Central infusion of aliskiren prevents sympathetic hyperactivity and hypertension in Dahl salt-sensitive rats on high salt intake

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Central infusion of aliskiren prevents sympathetic hyperactivity and hypertension in Dahl salt-sensitive rats on high salt intake. Am J Physiol Regul Integr Comp Physiol 302: R825–R832, 2012. First published January 18, 2012; doi:10.1152/ajpregu.00368.2011.—Central infusion of an angiotensin type 1 (AT1) receptor blocker prevents sympathetic hyperactivity and hypertension in Dahl salt-sensitive (S) rats on high salt. In the present study, we examined whether central infusion of a direct renin inhibitor exerts similar effects. Intracerebroventricular infusion of aliskiren at the rate of 0.05 mg/day markedly inhibited the increase in ANG II levels in the cerebrospinal fluid and in blood pressure (BP) caused by intracerebroventricular infusion of rat renin. In Dahl S rats on high salt, intracerebroventricular infusion of aliskiren at 0.05 and 0.25 mg/day for 2 wk similarly decreased resting BP in Dahl S rats on high salt. In other groups of Dahl S rats, high salt intake for 2 wk increased resting BP by ~25 mmHg, enhanced pressor and sympathoexcitatory responses to air-stress, and desensitized arterial baroreflex function. All of these effects were largely prevented by intracerebroventricular infusion of aliskiren at 0.05 mg/day. Aliskiren had no effects in rats on regular salt. Neither high salt nor aliskiren affected hypothalamic ANG II content. These results indicate that intracerebroventricular infusions of aliskiren and an AT1 receptor blocker are similarly effective in preventing salt-induced sympathetic hyperactivity and hypertension in Dahl S rats, suggesting that renin in the brain plays an essential role in the salt-induced hypertension. The absence of an obvious increase in hypothalamic ANG II by high salt, or decrease in ANG II by aliskiren, suggests that tissue levels do not reflect renin-dependent ANG II production in sympathoexcitatory angiotensinergic neurons.

Central angiotensinergic pathways play a pivotal role in salt-induced hypertension. In Dahl salt-sensitive (S) rats, high salt intake increases mRNA expression or binding densities of angiotensin type 1 (AT1) receptor and angiotensin-converting enzyme (ACE) in brain nuclei involved in cardiovascular regulation such as the paraventricular nucleus (PVN) and supraoptic nucleus (SON) (34, 39, 44). Chronic intracerebroventricular infusion of the AT1 receptor blockers losartan, irbesartan, or candesartan prevents sympathetic hyperactivity and hypertension in Dahl S rats on high salt intake (15, 25, 36). Central infusion of Na+–rich artificial cerebrospinal fluid (aCSF) also causes sympathetic hyperactivity and hypertension (12, 17), and this pressor effect is markedly attenuated in transgenic rats with absent glia-derived angiotensinogen (12), suggesting that angiotensins locally produced in the brain are essential for the pressor effect of CSF Na+. Whether renin locally produced in the brain or other enzymes contribute to production of angiotensin peptides in angiotensinergic pathways and contributes to salt-induced hypertension in Dahl S rats has not yet been assessed.

Circulating renin is unable to cross the blood-brain barrier, but renin-like activity and immunoreactivity have been demonstrated in hypothalamic nuclei such as the PVN and SON (6, 11), suggestive for local production. Human renin and angiotensinogen double-transgenic mice expressing intracellular or secreted renin in the brain show an increase in blood pressure (BP) that is rapidly decreased by intracerebroventricular injection of losartan, indicating that renin in the brain can play a functional role in BP regulation (21). If endogenous renin plays a functional role, inhibition of renin activity in the brain should reduce local angiotensin production and achieve results similar to central blockade of AT1 receptors.

Aliskiren is a recently developed direct human renin inhibitor that blocks the first rate-limiting catalytic step of the renin-angiotensin system and prevents the cleavage of angiotensinogen to form ANG I (40). Aliskiren inhibits human, dog, and rat plasma renin with IC50 values of 0.6, 7, and 80 nM, respectively (40). In rats overexpressing human renin and angiotensinogen (29), subcutaneous infusion of aliskiren at 0.3–3 mg·kg–1·day–1 for 3 wk markedly decreased circulating ANG I, ANG II, and BP. In spontaneously hypertensive rats, subcutaneous infusion of aliskiren at higher rates (10–100 mg·kg–1·day–1) decreased BP in a dose-dependent manner (41) and at 100 mg·kg–1·day–1 decreased MAP by 30 mmHg associated with decreases in plasma renin activity and ANG I and II levels (37). So far, there have been no studies reported regarding effects of aliskiren on renin activity and angiotensin levels in the brain.

The main purpose of the present study was to examine the effects of chronic intracerebroventricular infusion of aliskiren on salt-induced sympathoexcitation and hypertension in Dahl S rats. To evaluate whether in the brain aliskiren is able to inhibit rat renin, we tested in Wistar rats whether intracerebroventricular infusion of aliskiren can prevent the increases in CSF ANG II as well as in BP and heart rate (HR) caused by intracerebroventricular infusion of rat renin. In Dahl S rats, we evaluated the effects of high salt diet with or without concomitant intracerebroventricular infusion of aliskiren on BP, HR, and renal sympathetic nerve activity (RSNA) at rest and in response to air-stress, as well as arterial baroreflex control of RSNA and HR, and on ANG II content in the whole hypothalamus.

Methods

Male Dahl S rats (SS/Mcw) and Wistar rats (Charles River, Montreal, Canada), 5–6 wk of age, were fed with standard rat chow (120 μmol Na+/g) and water ad libitum. The study was approved by the University of Ottawa Animal Care Committee and conforms with
Dose-response study for aliskiren in Dahl S rats. In four groups of Dahl S rats (n = 7–9/group), under isoflurane inhalation anesthesia, an “L”-shaped stainless steel cannula was placed in the right lateral cerebroventricle (coordinates with respect to bregma: 0.4 mm posterior and 1.4 mm lateral, and depth from dura: 3.3 mm) (15). Via polyethylene tubing, the cannula was connected to an osmotic mini-pump (model 2002; Alzet) placed subcutaneously for intracerebroventricular infusion for 2 wk of aCSF or aliskiren at 0.05, 0.25, or 1.25 mg/day. Aliskiren was dissolved in aCSF followed by sterilization with an Acrodisc syringe filter. The rates of intracerebroventricular infusion of aliskiren were extrapolated from studies using oral or subcutaneous administration of aliskiren (27, 37, 41). At a CSF production of 6 ml/day and total CSF volume of 0.5 ml (9), intracerebroventricular infusion of aliskiren at the rates of 0.05, 0.25, or 1.25 mg/day results in a concentration of 6 ml/day and total CSF volume of 0.5 ml (9), intracerebroventricular infusion of aliskiren at the rates of 0.05, 0.25, or 1.25 mg/day, respectively, but likely a factor lower accounting for its distribution into brain tissue and clearance in the central nervous system (CNS). After the surgery, all rats were provided with high salt diet (1,370 mmol Na+/H11001/g; Harlan Teklad, Madison, WI). A fifth group of rats stayed on regular salt diet and received an intracerebroventricular infusion of aCSF.

Two weeks after the start of the treatments, under isoflurane anesthesia, a polyethylene PE-10/PE-50 tubing was placed in the right femoral artery and was tunneled to the back of the neck. The following morning about 18 h after the surgery, the catheter was connected to a pressure transducer linked to a Grass polygraph (model 7E) and a Grass 7P44 tachograph. Real-time digital data were obtained using a personal computer equipped with a Grass data acquisition and analysis program (Polyview 2.0). Rats were allowed to rest for 30 min, and thereafter resting BP and HR were recorded for 10 min in conscious animals. Rats were then killed with an overdose of phenylephrine was infused intravenously at 5–50 µg/kg H11001/min in conscious animals. A 30-gauge stainless steel needle was then inserted in the guide cannula so that its tip protruded into the lateral ventricle. The upper end of the needle was connected to a 10-µl Hamilton microsyringe or 1-ml syringe for intracerebroventricular injection or infusion via a Sage 355 infusion pump. The experiment was conducted in three parallel groups (n = 4/group). In the first group, 2 µl aCSF were injected intracerebroventricularly followed by intracerebroventricular infusion of aCSF at 100 µl/h for 1 h. Next, renin at the pretested arbitrary rate was infused intracerebroventricularly for 30 min. In the second group, aliskiren (10 µg/2 µl aCSF) was first injected intracerebroventricularly as a loading dose, followed by intracerebroventricular infusion of aliskiren at 2 µg/100 µl H-1 (equivalent to 0.05 mg/day) for 1 h. Next, aliskiren (2 µg/100 µl H-1) plus renin at the same rate as in group 1 was infused intracerebroventricularly for 30 min. In the third group of rats, aCSF alone was injected and infused at the same rates and for the same durations. For all groups, at the end of the final 30-min intracerebroventricular infusion, the intracerebroventricular infusions continued, and rats were anesthetized with intravenous pentobarbital (50 mg/kg) and mounted on a stereotaxic frame, and CSF was withdrawn from the cisterna magna (16) at <10 µl/s. The CSF samples were stored at −80°C for ANG II assay.

Effects of aliskiren on salt-induced sympathetic hyperactivity and hypertension. Dahl S rats underwent surgery for intracerebroventricular or subcutaneous infusion and were divided into five groups (n = 7–11/group): 1) intracerebroventricular infusion of aCSF and on regular salt diet; 2) intracerebroventricular infusion of aliskiren at 0.05 mg/day and on regular salt diet; 3) intracerebroventricular infusion of aCSF and on high salt diet; 4) intracerebroventricular infusion of aliskiren at 0.05 mg/day and on high salt diet; and 5) a control to assess for possible peripheral effects of centrally administered aliskiren, subcutaneous infusion of aliskiren at 0.05 mg/day and on high salt diet. Diets and infusions continued for 2 wk. To accustom the animals to the experimental environment, all rats were trained to stay in a small testing cage for 30 min three times per week during the 2-wk dietary period.

At the end of the 2-wk treatments, under isoflurane anesthesia, catheters were placed in the right femoral artery and vein, and a pair of silver electrodes was fixed to the left renal nerve with silicone rubber (15). About 4 h after recovery from the anesthesia, the rat was placed in a testing cage that permitted movement back and forth. The electrodes were linked to a Grass P511 bandpass amplifier and a rectifying voltage integrator (model 7P10; Grass) and recorded through the polygraph. The RSNA signals (mV) together with BP and HR were fed into an online computer equipped with Polyview 2.0. Changes in RSNA were assessed as percent changes from the resting RSNA for each rat. After MAP was raised by >50 mmHg, RSNA reached low levels that were very close to noise level. The actual noise level was determined for each rat after it had been killed at the end of the study and was subtracted from the total activity (15).

After a 30-min rest, RSNA, MAP, and HR were recorded for 5 min. A standardized air-stress was then applied for 30 s two times at 10-min intervals, using an air-jet stream (1–1.5 lb/in.2) directed to the face of the rat (15). Twenty minutes after the responses to air stress had subsided and all parameters had returned to baseline levels, phenylephrine was infused intravenously at 5–50 µg/kg H-1·min-1 to induce a ramp increase in MAP up to +50 mmHg over 0.5–1 min.
After all parameters had returned to baseline levels and a further 20-min rest, sodium nitroprusside was infused intravenously at 5–100 
μg·kg⁻¹·min⁻¹ to induce a ramp decrease in MAP down to −50 
mmHg over 0.5–1 min. To evaluate the arterial baroreflex function, 
changes in RSNA and HR in response to changes in MAP were 
analyzed as a logistic model (10). HR responses to increases in MAP 
induced by phenylephrine and decreases in MAP induced by nitro-
prusside were also separately analyzed by linear regression. About 30 
min after the experiment, the rats were killed, and the whole brains 
were collected and stored at −80°C for ANG II assay, as described 
above.

For all experiments, the accuracy of the position of the intracere-
broventricular cannula was verified by visual examination during 
tissue collection. A few rats (4 out of a total of 35) were excluded 
from the dose-response study because of unsuccessful intracerebro-
ventricular cannulation or broken pump catheters, but none from the 
other experiments.

Statistical analysis. One-way ANOVA or one-way repeated-meas-
ures ANOVA was used to analyze data for responses to renin and 
aliskiren, as well as the dose-response experiment for aliskiren. In the 
other experiments, two-way ANOVA considering diet and drug as 
facors was performed. When F ratios were significant, a Bonferroni’s 
multirange test followed to locate the significant differences. Statis-
tical significance was defined as P < 0.05.

RESULTS

Responses to intracerebroventricular infusion of renin. In 
normal Wistar rats, infusion of aCSF or intracerebroventricular 
infusion of aliskiren at 2 μg·100 μl⁻¹·h⁻¹ for 1 h did not 
change BP or HR significantly. In rats with intracerebroven-
tricular infusion of aCSF, subsequent intracerebroventricular 
intron of renin increased MAP and HR significantly, up to 
26 ± 3 mmHg and 60 ± 5 beats/min, respectively (Fig. 1A). In 
rats with preintracerebroventricular infusion of aliskiren, sub-
sequent intracerebroventricular infusion of renin plus aliskiren 
increased MAP by only 10 ± 2 mmHg and HR by 24 ± 4 
beats/min (P < 0.05 for both increases vs. renin alone). BP did 
not change in rats with intracerebroventricular aCSF alone.

ANG II was not detected in CSF of rats with intracerebroven-
tricular infusion of aCSF alone. In rats preinfused with 
aCSF, intracerebroventricular infusion of renin markedly in-
creased CSF ANG II levels. Intracerebroventricular infusion of 
aliskiren inhibited the increase in CSF ANG II by intracere-
broventricular infusion of renin by 80–90% (Fig. 1B).

Dose-response study for intracerebroventricular infusion of 
aliskiren in Dahl S rats. Most of the rats treated with intracere-
broventricular infusion of aliskiren at 1.25 mg/day developed 
seizures and were killed within a few days, and infusion at this 
rate was discontinued. No adverse effects or behavioral abnor-
malities were observed in rats treated with intracerebroventric-
ular infusion of aCSF or aliskiren at 0.05 or 0.25 mg/day. After 
2 wk of treatments, gains of body weight in rats on high salt 
and treated with intracerebroventricular aCSF or aliskiren at 
0.05 and 0.25 mg/day were similar (70 ± 5, and 68 ± 4, and 
67 ± 5 g, respectively).

After 2 wk of high salt diet, in Dahl S rats treated with 
intrad cerebroventricular infusion of aCSF, resting MAP was 
significantly increased compared with rats on regular salt (Fig. 2). Intracerebroventricular infusion of aliskiren at 0.05 
and 0.25 mg/day similarly prevented the salt-induced increase 
in MAP. Resting HR was significantly increased in rats on high 
salt compared with rats on regular salt, and intracerebroven-
tricular infusion of aliskiren at 0.05 and 0.25 mg/day also 
prevented the salt-induced increases in HR (Fig. 2).

Aliskiren and salt-induced sympathetic hyperactivity in Dahl 
S rats. Intracerebroventricular infusion of aliskiren at 0.05 
mg/day had no effects on resting MAP and HR in rats on 
regular salt diet (Fig. 3). High salt diet significantly increased 
resting MAP and HR in rats treated with vehicle. These 
increases were prevented by intracerebroventricular infusion of 
aliskiren. Subcutaneous infusion of aliskiren at the same rate 
did not affect the increases in MAP and HR.

In rats on regular salt diet treated with intracerebroventricular 
vehicle or intracerebroventricular aliskiren, air-jet stress 
elicted similar mild increases in resting MAP, RSNA, and HR 
(Fig. 4). In rats on high salt diet, the extent of excitatory MAP, 
RSNA, and HR responses to air-jet stress was significantly 
enhanced by 100–150%. Intracerebroventricular infusion of 
aliskiren prevented the enhancement of MAP, RSNA, and HR
responses to stress, whereas subcutaneous infusion of aliskiren had no effects.

In rats on regular salt diet, intracerebroventricular infusion of aliskiren had no effect on baroreflex control of RSNA and HR (Fig. 5 and Table 1). High salt diet significantly decreased the maximal gain and range of responses of reflex control of RSNA, and maximal gain of control of HR. Intracerebroventricular infusion of aliskiren prevented salt-induced decreases in these parameters, whereas subcutaneous infusion of al-

Fig. 2. Resting MAP and HR in Dahl salt-sensitive (S) rats on regular salt (RNa) treated with icv infusion of aCSF (veh) or on high salt (HNa) treated with icv infusion of vehicle or aliskiren at 0.05 or 0.25 mg/day for 2 wk (n = 6–9/group). Values are means ± SE analyzed by one-way ANOVA. For MAP, F = 4.23; P = 0.005; HR: F = 2.44; P = 0.03. d, Day. *P < 0.05 vs. others.

Fig. 3. Resting MAP, HR, and hypothalamic ANG II content in Dahl S rats on RNa or HNa and icv infusion of vehicle (veh: aCSF) or icv or sc infusion of aliskiren at 0.05 mg/day for 2 wk. Values are means ± SE (n = 7–11/group) analyzed by 2-way ANOVA. For MAP, F = 21.32, P = 0.0001 between diets; F = 11.68, P = 0.002 between treatments; and F = 5.89, P = 0.02 for diets × treatments. For HR, F = 23.89, P = 0.0001 between diets; F = 0.02, P = 0.89 between treatments; and F = 5.54, P = 0.03 for diets × treatments. For ANG II content, F = 1.89, P = 0.71 between diets; F = 0.02, P = 0.89 between treatments; and F = 1.22, P = 0.73 for diets × treatments. *P < 0.05 vs. rats on RNa. *P < 0.05 vs. rats on HNa with icv vehicle or sc aliskiren.

Fig. 4. Increases in MAP, renal sympathetic nerve activity (RSNA), and HR in response to air-stress in Dahl S rats on RNa or HNa intake and icv infusion of vehicle (aCSF) or icv or sc infusion of aliskiren at 0.05 mg/day for 2 wk. Values are means ± SE (n = 7–11/group) analyzed by 2-way ANOVA. For MAP, F = 17.6, P = 0.0003 between diets; F = 5.54, P = 0.03 for diets × treatments. For HR, F = 21.0, P = 0.0001 between diets; F = 23.0, P = 0.0001 between treatments; and F = 16.63, P = 0.0004 between diets; F = 4.66, P = 0.04 between treatments; and F = 10.5, P = 0.004 for diets × treatments. *P < 0.05 vs. rats on RNa. *P < 0.05 vs. rats on HNa with icv vehicle or sc aliskiren.
Aliskiren had no effects. Overall, the reflex curves were flatter and shifted to higher pressure levels in rats on high salt diet treated with either intracerebroventricular vehicle or subcutaneous aliskiren compared with the other groups (Fig. 5). Changes in gain for HR in response to BP changes induced by nitroprusside or phenylephrine, respectively, or combined gain induced by nitroprusside and phenylephrine were similar to those obtained by logistic analysis for combined HR responses (Table 1).

**Hypothalamic ANG II.** In the dose-response study, no significant differences in hypothalamic ANG II were observed in rats on high salt diet treated with intracerebroventricular infusion of aliskiren at 0.05 and 0.25 mg/day vs. intracerebroventricular infusion of vehicle (27 ± 5 and 42 ± 5 vs. 33 ± 6, P = 0.7). In the second group of Dahl S rats, rats on regular and high salt diet had similar hypothalamic ANG II levels (Fig. 3) that were not affected by intracerebroventricular infusion of aliskiren at 0.05 mg/day (Fig. 3).

**DISCUSSION**

The present study demonstrates that, in Dahl S rats, intracerebroventricular infusion of aliskiren prevents salt-induced increases in resting BP and HR, the salt-induced enhancement in sympathoexcitatory responses to air stress, as well as the impairment in arterial baroreflex control of RSNA and HR. However, neither high salt nor aliskiren caused obvious changes in hypothalamic ANG II content.

Dahl S rats on high salt intake demonstrate sympathetic hyperactivity as well as impaired baroreflex function for both RSNA and HR control (13, 15). In Dahl S rats, both Na⁺ entry from the blood into the CSF and sympathoexcitatory pressor responses to an increase in CSF Na⁺ concentration ([Na⁺]) are enhanced compared with Dahl salt-resistant (R) or Wistar rats (16, 17). These responses to high salt intake and increased CSF [Na⁺] can be prevented by central infusion of an AT1 receptor blocker or ACE inhibitor (14, 15, 25, 36, 44). Intracerebroventricular infusion of aliskiren also prevents the salt-induced sympathetic hyperactivity and hypertension as well as desensitization of arterial baroreflex. Subcutaneous infusion of aliskiren at the same rate had no effects, indicating that peripheral effects of the central infusion by transfer of aliskiren from the brain into the circulation do not play a role. Similar prevention of the hypertension by central blockade of renin, ACE, or AT1 receptors would suggest that renin and...
ACE-dependent ANG II generation in the brain and resultant AT1 receptor stimulation are essential for the high salt-induced hypertension. An increase in CSF [Na+] by high salt intake may be sensed in the SFO/OVLT and lead to enhanced activity in angiotensinergic projections to the PVN and SON (8, 26, 38), as well as RVLM (3). Intracerebroventricular infusion of aliskiren will cause blockade throughout the hypothalamus and brain stem, and alternative approaches with, e.g., small interfering RNA are needed to ascertain the actual sites of action.

Higher AT1 receptor stimulation may result from increased ANG II release and/or increased responsiveness to ANG II. Effects of high salt intake on ANG II release per se have not been studied in any of these nuclei. We previously (44) reported that Dahl S rats on regular salt have significantly (50%) lower hypothalamic ANG II levels than Dahl R rats and that these levels remain at the lower levels after 2 and 5 wk of high salt intake. In the present study, high salt intake also did not cause an obvious increase in hypothalamic ANG II levels in Dahl S rats. However, content in the whole hypothalamus is likely not representative of levels in individual nuclei. The current RIA is not sensitive enough to measure the ANG II content of specific nuclei. Moreover, high salt intake may cause parallel increases in ANG II production and release and therefore no increase in “steady-state” ANG II content. Alternatively or in addition, high salt intake may enhance AT1 receptor-mediated signaling. In Dahl S rats, high salt intake activates an aldosterone-“ouabain” pathway (22), which can contribute to an increase in ACE mRNA and activity in the hypothalamus (44) and AT1 receptor mRNA (34) and binding densities in the PVN and SON (39). This pathway may also increase NADPH oxidase subunits mRNA and protein and thereby reactive oxygen species in the PVN (18). Altogether, this pattern of changes may be sufficient for enhanced ANG II-mediated intracellular signaling. In rats with cold-induced hypertension, hypothalamic ANG II levels also do not change, but AT1 receptor mRNA showed a fourfold increase, and intracerebroventricular losartan prevents the hypertension as well as the increase in plasma renin and urinary catecholamines (35). Sun et al. (35) concluded that upregulation of brain AT1 receptors contributes to the cold-induced hypertension.

In the present study, in Wistar rats, intracerebroventricular infusion of rat renin caused clear increases in CSF ANG II and in BP. In previous studies in rats (43) or dogs (30, 31), intracerebroventricular injection of renin also caused increases in CSF ANG II, BP, and water intake, which were markedly prevented by intracerebroventricular administration of an AT1 receptor blocker or ACE inhibitor. Therefore, in the brain, renin indeed can activate the cascade of the local renin angiotensin system, increase brain ANG II, and elicit sympathoexcitatory and pressor responses. In the present study, acute intracerebroventricular infusion of aliskiren at an equivalent rate to chronic infusion markedly prevented increases in CSF ANG II and in BP in response to intracerebroventricular infusion of rat renin, indicating that the infusion rate of aliskiren used in the present study is sufficient to inhibit renin and decrease ANG II formation in the CNS. However, intracerebroventricular infusion of aliskiren did not change hypothalamic ANG II content in Dahl S rats on high salt. The persistence of hypothalamic ANG II content after intracerebroventricular treatment with aliskiren may reflect nonhomogenous ANG II production. ANG II may exist in two pools in the brain: one is perhaps a storage pool and/or produced by other enzymes, such as tonin, cathepsin G, or chymase (2), which is not affected by aliskiren while the other one is a small functional pool in sympathoexcitatory angiotensinergic neurons in which renin produces ANG II, and this ANG II production can be reduced by aliskiren. Alternatively, aliskiren may only inhibit secreted (pro)renin and not nonsecreted intracellular renin (21, 42).

Together, our findings would suggest that aliskiren inhibits renin, which contributes to cardiovascular regulation in the CNS, but that it may prevent ANG II production and release only in a small functional pool contributing to the salt-induced hypertension. However, the present data do not exclude that the central effects of aliskiren may not or not only relate to inhibition of ANG II production but may relate to other actions independent of ANG II formation such as blockade of (pro)renin receptor-mediated signaling in the brain, which may also play a functional role in neuronal cardiovascular control (32, 33).

In Dahl S rats on regular salt, intracerebroventricular infusion of aliskiren did not change resting BP and HR and had no effects on excitatory responses to air-stress, arterial baroreflex function, or RSNA. Intracerebroventricular infusion of an AT1 receptor blocker or ACE inhibitor is similarly ineffective in Dahl S or Wistar rats on regular salt intake (28, 44). Together, it appears that baseline angiotensin production in Dahl S rats on regular salt plays only a minor role in regulation of sympathetic activity and BP.

Whereas intracerebroventricular infusion of aliskiren at 0.05 and 0.25 mg/day appears well tolerated, aliskiren at 1.25 mg/day induced marked adverse effects, i.e., disorientation and seizures. Calculated CSF concentrations of aliskiren at this infusion rate were very high relative to the IC50 for rat renin, and off-target effects, e.g., on bradykinin levels in the CNS, as reported in the heart (4), may also play a role. High bradykinin levels may further increase sympathetic activity and BP (44), causing the neurological symptoms noted with the high dose of aliskiren.

Limitations of the present study. The RIA for ANG II in the present study is clearly specific for ANG II and can detect changes of 30–50% in hypothalamic ANG II levels (e.g., Refs. 12 and 44). Smaller increases and/or increases by high salt intake in specific nuclei would not have been detected by RIA for the whole hypothalamus. On the other hand, if hypothalamic ANG II generation was solely dependent on renin, one would expect “undetectable” levels by RIA after chronic intracerebroventricular treatment of aliskiren. This was clearly not the case, consistent with additional non-renin-dependent ANG II generation.

Assessments of baseline parameters, the responses to air-stress, or baroreflex function were performed 4–18 h after the surgeries. Although the rats were trained to stay quietly in the testing cages, they were still under some influence of postoperative stress during the experiment, which may affect the actual baseline values and their responses.

Perspectives and Significance

In conclusion, intracerebroventricular infusion of the renin inhibitor aliskiren or an AT1 receptor blocker similarly prevents sympathetic hyperactivity and hypertension and prevents desensitization of arterial baroreflex function in Dahl S rats on
high salt. These findings suggest that the effects of high salt diet on sympathetic hyperactivity and hypertension in Dahl S rats are mediated by brain renin-mediated production of local ANG II. Because neither high salt nor aliskiren changed hypothalamic ANG II content, tissue levels of ANG II appear not to reflect the extent of AT1 receptor-mediated central responses.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: B.S.H. and F.H.L. conceived and designed the research; B.S.H., R.A.W., and L.B. performed experiments; B.S.H., R.A.W., and L.B. prepared figures; B.S.H., R.A.W., and L.B. analyzed data; B.S.H., R.A.W., and L.B. interpreted results of experiments; B.S.H., F.H.L. and L.B. edited the revised manuscript; B.S.H., R.A.W., and F.H.L. approved the final version of manuscript.

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