HYPOTENSIVE ACTION OF TAURINE IN DOCA-SALT RATS
— Involvement of Sympathoadrenal Inhibition and Endogenous Opiate —

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We studied the roles of the sympathoadrenal system and endogenous opiate in the antihypertensive effects of supplementation of dietary taurine, a sulfur amino acid, in deoxycorticosterone acetate (DOCA)-salt rats. Supplementation of 1% taurine in drinking water for 2 weeks was found to prevent the increase in systolic blood pressure of DOCA-salt rats $(116 \pm 2 \text{ vs } 138 \pm 2 \text{ mmHg, } p < 0.01)$, but failed to effect the systolic blood pressure of vehicle-treated control rats $(115 \pm 2 \text{ vs } 112 \pm 3 \text{ mmHg})$. Taurine supplementation restored to normal increased plasma norepinephrine $(326 \pm 32 \text{ vs } 531 \pm 67 \text{ pg/ml, } p < 0.01)$ and epinephrine $(204 \pm 19 \text{ vs } 304 \pm 43 \text{ pg/ml, } p < 0.05)$ concentrations in DOCA-salt rats, but had no effect on norepinephrine $(346 \pm 23 \text{ vs } 338 \pm 33 \text{ pg/ml})$ or epinephrine $(198 \pm 17 \text{ vs } 224 \pm 26 \text{ pg/ml})$ concentrations in control rats. Accordingly, the increased epinephrine content in the adrenals of DOCA-salt rats was normalized with the supplementation of taurine, associated with a markedly increased adrenal taurine content. In conscious rats, moreover, intraperitoneal injection of naloxone (2 mg/kg), a specific opiate antagonist, increased systolic blood pressure only in taurine-supplemented DOCA-salt rats. Evidence presented suggests, therefore, that both the suppression of the increased sympathoadrenal activity and the activation of endogenous opiate might contribute to the antihypertensive effect of taurine in DOCA-salt rats.

Our recent studies$^{1-3}$ have indicated that supplementation of the diet with taurine, a sulfur amino acid, could attenuate the development of deoxycorticosterone acetate (DOCA)-salt hypertension in rats and that this hypotensive effect may be attributable to suppression of sympathetic overactivity, possibly mediated by changes in the central noradrenergic system. More recently, we have demonstrated that endogenous opiate activation, which is intimately related to sympathetic nervous system (SNS) suppression, may contribute to the antihypertensive effect of taurine in DOCA-salt hypertensive rats$^4$

Several investigators have suggested that increased adrenomedullary activity or reactivity, in addition to SNS overactivity, contributes to the development and maintenance of high blood pressure in DOCA-salt hypertensive rats. For example, the DOCA-salt hypertensive model has been associated with an increased catecholamine synthesis in the adrenal medulla$^{5-7}$ Increased plasma epinephrine (E) and norepinephrine (NE) levels were observed in these animals$^8$ Also, the E increase following carotid occlusion was found to be markedly potentiated in DOCA-salt rats, suggesting adrenal medullary hyperreactivity to baroreflex activation.

Key words:
Taurine
DOCA-salt hypertension
Sympathoadrenal tone
Opiate
Naloxone

(Received December 25, 1989; accepted August 4, 1990)
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There is a growing body of recent evidence that endogenous opiate may be involved in sympathoadrenal activity and the resultant blood pressure control. For example, one of the two major endogenous opioid peptides, \( \beta \)-endorphin, administered intraventricularly or into the nucleus tractus solitarii (NTS), results in a dose-related fall in blood pressure\(^{10–12} \). Also, it is suggested that opiate receptors on chromaffin cell membranes of the bovine adrenal medulla modulate the function and availability of acetylcholine (Ach) receptors\(^{13} \) and endogenous opioid peptides such as \( \beta \)-endorphin and methionine-enkephalin (Met-enkephalin) inhibit Ach-induced catecholamine release from chromaffin cells more potently than do other enkephalins\(^{14} \). Moreover, interaction between taurine and morphine-like peptides such as \( \beta \)-endorphin or enkephalin has been suggested. Morphine-treated rats have increased taurine in their spinal cords\(^{15} \) and the administration of taurine inhibits the development of tolerance to morphine-like peptides in rats\(^{16} \). These observations led us to test the possibility that endogenous opiate activation with taurine supplementation in DOCA-salt rats might contribute to not only inhibition of adrenomedullary activity but also suppression of SNS activity. It might also be involved in the antihypertensive action of taurine in DOCA-salt rats. Thus present study was designed to examine the effects of taurine supplementation in DOCA-salt rats on circulating NE and E levels, adrenal E content, and the pressor response to naloxone, a specific opiate antagonist.

**METHODS**

**Animal Preparation**

Sixty male Sprague-Dawley rats (Charles-River Japan, Atsugi, Japan) weighing 120–130 g were subjected to unilateral left nephrectomy at 5 weeks of age. The left adrenal gland was left in place. After 10–14 days they were randomly divided into 4 groups as follows. The DOCA-salt group (n=15) received weekly subcutaneous injections of 0.4 ml of a suspension containing, per milliliter of water: 25 mg of DOCA (Sigma Chemical, St. Louis, MO, USA), 10.5 mg methylcellulose (Wako Pure Chemical,
Osaka, Japan), 3 mg carboxymethyl cellulose (Wako), 1 mg polysorbate 80 (Wako), and 8 mg NaCl (Wako) and was given 1% NaCl in tap water ad libitum for 2 weeks. The 1% taurine-supplemented DOCA-salt group (n=16) received injections of DOCA suspension and was given a mixed solution of 1% NaCl and 1% taurine (Wako) as drinking water ad libitum. The control group (n=14) received weekly injections of the vehicle suspension without DOCA and was given tap water to drink ad libitum. The 1% taurine-supplemented control group (n=15) received weekly injections of the vehicle suspension without DOCA and was given 1% taurine in tap water to drink ad libitum.

Throughout the study, animals were housed at constant temperature (23 ± 1°C) and humidity (60 ± 5%) and with light from 0600 to 1800h. All rats received a standard laboratory rat chow (MF: Oriental Yeast Co., Tokyo, Japan). Systolic blood pressure was measured weekly in conscious, prewarmed rats (10 rats of each group) using an occlusive tail cuff and a pneumatic pulse transducer attached to a programmed electrophygmomanometer (Model PE-300; N:*, Co Biosystems, Houston, TX, USA)!−3 Body weight was also measured once a week. After 2 weeks of each treatment, the following experiments were performed.
Fig. 4. The effects of naloxone on systolic blood pressure in control and DOCA-salt rats with or without taurine supplementation. Naloxone (2 mg/kg) or vehicle (saline; 0.5 ml/kg) was injected i.p. at 0 min into the same animal on a different day. Points and vertical bars represent mean ± SEM for changes following drug administration. Daggers indicate significant changes (*p<0.05; **p<0.01) from basal (0 min) values.

Measurements of basal Cardiovascular Activity and Plasma Catecholamine Levels

Eight rats of each group were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Tip-tapered polyethylene catheters (PE-50) were inserted into the lower abdominal aorta through the femoral artery. The catheter was tunneled under the skin, and externalized behind the neck. Forty-eight hours after catheter placement, the catheter was connected to a pressure transducer (Model TP-101T; Nihonkohden, Tokyo, Japan). Mean blood pressure and heart rate were recorded directly on a polygraph (Model WS-641G; Nihonkohden). After a stable mean arterial pressure and heart rate were obtained (at least 30 min was allowed to pass after the start of recording), 0.5 ml of blood was withdrawn from the arterial line into iced-tubes containing 20 µl of EGTA (95 mg/ml) and glutathione (60 mg/ml) for NE and E assay. Blood samples were immediately centrifuged at 4°C and the plasma was frozen at −40°C until the samples were assayed. Plasma NE and E were measured using a modification of the radioenzymatic method of Peuler and Johnson (Cat-A-Kit, Upjohn, Kalamazoo, Michigan, USA)\(^7\).

Preparation of Adrenal Glands

After blood sampling, the animals were killed by decapitation. The adrenal glands were removed immediately, frozen on dry ice and kept at −40°C until homogenized for assay. Whole adrenal glands were homogenized in 3 ml of ice-cold 0.4N HClO\(_4\) and the homogenates were centrifuged at 40,000 g max for 10 min. An aliquot of supernatant fluid was used for E assay. E was analyzed by trihydroxyindole methods after high performance liquid chromatography separation\(^8\). In addition, an aliquot of supernatant fluid was assayed for taurine content in adrenals using an amino acid analyzer\(^2,3\). E and taurine contents in adrenals were expressed as µg/pair or µmoles/pair, respectively.

Effect of Naloxone on Systolic Blood Pressure

The remainder of the rats in each group were gently heated with a warming plate, a procedure to which the animals had become accustomed over a 2-week period preceding the experiment. Base-line systolic blood pressure in each animal was taken as the mean of 5 to 8 measurements. Each animal was injected intraperitoneally with naloxone HCl (2 mg/kg in 0.5 ml of saline) or an equal volume of saline (vehicle) and the effects of the drugs were assessed from readings (mean of 3) carried out at 5 min intervals for a period up to 30 min after drug administration. The experiment was repeated in accordance with crossover design with a 1-day interval. For example, the rats in each group that received naloxone in the first experiment were given vehicle in the second experiment.

Statistics

All data are expressed as means ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) for repeated measurements was used to determine the effects of line (F\(_{A}\); control vs DOCA-salt), taurine (F\(_{B}\)) and interaction (F\(_{AB}\); effects of taurine in control and DOCA-salt rats).
Simultaneous comparisons between groups were made by the modified t-test, using Bonferroni's method to adjust the level of significance. Student's t-test for paired comparison was used to assess the effects of naloxone or vehicle on systolic blood pressure. Statistical significance was noted when the p value was equal to or less than 0.05.

RESULTS

Blood Pressure and Body Weight
Changes in systolic blood pressure and body weight in control and DOCA-salt rats with and without taurine supplementation are shown in Fig. 1. One week after each treatment, the systolic blood pressure of DOCA-salt rats (128±2 mmHg) was elevated and differed significantly from that of all other groups. At weeks 1 and 2, two-way ANOVA of systolic blood pressure revealed significant main effects for line (control vs DOCA-salt), taurine supplementation and line times taurine interaction; taurine supplementation resulted in the attenuation of blood-pressure rise only in DOCA-salt rats.

There were no significant between-group differences in body weight at weeks 0, 1, and 2 (Fig. 1).

Basal Values for Mean Blood Pressure and heart Rate
Mean blood pressure of rats treated with DOCA and salt for 2 weeks (136±4 mmHg) was elevated compared to those of the other 3 groups (Control, 116±2 mmHg; Control +1% taurine, 116±1 mmHg; DOCA-salt +1% taurine, 122±4 mmHg). These results are consistent with those of systolic blood pressure levels described in Fig. 1.

The mean heart rate of taurine-supplemented DOCA-salt rats (361±11 beats/min) was significantly reduced and differed from that of all other groups (Control, 409±8 beats/min; Control +1% taurine, 411±8 beats/min; DOCA-salt, 397±8 beats/min). Two-way ANOVA revealed that taurine supplementation reduced heart rate only in DOCA-salt rats.

Adrenal Taurine and E Contents
As shown in the upper panel of Fig. 3, the adrenal taurine content of rats treated with DOCA and salt for 2 weeks (1114±107 nmoles/pair) was increased compared to that of control rats (691±61 nmoles/pair) and 2-week taurine supplementation in DOCA-salt rats further increased taurine content in the adrenals (1600±174 nmoles/pair). However, 2-week taurine supplementation in control rats had no effect on adrenal taurine content (765±40 nmoles/pair).

In contrast, as shown in the lower panel of Fig. 3, the adrenal E content of DOCA-salt rats (26.39±1.30 μg/pair) was increased compared to that of control rats (22.08±0.94 μg/pair) but taurine supplementation restored the increased adrenal E content in DOCA-salt rats (19.55±1.62 μg/pair). Taurine supplementation in control rats did not affect E content in the adrenals (22.07±0.79 μg/pair).

Effect of naloxone on Blood Pressure
There were no significant differences in basal systolic blood pressure levels of rats treated with each regimen for 2 weeks before the administration of naloxone or vehicle (109±4 vs 111±2 mmHg in control group, 108±3 vs 109±2 mmHg in taurine-supplemented control group, 135±3 vs 137±5 mmHg in DOCA-salt group, and 109±2 vs 113±2 mmHg in taurine-supplemented DOCA-salt group, respectively). As shown in Fig. 4, the administration of saline (vehicle) did not affect blood pressure in any group up to 30 min. Naloxone administered to taurine-supplemented DOCA-salt rats caused a rapid increase in blood pressure that lasted up to 30 min: the maximal increase in systolic blood pressure (10 min) caused by naloxone was 19±5 mmHg. In contrast, the injection of naloxone had no effect on blood pressure in the other 3 groups.

Also, naloxone did not affect the heart rates of rats in any group.

DISCUSSION
Our first relevant observation, in keeping with previous reports, is that both plasma NE, E and adrenal E levels were increased in DOCA-salt rats compared to control rats, when the mean blood pressure was already increased. Grobecker et al7 reported that after only 1 week of treatment with DOCA and 1% saline tyrosine hydroxylase-activity,
the rate-limiting enzyme in catecholamine synthesis, in adrenal glands was significantly increased compared to that in controls and the increased enzyme activity was still increased after 4 weeks of DOCA-salt treatment associated with the increased adrenal E levels. Bouvier and de Champlain also demonstrated that baseline plasma NE and E levels of DOCA-salt rats under light chloralose anesthesia were significantly higher than those of control rats and resting plasma NE concentrations were significantly correlated with mean arterial pressure levels. These observations are consistent with the present findings and suggest that increased liberation of adrenal E into the circulation as well as increased SNS activity may contribute to the development of DOCA-salt hypertension. De Champlain and van Ameringen observed that after the combination of chemical sympathectomy with 6-hydroxydopamine and bilateral adrenalectomy, blood pressure levels of DOCA-salt rats and normotensive control rats became identical. This observation suggests that the most likely factor accounting for the elevated blood pressure of DOCA-salt rats is a synergic hyperactivity of the sympathetic nerve fibers and adrenal medulla and supports the issue mentioned above. Interestingly, in the present study, the adrenal taurine content of DOCA-salt rats was significantly higher than that of control rats. We have previously observed that the taurine content in the hearts of DOCA-salt rats was significantly higher than that in the hearts of control rats and indicated some possibility that increased sympathetic nervous system activity may participate in this mechanism. 

Although the physiological role of adrenal taurine is as yet unknown, the current result sheds intriguing light on the relationship between taurine metabolism and adrenomedullary activity.

The second observation is that taurine supplementation effectively prevented the development of DOCA-salt hypertension but did not affect blood pressure in normotensive control rats, which is consistent with our previous reports. This was also confirmed by direct measurement of arterial blood pressure. In addition, in the present study, taurine supplementation restored to normal increased plasma NE and E levels, and adrenal E content, of DOCA-salt rats, associated with the markedly increased taurine content in adrenals. Since plasma catecholamine levels have been proposed to be a valid indirect index of sympathoadrenal activity the finding that taurine supplementation normalized the increased plasma NE and E levels as well as the increased adrenal E content of DOCA-salt rats suggests the preventive or suppressive actions of taurine against the increased sympathoadrenal tone and E release from adrenal glands. Kuriyama et al demonstrated that the administration of taurine (4 to 7 g/kg/day for 3 days, orally) abolished the decrease in E content of the adrenal glands induced by immobilized cold stress and its effect may be attributed to the inhibition of E release from chromaffin tissue by direct interaction with chromaffin granules. Our recent study also showed that oral administration of taurine (6 g for 7 days) in young patients with borderline hypertension not only reduced basal plasma E but also attenuated the increased response of plasma E to glucagon stimulation. Thus, it is speculated that the markedly increased taurine in the adrenal glands may contribute both to the suppression of increased E release from adrenal medullary granules of DOCA-salt rats and to the attenuation of stress-induced E release. In turn, these suppressive effects of taurine could not be observed in taurine-supplemented control rats, because of the absence of the increase in adrenal taurine content.

Thirdly, in the present study, taurine supplementation significantly reduced the heart rates of DOCA-salt rats. It is shown that taurine has a negative chronotropic effect: intracerebroventricular injection of taurine produces dose-related bradycardia concurrent with hypotension. In addition, taurine administered intraventricularly blocked the central actions of angiotensin II, pressor and positive chronotropic actions, but when administered directly into the ganglia, had no effect on the ganglionic stimulatory effect of angiotensin II. These pieces of evidence suggest that taurine-induced hypotensive and bradycardiac effects might be exerted via changes in the central nervous system. Moreover, the present results suggest that those effects could be potentiated in the pre-

sence of increased SNS activity or the resultant hypertension.

The fourth observation is that the injection of naloxone, an opiate antagonist, induced a significant elevation of blood pressure in taurine-supplemented DOCA-salt rats but not in control or DOCA-salt rats. Naloxone is a specific receptor antagonist to the endogenous opiates and is promptly absorbed (~15 min) into brain tissues following subcutaneous administration. Two major endogenous opioid peptides, β-endorphin and enkephalin, differ in their distribution in the brain, and both have been shown to be implicated in central cardiovascular regulation by separate systems: β-endorphin is predominantly located in the medial basal hypothalamus, with axons widely distributed throughout the brain including the NTS, while enkephalin is seen in many local cell groups throughout the neuraxis, especially the lower brain stem. Whereas the administration of enkephalin or D-Ala2-Met- enkephalinamide (DAME), a potent enkephalin analogue resistant to enzymatic breakdown, intraventricularly or into the NTS produces a dose-related pressor response and tachycardia, similarly administered β-endorphin results in a dose-response relationship with a fall in blood pressure and heart rate. Moreover, these cardiovascular effects caused by the administration of opioids are prevented or reduced by pretreatment with naloxone. From this evidence, the present finding that the injection of naloxone increased blood pressure only in taurine-supplemented DOCA-salt rats appears to result from the blockade of endogenous endorphinergic inhibition of SNS activity in the brain. In agreement with this view, recent results from our laboratory have shown that β-endorphin-like immunoreactive material content in the hypothalamus was significantly increased in taurine-supplemented DOCA-salt rats, although its content is not increased in taurine-supplemented control rats. The evidence strongly suggests that overproduction of β-endorphin in the hypothalamus might contribute to the taurine-induced normalization of the sympathetic overactivity and the resultant hypertension in DOCA-salt rats. In addition, the increased endorphinergic activity of taurine-supplemented DOCA-salt rats might be the increased adrenomedullary activity observed in this study, since it is shown that β-endorphin inhibits Ach-induced E release from chromaffin cells.

Whereas the mechanism for the activation of the endogenous endorphinergic system by taurine supplementation could not be clarified by the present study, several reports suggest an interaction between taurine and morphine or morphine-like peptides. Rats rendered dependent by the implantation of a morphine pellet showed significantly increased taurine content in the spinal cord. Treatment with taurine suppressed the development of tolerance to both analgesia and akinesia effects of DAME. Both taurine and β-endorphin inhibit K+-induced release of 3H-NE from a variety of neural tissues and the alteration of Ca2+ influx has been suspected as the common mechanism for this. Moreover, taurine has been shown to antagonize the reductions of synaptic vesicular Ca2+ content induced by morphine administration in mice. Taken together, it seems reasonable to assume that possible taurine-induced alteration of Ca2+ transport, which may modulate neurotransmission within the central nervous system, could be partially restored by treatment with naloxone in taurine-supplemented DOCA-salt rats, resulting in the elevation of blood pressure due to increased sympathoadrenal activity. It is suggested, therefore, that taurine and β-endorphin in the brain are interdependent and may exert suppressive effects on the increased sympathoadrenal activity cooperatively.

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*Japanese Circulation Journal  Vol.55, May 1991*