Inhibition of Hypertension and Salt Intake by Oral Taurine Treatment in Hypertensive Rats

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SUMMARY Effects of oral treatment with taurine on fluid intakes produced by renin were assessed in spontaneously hypertensive rats of the Okamoto strain (SHR). Renin injected into the preoptic area increased water intake and evoked salt (2.7% NaCl solution) intake, and angiotensin II injected into this area increased water intake, but not salt intake, in both SHR and control normotensive Wistar-Kyoto rats (WKY). The salt intake elicited by renin, but not water intake produced by renin or angiotensin II, was potentiated in SHR. These effects of renin and angiotensin II on fluid intakes were antagonized by previous administration of taurine or β-aminobutyric acid into the cerebral ventricles in both strains. When SHR received water containing 3% taurine from 32 to 105 days of age, development of hypertension was inhibited. Renin administered into the preoptic area at 105 days of age caused an increase in salt intake, but the increase was markedly inhibited by the oral administration of taurine as well. These results show that salt appetite produced by centrally administered renin is exaggerated in SHR and that development of hypertension as well as renin-induced salt appetite in SHR is inhibited by dietary taurine. (Hypertension 10: 383-389, 1987)

KEY WORDS • taurine • hypertensive rats • renin • blood pressure • salt intake • spontaneously hypertensive rats

ALL the components of the renin-angiotensin system (RAS) necessary for the local formation of angiotensin II (ANG II) have now been demonstrated to be present in the brain tissue of dog, human, and rat. A functional brain RAS, independently of the peripheral circulating RAS, plays a major role in the regulation and maintenance of blood pressure and water as well as sodium balance of the body fluid. ANG II acts on the central nervous system (CNS) to produce a wide variety of physiological effects, including elevation of arterial blood pressure by increased adrenergic activity with a consequent increase in cardiac output and total peripheral resistance, increased release of vasopressin and adrenocorticotropic hormone from the pituitary, and stimulation of thirst, sodium appetite, and natriuresis.

Large populations of angiotensin receptors in the rat brain are localized in the circumventricular organs, subfornical organ, organum vasculosum of the lamina terminalis, median eminence, and area postrema, all highly vascular structures located outside the blood-brain barrier and accessible to circulating ANG II. ANG II-sensitive sites, such as the preoptic area (POA) inside the blood-brain barrier, at some distance from the ventricular system. Most recently, ANG II-immunoreactive cells and fibers in the rat CNS have been well identified and mapped in detail. Furthermore, spontaneously hypertensive rats (SHR), believed to be a model for at least some of the genetic factors that may be involved in human essential hypertension, had a greater pressor response than did the normotensive control animals after intracerebroventricular administration of ANG II. It has been also suggested that the intrinsic brain RAS in SHR may be hyperactive because of increased production of central ANG II compared with that in Wistar-Kyoto rats (WKY). On the other hand, taurine is a simple, low molecular weight amino acid with a high degree of chemical stability and very low metabolic reactivity. This sulfur-containing amino acid has a discrete regional distribution in the brain. In the rat, the greatest concentration is found in the olfactory bulb, and high levels also...
exist in the cerebellum, cerebral cortex, and corpus striatum. In addition, taurine is now generally accepted as an inhibitory amino acid that hyperpolarizes neurons in the mammalian CNS.

In our previous studies with Wistar rats, ANG II injected into the posterior hypothalamus and renin administered into the cerebral ventricles elicited both pressor and positive chronotropic responses. Taurine and γ-aminobutyric acid (GABA) administered i.e.v. produced dose-dependent decreases of blood pressure and heart rate and antagonized those cardiovascular responses to ANG II and renin.

It is widely recognized that a high level of salt intake constitutes a major risk factor in the development and maintenance of human essential hypertension. As mentioned, RAS in the brain seems to be involved in stimulation of thirst and sodium appetite. Furthermore, SHR possess greater sodium acceptance and preference as compared with WKY. Accordingly, the present study was undertaken to study differences in water and salt intakes caused by centrally administered renin between WKY and SHR and to investigate whether orally administered taurine can inhibit fluid intakes induced by renin in SHR.

### Materials and Methods

Male SHR and age-matched WKY (Charles River Japan, Atsugi, Japan) were used in this study. Unless otherwise stated, the rats were housed three to a cage and had free access to standard laboratory food pellets (CE-2; Clea Japan, Tokyo, Japan) containing sodium, 0.15 mmol/g, and drinking solution. The room lighting was on a 12-hour light/dark cycle, with the lights coming on at 0700, and the temperature was maintained at approximately 24°C. At about 1100 each morning, the rats were weighed and daily food intake was estimated by weighing uneaten and spilled food. Fluid intake was automatically recorded at regular intervals using drinkometers (Model LA-1; Ohara, Tokyo, Japan) according to a method previously described. Fresh water containing 3% taurine and 2.7% NaCl solution was prepared every day. The position of the two fluids was reversed each day to control for any possible position preference when the rats were given the choice of drinking either water (with or without taurine) or 2.7% NaCl solution.

Before operation, the rat was anesthetized with sodium pentobarbital (50 mg/kg) injected intraperitoneally and the rat’s head was mounted horizontally in a stereotaxic apparatus (Model SR-6; Narishige, Tokyo, Japan). A stainless steel cannula (outside diameter, 0.75 mm; length, 15 mm; Takahashi Shoten, Tokyo, Japan) was implanted into the left medial POA (stereotaxic coordinates: horizontal skull, anteroposterior (AP), 7.8 mm from the interaural line; lateral (L), 0.8 mm; ventral (V), 7.5 mm down from the dura) and the right lateral cerebral ventricle (stereotaxic coordinates: horizontal skull, AP, 5.6 mm; L, 1.7 mm; V, 4.0 mm) according to the method described by Epstein et al.

After implantation of cannulas, all animals were allowed a week to recover from the operation before the intracranial injections of test solutions. Drug administrations into the brain were undertaken at about 1100 when unstimulated or control rats would be inactive. For the intracranial injections, the rats were hand-held and test solutions were injected into the POA in a volume of 1 µl or into the cerebral ventricles in a volume of 10 µl during a 60-second period. Central pretreatments (i.e.v.) with various drugs were done 5 minutes before POA injection of renin or ANG II. After the injection of ANG II or renin, the rats were immediately returned to their cages for the measurements of fluid intakes.

Systolic blood pressure of the rat was measured by tail plethysmography using a programmed electro- sphygmomanometer (Model PE-300, Narco Bio-Systems, Houston, TX, USA) and a rat holder temperature control unit (Model MK IV; Narco Bio-Systems). The output signals from the sphygmomanometer were recorded on a polygraph (Model TI-101; Tokai Irika, Fukuoka, Japan). Plethysmography was selected for indirect determination of systolic blood pressure to facilitate repetitive measures of pressure and to avoid the additional stress inherent in vascular cannulation. Each animal was placed in a specially constructed box (with an ambient temperature of 37°C; Model 137; Ueda Seisakusyo, Tokyo, Japan) before measurement of blood pressure in order to obtain reproducible tail pulse recordings. The median of five to eight consecutive readings was taken as the data point.

Rats were handled daily to minimize stress due to handling on the day of experiment and were fully habituated to the test cages. During the experiment, special care was taken to provide a calm environment free of external disturbing influences.

### Study of Fluid Intakes Produced by ANG II and Renin

The rats received only tap water to drink from birth to 96 days of age. At 97 days of age the brain cannula was implanted stereotaxically into the POA in each study animal. After the intracranial cannulation, the rats were housed individually and given the choice of drinking either tap water or 2.7% NaCl solution. Following a 4-day acclimatization period, a further 3-day period was used to establish baseline intakes of fluids and food. ANG II (100 ng) was administered into POA at 105 days of age in one group of rats of both strains, and renin (2 mU) was given twice at weekly intervals (at 105 and 112 days of age) in the other group.

### Study of Effects of Oral Administration of Taurine

SHR were separated into two groups; one group received tap water to drink and the other group received water containing 3% taurine to drink. Oral treatment with taurine was initiated at 32 days of age and continued until the animals were 115 days old. This dose and timing of oral taurine treatment were chosen according to the method previously described by Nara et al. To confirm the antihypertensive effect of taurine, systolic blood pressure of the taurine-treated and untreated rats was measured repeatedly. After POA cannulation at 97 days of age, the rats of both groups were housed individually and examined as for...
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the experiment on fluid intake. At 105 days of age renin (1 mU) was administered into the POA in each animal. Rats were then given a choice between water (with or without taurine) and 2.7% NaCl solution.

Histological Verification
At the end of each experiment, rats were deeply anesthetized and perfused intracardially with isotonic saline followed by 10% formalin for fixation. After fixation, the brains were sliced at 40 μm using a cryostat (Model 5030; Bright, Huntingdon, England) and selected sections through the regions of cannula tips were stained with hematoxylin and eosin. The location of the injection cannula was then determined from magnified tracings using the stereotaxic atlas of König and Klippel.34 Only data collected from the experiments in which the correct insertion of the cannula was verified are reported.

Drugs
Drugs used were ANG II (human type; Peptide Institute, Osaka, Japan), hog renin (Sigma Chemical, St. Louis, MO, USA), taurine (Taisho Pharmaceutical, Tokyo, Japan), and GABA (Nakarai Chemical, Kyoto, Japan). These drugs were dissolved in 0.9% NaCl solution (saline) and administered by intracranial injection.

Statistical Analysis
Results are expressed as means ± SEM for each group. Statistical analysis was performed using two-way analysis of variance for repeated measures. The remaining data were compared using one-way analysis of variance followed by Dunnett’s t test. Differences with a p value less than 0.05 were considered significant.

Results

Effects of Renin on Fluid Intakes in WKY and SHR
At 100 days of age, systolic blood pressure in SHR (189.42 ± 7.40 mm Hg; n = 18) was significantly higher than that in WKY (119.28 ± 5.81 mm Hg; n = 20, p < 0.01). There was no difference in the daily food intakes between WKY and SHR, but body weight in SHR was significantly lighter throughout the experimental period than that in WKY (F = 24.2, p < 0.01). The unstimulated SHR and WKY, when water and 2.7% NaCl solution were available, usually drank water, but rarely 2.7% NaCl. The daily salt (2.7% NaCl) intake in unstimulated SHR was a little greater than that in WKY, whereas the daily intakes of water were not different between rat strains.

Figure 1 shows the time course of daily intakes of water, 2.7% NaCl, and food as well as body weight after weekly POA administration of renin (2 mU) in WKY and SHR when they were 105 and 112 days old. Mean (± SEM) intakes of water and 2.7% NaCl on the day before the first injection of renin were 10.51 ± 0.89 and 0.02 ± 0.01 ml/100 g body weight/day in WKY and 10.89 ± 0.78 and 0.58 ± 0.28 ml/100 g body weight/day in SHR, respectively; the differences were insignificant.

The control injections of saline into POA in both

![Figure 1](http://hyper.ahajournals.org/Downloaded from http://hyper.ahajournals.org/)
WKY and SHR at 105 days of age did not cause drinking, though all rats had free access to water and 2.7% NaCl solution. However, renin administered into the POA caused a copious increase of water intake that began within several minutes after the administration and lasted for about 2 days in both strains. The increase of water intake induced by renin was significant in both strains \((F = 69.6, p < 0.01)\) but was not significantly different between both strains \((F = 1.88, p > 0.05)\). Salt (2.7% NaCl) intake was also increased, but this increase was slower in onset and lasted for about 2 days in WKY and 7 days in SHR. The renin-induced increase of salt intake was also significant in both strains \((F = 18.6, p < 0.01)\) and was significantly greater in SHR than in WKY \((F = 76.3, p < 0.01)\). This between-strain difference of salt intake became significantly smaller as the number of days after the administration increased \((F = 7.7, p < 0.01)\).

Mean \((\pm SEM)\) intakes of water and 2.7% NaCl on the day after the first POA injection of renin were 45.25 ± 3.47 and 2.98 ± 0.96 ml/100 g body weight/day in WKY and 42.36 ± 2.84 and 11.74 ± 1.44 ml/100 g body weight/day in SHR, respectively. The responses of salt intakes to the second preoptic injections of renin in both strains were potentiated to a certain extent, and the onset of the action became a little faster. However, the effects of the second injection of renin were essentially similar to those of the first injection.

After POA administration of renin (2 mU), temporal decrease of food intake and consequent decrease of body weight appeared in both WKY and SHR and continued for 1 to 2 days.

Effects of Intracerebroventricular Administration of Taurine or γ-Aminobutyric Acid on Renin-induced Water and Salt Intakes in WKY and SHR

Effects of renin are long-lasting, whereas effects of amino acids administered into the brain may not be long-lasting. Accordingly, to determine possible influences of taurine and GABA on renin-induced water and salt (2.7% NaCl) intakes, these amino acids were administered i.c.v. 5 minutes before POA injections of renin (2 mU) in WKY and SHR, and effects of these amino acids were observed for 60 minutes after renin administration, when both solutions, but not food, were available. Taurine and GABA administered i.c.v. did not cause drinking per se. As seen in Figure 2, in SHR, i.c.v. pretreatment with taurine (50, 100 μg) and GABA (50, 100 μg) inhibited in a dose-dependent manner water and salt intakes elicited by renin. In WKY, a similar but slightly weaker inhibition by these agents was also observed for water intake, but not for salt intake.

Effects of ANG II on Fluid Intakes in WKY and SHR

Figure 3 shows time course for 60 minutes of water and salt (2.7% NaCl solution) intakes induced by POA injections of ANG II in WKY and SHR. Water intake, but not salt intake, occurred within a few minutes after injection of ANG II (100 ng) into POA, gradually increasing and ceasing in about 30 minutes (see Figure 3). For 60 minutes after ANG II administration, water and 2.7% NaCl intakes were 4.18 ± 0.37 and 0.04 ± 0.02 ml/100 g body weight in WKY and SHR.
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3.87 ± 0.35 and 0.14 ± 0.06 ml/100 g body weight in SHR, respectively. Thus, salt intake was slightly greater in SHR than in WKY. However, there was no significant between-strain difference in water and salt intakes after ANG II.

Effects of Intracerebroventricular Administration of Taurine or γ-Aminobutyric Acid on ANG II-induced Water Intake in WKY and SHR

As water intake produced by POA injection of ANG II ceased in about 30 minutes, the effects of amino acids were assessed by determining the cumulative amount of water intake for 60 minutes after ANG II, when tap water, but not 2.7% NaCl solution and food, was available. As seen in Figure 4, i.c.v. pretreatment with taurine (25, 50, 100 μg) or GABA (25, 50, 100 μg) 5 minutes before ANG II inhibited in a dose-dependent manner water intake elicited by ANG II.

Effects of Oral Taurine Treatment on Blood Pressure in SHR

From 32 days of age, one group of SHR received tap water to drink and another group of SHR received water containing 3% taurine. Taurine had no significant effect on daily intake of water with or without 3% taurine, food intake, and weight gain (growth rate). The effects of oral taurine treatment on blood pressure in SHR are shown in Figure 5. Blood pressure in taurine-treated SHR was significantly lower throughout the period than that in age-matched, control SHR (F = 70.1, p < 0.01).

Effects of Oral Taurine Treatment on Renin-induced Fluid Intakes in SHR

After POA injection of renin (1 mU), water intake was significantly increased in both control and taurine-administered SHR (F = 18.2, p < 0.01), but these values were not significantly different from each other (F = 3.2, p > 0.05; Figure 6). However, renin elicited a significant increase of 2.7% NaCl intake in both groups (F = 3.1, p < 0.01), and the daily intake of 2.7% NaCl during 10 days after renin in taurine-treated SHR was significantly lower than that in control SHR (F = 9.8, p < 0.01). Thus, 2.7% NaCl intake, but not water intake, was markedly reduced by daily taurine treatment in SHR.

After the renin administration, food intake and body weight in both groups of rats were decreased temporarily but were restored the next day.

Discussion

The possibility that SHR possess an exaggerated salt appetite compared with WKY has been proposed. In continuous-preference situations allowing choice between NaCl solutions (0.5–3.0% NaCl) and water, 1.2 to 2.0% NaCl solutions are more accepted and preferred by the SHR strains than by the control WKY. In this study, when we offered water and a higher concentration of NaCl solution (2.7%), although the rats usually drank water and rarely drank NaCl solution, the baseline intakes of NaCl solution...
It has been proposed that the intrinsic brain RAS may be involved in the genesis of the genetically hypertensive state in SHR through overproduction of central ANG II or hyperactivity of the brain RAS. In fact, i.c.v. administration of captopril, an angiotensin converting enzyme inhibitor, reduced blood pressure in SHR, but not in WKY, suggesting that the brain RAS may be involved in the maintenance of the elevated blood pressure in SHR. In addition, the pressor response to ANG II administered into either POA or the cerebral ventricles was also potentiated in SHR as compared with WKY (unpublished observation, 1986). In this experiment, ANG II injected into POA induced an increase of water intake, but not salt intake, in both WKY and SHR. Enhanced intakes of hypertonic NaCl solution can only be evoked by repeated injections or continuous infusion of ANG II into the brain. The increased water intake after ANG II occurred in both strains of rats. Administration of renin into POA, in contrast, produced marked and prolonged increases of both water and salt intakes. There was no difference in water intake between WKY and SHR; however, salt intake was markedly potentiated in SHR as compared with WKY. The greater stimulating effect of renin on the salt appetite in the SHR may be due to an increase of brain converting enzyme activity or of sensitivity of brain ANG II receptor (or both).

On the other hand, our previous studies with Wistar rats showed that taurine and GABA given i.c.v. produced dose-dependent decreases of blood pressure and heart rate per se but did not evoke thirst and salt appetite. Additionally, taurine and GABA antagonized central stimulating effects of ANG II and renin on blood pressure and fluid intakes. Other amino acids, such as methionine, cysteine, cysteine sulfenic acid, cysteic acid, and alanine, did not exert such actions. Moreover, the antagonisms between ANG II or renin and taurine or GABA were not observed when these were administered peripherally. These amino acids did not exert such antagonistic effects on the pressor response to centrally administered prostaglandin \( P_{2} \), histamine, or carbachol. Thus, the antagonism is selective for taurine and GABA among amino acids tested. The antagonism is also selective for ANG II and renin. As for the possible site of action in the brain, water intake elicited by POA injection of ANG II was antagonized by an ANG II receptor antagonist injected into POA through the same brain cannula. Similarly, water and salt intakes produced by POA injection of renin were antagonized by POA treatment with an ANG II receptor antagonist or an angiotensin converting enzyme inhibitor. In addition, although antagonism by GABA and taurine of the effects of ANG II and renin on fluid intakes appeared after both i.c.v. and POA treatment, the acids are effective at one-tenth smaller doses when administered into POA. On the basis of such findings, POA seems to be one site of action, at least for fluid intakes. In this experiment with both WKY and SHR, i.c.v. pretreatment with taurine or GABA inhibited in a dose-dependent manner the fluid intakes induced by POA administration of ANG II or renin.

It has been previously demonstrated that long-term oral administration of taurine lowers blood pressure slightly in SHR, moderately in the stroke-prone sub-strain of SHR, and strikingly in deoxycorticosterone acetate–salt hypertensive rats but has no effect on blood pressure in control WKY. In addition, even in patients with essential hypertension, long-term taurine treatment has been shown to reduce blood pressure significantly. Furthermore, addition of taurine (3%) to the drinking water of rats during the period from 4 to 14 weeks of age slightly retarded development of hypertension in SHR, had little effect on blood pressure in WKY, and significantly increased tissue taurine content in the brain of SHR.
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On the other hand, it has been proposed that GABA does not enter the brain through the blood-brain barrier. In this experiment, oral treatment with taurine in SHR from 4 to 14 weeks of age caused a reduction in the development of hypertension, without affecting food intake and weight gain. Additionally, this oral taurine treatment in SHR markedly inhibited the stimulating effect of centrally administered renin on salt appetite, without affecting that on water intake. These results suggest that an intrinsic brain RAS activated by injection of renin into the brain evokes an exaggerated salt appetite in SHR, which is inhibited by oral taurine treatment, and that the development of hypertension in SHR is attenuated by oral taurine treatment.

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References

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