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 an AT$_1$ 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 (icv) 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 BP caused by icv infusion of rat renin. In Dahl S rats on high salt, icv infusion of aliskiren at 0.05 and 0.25mg/day for 2 weeks similarly decreased resting BP in Dahl S rats on high salt. In other groups of Dahl S rats, high salt intake for 2 weeks increased resting BP by ~25 mmHg, enhanced pressor and sympatho-excitatory responses to air-stress, and desensitized arterial baroreflex function. All these effects were largely prevented by icv 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 icv infusion of aliskiren and an AT$_1$ 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 sympatho-excitatory angiotensinergic neurons.

**Key words:** rat renin, aliskiren, salt-sensitive hypertension, baroreflex, air stress, hypothalamic Ang II.
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 AT_1 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 intra-cerebroventricular (icv) infusion of the AT_1 receptor blocker 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 contribute 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) which is rapidly decreased by icv 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 AT_1 receptors.
Aliskiren is a recently developed direct human renin inhibitor, which blocks the first rate-limiting catalytic step of the renin-angiotensin system (RAS) and prevents the cleavage of angiotensinogen to form Ang I (40). Aliskiren inhibits human, dog and rat plasma renin with IC\textsubscript{50} values of 0.6, 7 and 80 nM, respectively (40). In rats over-expressing human renin and angiotensinogen (29), subcutaneous (sc) infusion of aliskiren at 0.3-3 mg/kg/day for 3 weeks markedly decreased circulating Ang I, Ang II and BP. In spontaneously hypertensive rats (SHR) sc infusion of aliskiren at higher rates (10-100 mg/kg/day) decreased BP in a dose-dependent manner (41), and at 100 mg/kg/day 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 icv infusion of aliskiren on salt-induced sympatho-excitation 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 icv infusion of aliskiren can prevent the increases in CSF Ang II as well as in BP and HR caused by icv infusion of rat renin. In Dahl S rats, we evaluated the effects of high salt diet with or without concomitant icv infusion of aliskiren on BP, heart rate (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 weeks 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 the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication NO. 85-23, revised 1996).

Dose response study for aliskiren in Dahl S rats.

In 4 groups of Dahl S rats (n=7-9/group), under isoflurane inhalation anesthesia an “L” shaped stainless steel cannula was placed into the right lateral cerebro-ventricle (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 minipump (Alzet, Model 2002) placed subcutaneously for icv infusion for 2 weeks of aCSF or aliskiren at 0.05, 0.25 or 1.25 mg/day, respectively. Aliskiren was dissolved in aCSF followed by sterilization with an Acrodisc syringe filter. The rates of icv 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), icv infusion of aliskiren at the rates of 0.05, 0.25 or 1.25 mg/day results in a concentration of ~14, 70 or 350 μM in the CSF respectively, but likely a factor lower accounting for its distribution into brain tissue and clearance in the CNS.

After the surgery, all rats were provided with high salt diet (1370 μmol Na⁺/g, Harlan Teklad, Madison, WI, USA). A fifth group of rats stayed on regular salt diet and received an icv infusion of aCSF.
Two weeks after the start of the treatments, under isoflurane anesthesia, a polyethylene PE10/PE50 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 PC 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 sacrificed with an overdose of pentobarbital and brains collected for Ang II assays. Whole brains were frozen in dry-ice and stored at -80°C. The whole hypothalamus was dissected later according to Glowinski and Iversen (7). Angiotensin II content in the hypothalamus was measured by RIA after extraction on C18 Sep-Pak cartridges and separation by HPLC, as described previously (24). The antibody used in the RIA was a generous gift from Drs. Schalekamp and Danser (Erasmus University Rotterdam, The Netherlands) and its specificity and sensitivity have been extensively validated (1, 19, 23). The peaks for the other peptides are well separated from Ang II (see figure 1 of ref #24) and are not included in the calculations. The detection limit of our assay is 5-6 pg/gm tissue. The actual tissue levels are approximately 5 fold higher in the current study. Intra- and inter-assay variabilities were 5 and 13% respectively.

**Responses to icv renin in Wistar rats**

Partially purified renin was prepared according to the method of de Jong et al (20). All steps were performed on ice, or at 4°C, unless otherwise specified. Briefly, kidneys from male Wistar rats were homogenized in an equal volume of deionized water, followed by sonication. The homogenate was stirred at room temperature for 1.5 hours, and then
The supernatant was retained, and the pellet re-extracted. The combined supernatant was first adjusted to pH 2.6, stirred for 10 min, re-adjusted to pH 7.0 and centrifuged for 60 min at 800g. This supernatant was adjusted to pH 4.5, ammonium sulfate was added to a final concentration of 2.2M, then it was stirred for 15 min and re-spun at 800g. Ten volumes of water were added to the pellet, and the suspension was dialyzed twice (24 hrs each) against 50 volumes of water. The retentate was centrifuged at 20,000g for 10 min, and the supernatant reserved. The pellet was washed with the same volume of water and the combined supernatant lyophilized and stored at -80°C. For use, the renin was reconstituted with distilled water and diluted with aCSF to a concentration that caused a 20-30 mm Hg increase in BP when infused icv for 10 min at 100μl/h in Wistar rats in a preliminary experiment.

In Wistar rats, under isoflurane inhalation anesthesia, a 23 gauge stainless steel guide cannula was placed just above the right lateral cerebro-ventricle (2.8 mm deep from dura) and fixed on the skull with dental cement. After 2-3 days, under the same anesthesia the right femoral artery and vein were catheterized. The following morning about 18 h after the surgery, the catheter was connected to a pressure transducer linked to a Grass polygraph as described above. Rats were allowed to rest for 30 min, and thereafter resting MAP and HR were recorded for 5 min in conscious animals. A 30-gauge stainless steel needle was then inserted into 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 micro-syringe or 1 ml syringe for icv injection or infusion via a Sage 355 infusion pump. The experiment was conducted in 3 parallel groups (n=4/group). In the first group, 2 μl aCSF was injected icv followed by icv infusion of aCSF at 100 μl/h for 1 h. Then renin at the
pre-tested arbitrary rate was infused icv for 30 min. In the second group, aliskiren (10 μg/2 µl aCSF) was first injected icv as a loading dose, followed by icv infusion of aliskiren at 2 μg/100 µl/h (equivalent to 0.05 mg/day) for 1 h. Then aliskiren (2 μg/100 µl/h) plus renin at the same rate as in group 1 was infused icv 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 icv infusion, the icv infusions continued and rats were anesthetized with intravenous pentobarbital (50mg/kg), mounted on a stereotaxic frame and CSF was withdrawn from the cisterna magna (16) at <10 µl/sec. 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 icv or sc infusion and were divided into 5 groups (n=7-11/group): 1) icv infusion of aCSF and on regular salt diet; 2) icv infusion of aliskiren at 0.05 mg/day and on regular salt diet; 3) icv infusion of aCSF and on high salt diet; 4) icv infusion of aliskiren at 0.05 mg/day and on high salt diet; and 5) as a control to assess for possible peripheral effects of centrally administered aliskiren, sc infusion of aliskiren at 0.05 mg/day and on high salt diet. Diets and infusions continued for 2 weeks. In order to accustom the animals to the experimental environment, all rats were trained to stay in a small testing cage for 30 min, 3 times per week during the 2-week dietary period.

At the end of the 2-week treatments, under isoflurane anesthesia, catheters were placed into 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 hours 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 (Grass Model 7P10), 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 % changes from the resting RSNA for each rat. After MAP was raised by >50 mmHg, RSNA reached low levels which 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 seconds twice at 10 min intervals, using an air-jet stream (1-1.5 lb/in²) directed to the face of the rat (15). Twenty min after the responses to air stress had subsided and all parameters had returned to baseline levels, phenylephrine was infused iv at 5-50 µg/kg/min 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 iv at 5-100 µg/kg/min 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 nitroprusside were also separately analyzed by linear regression.

About 30 min after the experiment, the rats were sacrificed and the whole brains collected and stored at -80°C for Ang II assay, as described above.

For all experiments, the accuracy of the position of the icv 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 icv cannulation or broken pump catheters, but none from the other experiments.
Statistical analysis

One-way ANOVA or one-way repeated measures 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 factors was performed. When F ratios were significant, a Bonferroni’s multi-range test followed to locate the significant differences. Statistical significance was defined as $p < 0.05$. 
Results

Responses to icv infusion of renin

In normal Wistar rats, infusion of aCSF or icv infusion of aliskiren at 2 μg/100 μl /h for 1 h did not change BP or HR significantly. In rats with icv infusion of aCSF, subsequent icv infusion of renin increased MAP and HR significantly, up to 26±3 mmHg and 60±5 bpm, respectively (Fig 1A). In rats with pre-icv infusion of aliskiren, subsequent icv infusion of renin plus aliskiren increased MAP by only 10±2 mmHg and HR by 24±4 bpm (p<0.05 for both increases, versus renin alone). BP did not change in rats with icv aCSF alone.

Ang II was not detected in CSF of rats with icv infusion of aCSF alone. In rats pre-infused with aCSF, icv infusion of renin markedly increased CSF Ang II levels. Icv infusion of aliskiren inhibited the increase in CSF Ang II by icv infusion of renin by 80-90% (Fig 1B).

Dose response study for icv infusion of aliskiren in Dahl S rats

Most of the rats treated with icv infusion of aliskiren at 1.25 mg/day developed seizures and were sacrificed within a few days, and infusion at this rate was discontinued. No adverse effects or behavioral abnormalities were observed in rats treated with icv infusion of aCSF or aliskiren at 0.05 or 0.25 mg/day. After 2 weeks of treatments, gains of body weight in rats on high salt and treated with icv 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 weeks of high salt diet, in Dahl S rats treated with icv infusion of aCSF resting MAP was significantly increased compared to rats on regular salt (Fig 2). Icv 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 icv 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**

Icv 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 icv 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 icv vehicle or icv aliskiren, air-jet stress elicited 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%. Icv infusion of aliskiren prevented the enhancement of MAP, RSNA and HR responses to stress, whereas sc infusion of aliskiren had no effects.

In rats on regular salt diet, icv infusion of aliskiren had no effect on baroreflex control of RSNA and HR (Fig 5, 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. Icv infusion of aliskiren prevented salt-induced decreases in these parameters, whereas subcutaneous infusion of 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 icv vehicle or sc aliskiren, as compared to 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 icv infusion of aliskiren at 0.05 and 0.25 mg/day versus icv infusion of vehicle (27±5 and 42±5 versus 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), which were not affected by icv infusion of aliskiren at 0.05 mg/day (Fig 3).
Discussion

The present study demonstrates that in Dahl S rats icv infusion of aliskiren prevents salt-induced increases in resting BP and HR, the salt-induced enhancement in sympatho-excitatory 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 sympatho-excitatory and pressor responses to an increase in CSF [Na\(^+\)] are enhanced, compared to 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 AT\(_1\) receptor blocker or ACE inhibitor (14, 15, 25, 36, 44). Icv infusion of aliskiren also prevents the salt-induced sympathetic hyperactivity and hypertension as well as desensitization of arterial baroreflex. Sc 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 AT\(_1\) receptors would suggest that renin and ACE –dependent Ang II generation in the brain and resultant AT\(_1\) 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). Icv infusion of aliskiren will cause blockade throughout the hypothalamus
and brainstem, and alternative approaches with e.g. siRNA are needed to ascertain the actual sites of action.

Higher AT$_1$ 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 weeks 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 AT$_1$ 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 AT$_1$ 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 AT$_1$ receptor mRNA showed a 4 fold increase and icv 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 AT$_1$ receptors contributes to the cold-induced hypertension.

In the present study, in Wistar rats icv infusion of rat renin caused clear increases in CSF Ang II and in BP. In previous studies in rats (43) or dogs (30, 31), icv injection of renin also caused increases in CSF Ang II, BP and water intake, which were markedly prevented by icv administration of an AT$_1$ 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 sympatho-excitatory and pressor responses. In the present study acute icv infusion of aliskiren at an equivalent rate to chronic infusion markedly prevented increases in CSF Ang II and in BP in response to icv 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, icv 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 icv treatment with aliskiren may reflect non-homogenous Ang II production. Ang II may exist in 2 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 sympa-tho-excitatory 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 non-secreted 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 icv infusion of aliskiren did not change resting BP and HR and had no effects on excitatory responses to air-stress, arterial baroreflex function or renal sympathetic nerve activity. Icv infusion of an AT$_1$ 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 icv 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 IC$_{50}$ 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, 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 icv 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 post-operative stress during the experiment, which may affect the actual baseline values and their responses.

**In conclusion,** icv infusion of the renin inhibitor aliskiren or an AT₁ receptor blocker similarly prevent sympathetic hyperactivity and hypertension as well as prevent 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. Since 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.
Acknowledgement

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References


Legends for Figures

**Fig 1:** Changes in MAP and HR (A) and Ang II concentration in the CSF (B) by icv infusion of aCSF, renin or aliskiren plus renin for 30 min in conscious rats.

Values are means±SE (n=4/group) analyzed by one-way repeated measures ANOVA (A) or one-way ANOVA (B).

For (A), F=12.2, p=0.0005.
For (B), F=23.2, p=0.00001.

* p<0.05, versus aCSF; a: p<0.05, versus renin alone.

**Fig 2:** Resting MAP and HR in Dahl 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 weeks (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.

* p<0.05, versus others.

**Fig 3:** Resting MAP, HR and hypothalamic Ang II content in Dahl S rats on regular (RNa) or high (HNa) salt and icv infusion of vehicle (veh: aCSF), or icv or sc infusion of aliskiren (alisk) at 0.05 mg/day for 2 weeks.

Values are means±SE (n=7-11/group) analyzed by two-way ANOVA.

For MAP, F=21.32, p=0.0001 between diets; F=11.68, p=0.002 between treatments; F=5.89, p=0.02 for diets x treatments.
For HR, F=23.89, p=0.0001 between diets; F=0.02, p=0.89 between treatments; F=5.54, p=0.03 for diets x treatments.

For Ang II content, F=1.89, p=0.71 between diets; F=0.02, p=0.89 between treatments; F=1.22, p=0.73 for diets x treatments.

* p<0.05, versus rats on RNa.

a: p<0.05, versus 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 regular (RNa) or high salt (HNa) intake and icv infusion of vehicle (veh: aCSF), or icv or sc infusion of aliskiren (alisk) at 0.05 mg/day for 2 weeks. Values are means±SE (n= 7-11/group) analyzed by two-way ANOVA.

For MAP, F=17.6, p=0.0003 between diets; F=15.4, p=0.0003 between treatments; F=10.6, p=0.003 for diets x treatments.

For HR, F=21.0, p=0.0001 between diets; F=23.0, p=0.0001 between treatments; F=12.3, p=0.002 for diets x treatments.

For RSNA, F=16.63, p=0.0004 between diets; F=4.66, p=0.04 between treatments; F=10.5, p=0.004 for diets x treatments.

* p<0.05, versus rats on RNa.

a: p<0.05, versus rats on HNa with icv vehicle or sc aliskiren.

**Fig 5.** Arterial baroreflex control of RSNA (top panel) and HR (bottom panel) analyzed as a logistic model in Dahl S rats on regular (RNa) or high salt (HNa) intake and icv infusion of vehicle (veh: aCSF), or icv or sc infusion of aliskiren (alisk) at 0.05 mg/day for 2 weeks.

For statistics, see Table 1.
Table 1. Ranges and maximal gain of baroreflex control of RSNA and HR as a logistic model, and by linear regression (for HR) in Dahl S rats on regular (RNa) or high salt (HNa) intake and icv infusion of vehicle (aCSF), or icv or sc infusion of aliskiren at 0.05 mg/day for 2 weeks.

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<td>Icv veh</td>
<td>161±9 *</td>
<td>-2.8±0.2 *</td>
</tr>
<tr>
<td>Icv alisk</td>
<td>184±9 †</td>
<td>-3.7±0.2 †</td>
</tr>
<tr>
<td>Sc alisk</td>
<td>160±13 *</td>
<td>-2.7±0.2 *</td>
</tr>
</tbody>
</table>

Values are means±SE (n= 7-11/group), analyzed by two- way ANOVA.

For ranges:
control of RSNA, F=7.88, p=0.003 between diets; F=5.58, p=0.03 between treatments,
F=5.59, p=0.03 for diets x treatments.
control of HR, F=0.02, p=0.90 between diets; F=7.31, p=0.012 between treatments;
F=16.21, p=0.0005 for diets x treatments.
For maximal gains:

Control of RSNA, F=5.05, p=0.03 between diets; F=9.36, p=0.005 between treatments; F=12.6, p=0.002 for diets x treatments.

Control of HR, F=5.68, p=0.03 between diets; F=6.04, p=0.02 between treatments, F=4.19, p=0.04 for diets x treatments.

For gains of HR by linear regression:

To PE, F=4.02, p=0.04 between diets; F=7.23, p=0.01 between treatments; F=4.12, p=0.04 for diets x treatments.

To NP, F=7.03, p=0.001 between diets; F=6.25, p=0.005 between treatments; F=13.7, p=0.0002 for diets x treatments.

To both PE and NP together, F=4.41, p=0.02 between diets; F=8.54, p=0.005 between treatments; F=4.77, p=0.01 for diets x treatments.

* p<0.05, vs RNa plus icv veh. †: p<0.05, vs HNa plus icv veh or sc aliskiren.

‡: p<0.05, vs HNa plus sc aliskiren.
Fig 1
MAP (mmHg)

HR (bpm)

veh aliskiren 0.05 0.25 mg/d
veh aliskiren 0.05 0.25 mg/d

Fig 2
Fig 3
Air stress

Fig 4
Changes in RSN A (% resting)

Changes in HR (bpm)

MAP (mmHg)

Fig 5