 4, 18 and 24 hours. BP reached that of control rats at 18 and 24 hours at both 2 and 4 weeks (Figure 3). There was no significant change of HR (Figure 4).

In control rats, icv losartan had no effect on BP and HR. Icv aCSF did not significantly change MAP and HR in either the DOCA-salt hypertensive rats or the control rats (Figure 3).

Chronic injection

After 4 weeks of treatment, the resting MAP was higher in the DOCA+icv aCSF group than in the control (141.1 ± 5.5 mmHg vs 112.8 ± 5.6 mmHg, $p = 0.0028$), and the resting MAP was higher in the DOCA+sc losartan group than in the control, but not statistically significant (132.4 ± 8.6 mmHg vs 112.8 ± 5.6 mmHg, $p = 0.0725$). However, the resting MAP of the DOCA+icv losartan group was not significantly different from that of the control (127 ± 15.2 mmHg vs 112.8 ± 5.6 mmHg, $p = 0.373$). A strong trend marked the decrease of MAP in the DOCA+icv losartan group, which was more than

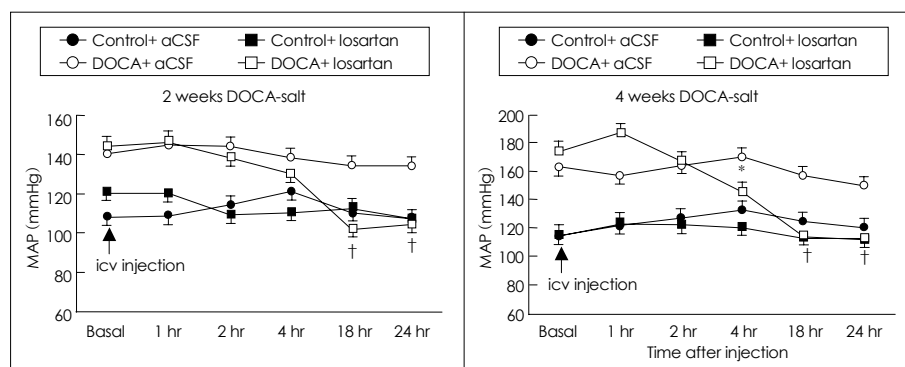


Figure 3. Acute injection. Time course of changes in mean arterial pressure (MAP) after injection of losartan 1 mg/4 μ L or artificial CSF into the lateral ventricle of the brain. Values represent mean \pm SEM. Asterisks below the line designate difference from basal in DOCA+losartan group. DOCA: deoxycorticosterone acetate, aCSF: artificial cerebral space fluid, CSF: cerebral space fluid. *: $p < 0.05$, † : $p < 0.01$.

Acute and Chronic Central Losartan Effect in DOCA-Salt Hypertension

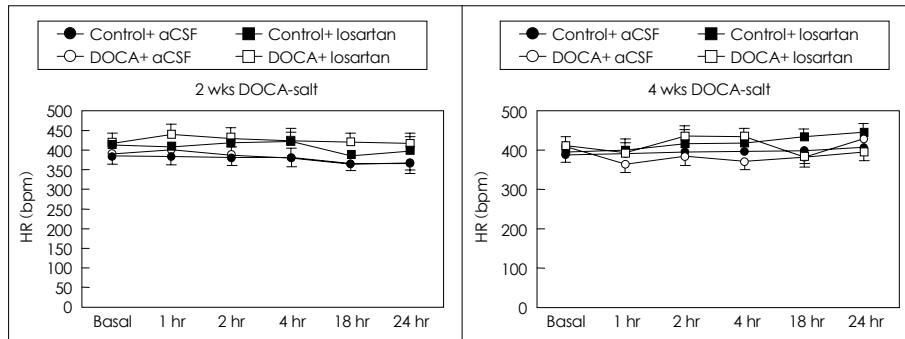


Figure 4. Acute injection. Time course of changes in heart rate (HR) after injection of losartan 1 mg/4 µL or artificial CSF into the lateral ventricle of the brain. Values represent mean ± SEM. bpm: beats per minute, DOCA: deoxycorticosterone acetate, aCSF: artificial cerebral space fluid, CSF: cerebral space fluid.

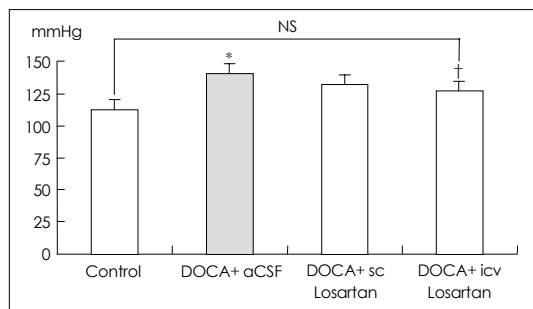


Figure 5. Changes in mean arterial pressure (MAP) after 4 weeks of chronic injection. The DOCA+icv losartan group showed no significant increase in MAP versus the control ($p=0.373$), but the DOCA+sc losartan group showed a trend of increasing MAP versus the control ($p=0.0725$). NS: not significant, DOCA: deoxycorticosterone acetate, aCSF: artificial cerebral space fluid. *: $p<0.05$ vs control, †: $p=.578$ vs DOCA+aCSF.

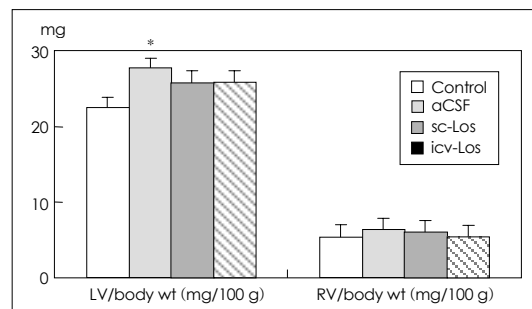


Figure 7. Changes in LV and RV weight after 4 weeks of chronic injection. The DOCA+aCSF group showed a significant increase in LV weight compared with the control, but the LV weights of both the DOCA+sc losartan or DOCA+icv losartan groups were not different from the control. LV: left ventricle, RV: right ventricle, DOCA: deoxycorticosterone acetate, aCSF: artificial cerebral space fluid. *: $p<0.05$ vs control.

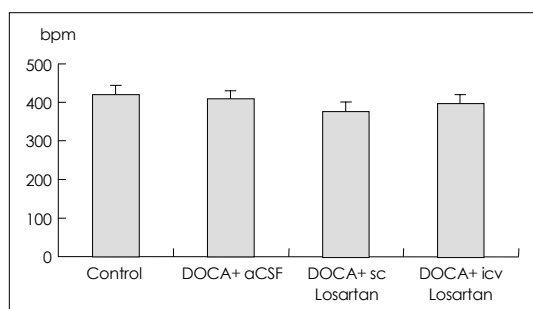


Figure 6. Changes in heart rate (HR) after 4 weeks of chronic injection. No significant difference was found in all study groups. DOCA: deoxycorticosterone acetate, aCSF: artificial cerebral space fluid, bpm: beat per minute.

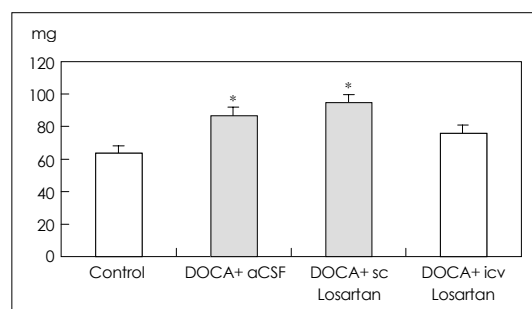


Figure 8. Changes in kidney weight after 4 weeks of chronic injection. In contrast to the other groups, which showed significant increases in kidney weight compared the control, the DOCA+icv losartan group showed no significant increase in kidney weight. DOCA: deoxycorticosterone acetate, aCSF: artificial cerebral space fluid. *: $p<0.05$ vs control.

that of the DOCA+icv aCSF group (127 ± 15.2 mmHg vs 141.1 ± 5.5 mmHg, $p=0.0578$) (Figure 5). Resting HR did not differ in all groups (Figure 6). LV and kidney weight increased significantly in the DOCA+icv aCSF group compared with the control group, but in the DOCA+icv losartan group, LV and kidney weight did not increase compared with the control. RV weight was the same in all groups (Figure 7, 8).

Discussion

The major parts of the brain relating to circulatory regulation include periventricular tissues, hypothalamic nuclei, periaqueductal gray matter of the midbrain, and nuclei of the medulla oblongata. These areas have AT₁ receptors that can be activated by angiotensin II (Ang II) and can induce hemodynamic change. Most of the brain tissues have the blood-brain barrier (BBB) separated from the circulatory system, but some circumventricular organs (CVO) and area postrema (AP) of the medulla oblongata have no BBB. These can be directly affected by circulatory active substances.

The brain renin-angiotensin system (RAS) contributes to the development and maintenance of certain forms of salt-sensitive hypertension. Chronic blockade of brain AT₁ receptors by icv losartan prevents both the sympathetic hyperactivity and exacerbation of hypertension in SHR on high sodium.¹⁵⁾ Chronic icv infusion of the AT₁ receptor blocker CV-11974 (active metabolite of candesartan) or losartan prevents the development of hypertension in Dahl-Iwai salt-sensitive rats¹⁶⁾ and Dahl S rats.¹⁷⁾ Therefore, activation of the brain AT₁ receptors seems to be essential for the development of salt-sensitive hypertension in SHR and Dahl S rats.

In unilaterally nephrectomized rats, blood pressure rose between 1–2 weeks after beginning DOCA-salt treatment.^{18–23)} After 4 weeks, blood pressure further increased and remained elevated even after stopping steroid treatment (post-DOCA-salt hypertension).²⁴⁾ Depending on the dose of steroid and sodium intake, rats with systolic blood pressure in excess of 200 mmHg entered a mali-

gnant phase and died with brain, vascular, and renal lesions as well as weight loss. In the present study, 1 among 15 rats after 2 weeks and 11 among 14 rats after 4 weeks of DOCA-salt treatment had systolic blood pressures over 200 mmHg. We used the 2-week rats to assess the role of Ang II in the development of DOCA-salt hypertension and the 4-week rats to evaluate the role of Ang II in the maintenance of DOCA-salt hypertension.

From a biochemical point of view, there is evidence for increased activity of the brain RAS during the development of DOCA-salt hypertension. After DOCA-salt treatment for 1 month, renin-like activity and Ang II in the hypothalamus and brain stem nuclei increased, while plasma renin activity was very low.^{7,8)} In rats with DOCA-salt hypertension for 4–8 weeks, angiotensin II receptor binding on autoradiography was also elevated in selected brain areas involved in cardiovascular regulation, such as the nucleus of the solitary tract, area postrema, median preoptic nucleus, subfornical organ, and solitary vagal area.^{4–6)} Moreover, BP responses to centrally administered Ang II were significantly augmented after 3 and 8–10 weeks of DOCA-salt treatment in rats.^{4,25)}

However, acute icv infusion of the Ang II antagonist saralasin led to a significant dose-dependent increase in BP 30 min after infusion in DOCA-salt hypertensive rats.¹²⁾ This pressor response might be due to partial agonist activity of saralasin²⁶⁾ in conjunction with increased number and sensitivity of brain Ang II receptors. Since nonpeptide AT₁ receptor blockers such as losartan lack agonist activity, they may be used to specifically examine the functional role of AT₁ receptors. In the present study, after 2 weeks of DOCA-salt treatment losartan decreased the MAP to control levels at 18 and 24 h after the injection. The decrease in BP developed slowly and did not occur until 4 h postinjection. These data indicate that brain AT₁ receptor stimulation plays a major role in the development of hypertension in DOCA-salt rats. Since icv injection of losartan also normalized the MAP once the hypertensive state is established (4 weeks of DOCA-salt), the brain RAS continues to play a major role in the

maintenance of DOCA-salt hypertension.

Injection of losartan into the lateral ventricle or the rostral parts of the third ventricle decreased BP in 4-week DOCA-salt hypertensive rats by 10–17 mmHg.¹⁴⁾ These depressor responses to losartan began within 1 min, reached a plateau within 20 min and lasted for more than 1 h.¹³⁾ In contrast, in the current study icv losartan decreased arterial pressure until 4 hours after injection, and control levels of BP were reached at 18–24 hours after injection. Others also observed delayed BP responses to AT₁ receptor blockers after icv administration. Continuous icv infusion of the AT₁ receptor blocker CV-11974 for 7 days lowered the BP only from the fourth day after the start of the infusion in 6-week DOCA-salt treated rats.¹⁴⁾ Pare et al. demonstrated that in SHR, losartan induced long-lasting (days) BP reductions (≤ 40 mmHg), only at 18 h after icv injection, but not EXP-3174, an active metabolite of losartan. They hypothesized that the slow development of BP reduction and its persistence might be due to the formation of an active metabolite, different from EXP-3174.²⁷⁾ However, these studies used rats under general anesthesia, which decreases vascular sympathetic tone and derange blood pressure; so studies using unconscious rats cannot reflect the precise effect of a drug. We used conscious rats to avoid this unexpected bias. In chronic injection study, but not in acute injection study, we observed some leakage from the icv cannulation site connected to the osmotic minipump in several rats during 4 weeks active ambulation. It might be less significant MAP decrease in the chronic DOCA+icv losartan group compared to the acute injection study. The chronic study showed no significant increase in the MAP of the DOCA+icv losartan group versus the control ($p=0.373$). In contrast, DOCA+sc losartan group, showed a statistically insignificant but slight increase in MAP versus the control ($p=0.0725$) (Figure 5). This indicates that blocking the peripheral AT₁ receptors by subcutaneous injection of losartan is not sufficient to decrease MAP and that intracerebroventricular injection of losartan is more potent in blocking the central AT₁ receptor. The protective effect on the heart and kidneys

observed in the DOCA+icv losartan group can also be explained by this MAP drop effect (Figure 7, 8).

Besides Ang II, vasopressin,²⁸⁾²⁹⁾ and the sympathoadrenal system³⁰⁾ may also contribute to the development and maintenance of DOCA-salt hypertension in rats. Central pathways involving stimulation of AT₁ receptors may contribute to increases in arginine vasopressin (AVP) and sympathetic activity, which contribute to the rise in BP of DOCA-salt rats.²⁵⁾ Acute icv injection of CV-11974 decreased the plasma concentration of AVP, and urinary excretion of AVP decreased from the fourth day of continuous icv infusion of CV-11974.¹⁴⁾ Therefore, it is possible that inhibiting the brain AT₁ receptor results in reduced release of AVP and, consequently, BP with some latency due to yet unclear reasons. Recent reports suggest that vasopressin stimulates arterial ET-1 production, resulting in increased superoxide levels in DOCA-salt hypertension. Vasopressin induces O₂⁻ production via its V₁ receptors, and the effect is ET-1 dependent.³¹⁾ ET-1 augments vascular superoxide production at least in part via an ET_A/NADPH oxidase pathway.³²⁾ It is known that circulating ET-1 level is increased in patients with low-renin essential hypertension.

In summary, centrally administered losartan normalized BP in DOCA-salt hypertensive rats. These results support the concept that the brain RAS and, specifically, stimulation of the brain AT₁ receptor contribute to the development and maintenance of hypertension in this model and also suggests that the brain AT₁ receptor blocker has a protective effect on cardiovascular regulatory organs, such as the heart and kidney.

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