

## Effects of Centrally Administered Losartan on Deoxycorticosterone-salt Hypertension Rats

To investigate whether brain AT<sub>1</sub> receptor stimulation contributes as a hypertensive mechanism to deoxycorticosterone acetate (DOCA)-salt hypertension, losartan (1 mg/4  $\mu$ L) or artificial cerebrospinal fluid (aCSF) was injected into the lateral cerebral ventricle in conscious control uninephrectomized Wistar rats or rats with DOCA-salt for 2 or 4 weeks, and mean arterial pressure (MAP) and heart rates (HR) were recorded. In rats with DOCA-salt treatment, resting MAP increased to  $144 \pm 6$  mmHg after 2 weeks and to  $170 \pm 5$  mmHg after 4 weeks versus 115-120 mmHg in controls. In rats with 2 week DOCA-salt treatment, MAP started declining at 4 hr after intracerebroventricular (icv) injection of losartan, and significant decreases in MAP were found at 18 and 24 hr. In rats with 4 week DOCA-salt treatment, MAP was significantly decreased at 4, 18 and 24 hr. In both groups MAP decreased to that of control rats. In control rats, icv losartan had no effect on MAP and HR. Icv aCSF did not significantly change MAP and HR in either DOCA-salt hypertensive rats or control rats. Normalization of MAP after icv administration of the AT<sub>1</sub> receptor antagonist suggests a significant role for brain AT<sub>1</sub> receptor stimulation in the development and maintenance of hypertension in the DOCA-salt hypertensive rat model.

**Key Words :** Losartan; Angiotensin II; Brain AT<sub>1</sub> Receptor; Deoxycorticosterone, Hypertension

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## 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) inhibitors (2) or angiotensin (Ang) II receptor antagonists (3) has no effect on blood pressure (BP). However, there is some evidence for 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 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 hypothalamus and brain stem nuclei (7, 8). 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 or into the rostral part of the third ventricle did lower BP for more than 1 hr in 4 weeks DOCA-salt hypertensive rats (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 4 weeks and 6 weeks DOCA-salt hypertensive rats, the role of brain Ang II in the development of the hypertension in the DOCA-salt hypertensive rat model is not known yet.

The aim of the present study was to determine the acute central effects of the type 1 Ang II (AT<sub>1</sub>) receptor antagonist losartan in conscious DOCA-salt hypertensive rats, at either 2 weeks during the development of the hypertension or at 4 weeks in the maintenance phase of the hypertension.

## MATERIALS AND 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 hr light/dark cycle, and 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 a left nephrectomy. After surgery they were randomized to either the control or DOCA-salt group. At twenty-four hours after surgery, rats in the DOCA-salt group received the first injection of DOCA (25 mg/kg subcutaneously in 0.1 mL sesame oil/100 g body weight) and 1% NaCl as drinking water. Rats in the control group (Control) received sesame oil only and distilled water as drinking water. Treatments continued at 3 times a week for 2 or 4 weeks in two separate experiments. For the 2-week treatment experiment, the following groups were studied: Control+icv aCSF (n=6); Control+icv losartan (n=9); DOCA+icv aCSF (n=7); DOCA+icv losartan (n=8), and for 4 week treatment: Control+icv aCSF (n=7); Control+icv losartan (n=6); DOCA+icv aCSF (n=6); DOCA+icv losartan (n=8).

### Intracerebroventricular cannulation

Intracerebroventricular cannulation was done 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 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 recovery from the anesthesia for 4-5 hr, in the afternoon the intra-arterial catheter was connected to a pressure transducer for recording 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, U.S.A.) 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. A 20- $\mu$ L volume Hamilton

microsyringe was used for icv injections. Injections consisted of a volume of 4- $\mu$ L delivered manually over a period of 2 min (losartan 1 mg dissolved in aCSF, or aCSF). Each rat received only a single injection.

The resting BP and HR were taken at baseline, and at 1, 2, 4, 18, and 24 hr after icv injection. The accuracy of the icv cannulation was checked at autopsy with an icv injection of methylene blue.

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.

## RESULTS

After 2 and 4 weeks of treatment, resting mean arterial pressure (MAP) increased significantly in rats with DOCA-salt hypertension versus control rats ( $144 \pm 6$  mmHg versus  $118 \pm 5$  mmHg and  $170 \pm 5$  mmHg versus  $115 \pm 3$  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 controls after 2 weeks, but was significantly less after 4 weeks (Table 1).

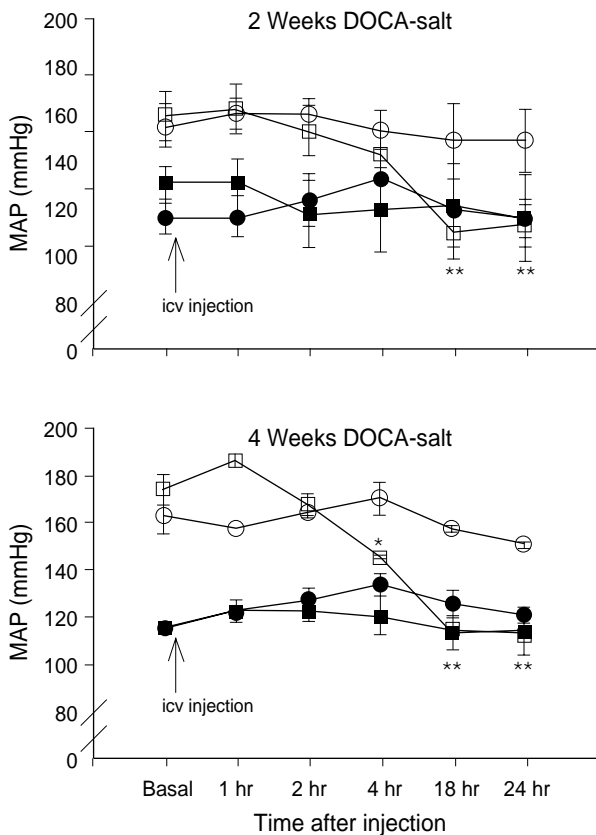
In rats with DOCA-salt for 2 weeks, MAP did not show a significant change at 1 and 2 hr after icv injection of losartan, but started declining at 4 hr, and significant decreases in MAP were found at 18 and 24 hr after icv losartan. In 4 weeks DOCA-salt hypertensive rats, MAP decreased significantly at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

In control rats, icv losartan had no effect on BP and HR. Icv aCSF did not significantly change MAP and HR in either DOCA-salt hypertensive or control rats (Fig. 1).

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

		MAP (mmHg)	HR (bpm)	BW (g)
<b>Two weeks</b>				
Control	aCSF (n=6)	110 $\pm$ 5	387 $\pm$ 13	301 $\pm$ 12
	Losartan (n=9)	122 $\pm$ 6	415 $\pm$ 7	302 $\pm$ 10
DOCA-salt	aCSF (n=7)	142 $\pm$ 8*	392 $\pm$ 16	303 $\pm$ 8
	Losartan (n=8)	146 $\pm$ 9*	418 $\pm$ 18	284 $\pm$ 8
<b>Four weeks</b>				
Control	aCSF (n=7)	115 $\pm$ 3	383 $\pm$ 8	342 $\pm$ 9
	Losartan (n=7)	115 $\pm$ 3	390 $\pm$ 16	357 $\pm$ 9
DOCA-salt	aCSF (n=6)	163 $\pm$ 8 <sup>†</sup>	403 $\pm$ 4	301 $\pm$ 12*
	Losartan (n=8)	174 $\pm$ 7 <sup>†</sup>	405 $\pm$ 20	289 $\pm$ 6*

Values are expressed as mean  $\pm$  SEM. \* $p < 0.05$  and <sup>†</sup> $p < 0.01$  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

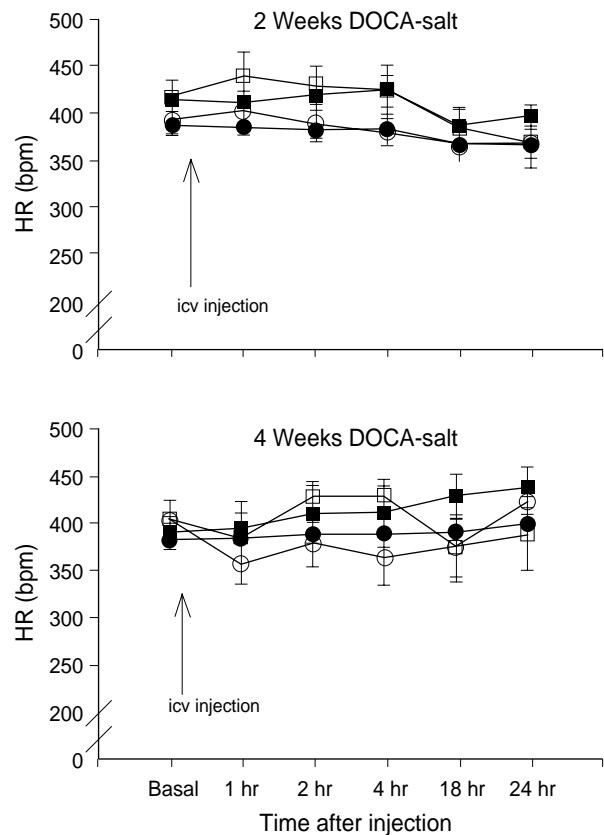


**Fig. 1.** Time course of changes in mean arterial pressure (MAP) after injection of losartan 1 mg/4  $\mu$ L or artificial CSF into the lateral ventricle of the brain. Values represent mean  $\pm$  SEM. Asterisks below the line designate difference from basal in DOCA+losartan group. \* $p < 0.05$ , \*\* $p < 0.01$ . (●); Control+aCSF, (■); Control+losartan, (○); DOCA+aCSF, (□); DOCA+losartan.

### DISCUSSION

The brain renin-angiotensin system (RAS) contributes to the development and maintenance of certain forms of salt-sensitive hypertension. Chronic blockade of brain AT<sub>1</sub> receptors by icv losartan prevents both the sympathetic hyperactivity and exacerbation of hypertension in SHR on high sodium (15). Chronic icv infusion of the AT<sub>1</sub> receptor blocker CV-11974 (active metabolite of candesartan) or losartan prevents the development of hypertension in Dahl-Iwai salt-sensitive rats (16) and Dahl S rats (17). Therefore, activation of brain AT<sub>1</sub> receptors seems to be essential for development of salt-sensitive hypertension in SHR and Dahl S rats.

In unilaterally nephrectomized rats, blood pressure rises between 1-2 weeks after start of DOCA-salt treatment (18-23). After 4 weeks, blood pressure has further increased and remains 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



**Fig. 2.** Time course of changes in heart rate (HR) after injection of losartan 1 mg/4  $\mu$ L or artificial CSF into the lateral ventricle of the brain. Values represent mean  $\pm$  SEM. bpm, beats per minute. (●); Control+aCSF, (■); Control+losartan, (○); DOCA+aCSF, (□); DOCA+losartan.

in excess of 200 mmHg enter a malignant phase and die with brain, vascular and renal lesions with 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 pressure over 200 mmHg. We used 2-week rats to assess the role of Ang II in the development of DOCA-salt hypertension and 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 hypothalamus and brain stem nuclei were elevated, but 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 nucleus of solitary tract, area postrema, median preoptic nucleus, subfornical organ, and solitary vagal area (4-6). Moreover, BP responses to centrally administered Ang II were significant-

ly 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 saralasin (26) in conjunction with increased number and sensitivity of brain Ang II receptors. Since nonpeptide AT<sub>1</sub> receptor blockers such as losartan lack agonist activity, they may be used to specifically examine the functional role of AT<sub>1</sub> receptors. In the present study, after 2 weeks of DOCA-salt treatment losartan decreased the MAP to control levels at 18 and 24 hr after the injection. The decrease in BP developed slowly and did not occur until 4 hr postinjection. These data indicate that brain AT<sub>1</sub> 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 weeks DOCA-salt hypertensive rats by 10-17 mmHg (14). These depressor responses to losartan began within 1min, reached a plateau within 20 min and lasted for more than 1 hr (13). In contrast, in the current study icv losartan decreased arterial pressure until 4 hr after injection, and control levels of BP were reached at 18-24 hr after injection. Others also observed delayed BP responses to AT<sub>1</sub> receptor blockers after icv administration. Continuous icv infusion of the AT<sub>1</sub> 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 ( $\leq 40$  mmHg), only at 18 hr 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).

Besides Ang II, vasopressin, endothelin (28, 29), and the sympathoadrenal system may also contribute to the development and maintenance of DOCA-salt hypertension in rats. Central pathways involving AT<sub>1</sub> receptor stimulation may contribute to increases in arginine vasopressin (AVP) and sympathetic activity, which contribute to the increases in BP in DOCA-salt rats (25, 40). Acute icv injection of CV-11974 decreased plasma concentration of AVP, and urinary excretion of AVP decreased from the fourth day of continuous icv infusion of CV-11974 (14). It is therefore possible that inhibition of brain AT<sub>1</sub> receptor results in a decrease of AVP release and therefore BP with some latency for as yet unclear reasons.

In summary, centrally administered losartan normalized BP in DOCA-salt hypertensive rats. These results support

the concept that the brain RAS and specifically brain AT<sub>1</sub> receptor stimulation may contribute to the development and maintenance of hypertension in this model.

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