Role of Sympathetic Nervous System in Hypotensive Action of Taurine in DOCA-Salt Rats

YUJI SATO, KATSUYUKI ANDO, AND TOSHIRO FUJITA

SUMMARY We tested the hypothesis that the antihypertensive effects of dietary taurine supplementation in deoxycorticosterone acetate (DOCA)–salt rats may be attributed to the suppression of sympathetic nervous system activity. In uninephrectomized rats treated with DOCA while receiving 1% NaCl solution for 2 weeks, systolic blood pressure was significantly increased as compared with that in control rats treated with vehicle suspension and tap water. Sympathetic nervous system activity was assessed by tissue norepinephrine turnover, which was determined from the rate of decline of tissue norepinephrine concentration after the administration of \( \alpha \)-methyl-p-tyrosine, a potent inhibitor of the rate-limiting step of catecholamine synthesis. Cardiac and splenic norepinephrine turnover during either normal conditions or cold exposure (4°C, 8 hours) were markedly increased in DOCA-salt rats as compared with control rats. Also, DOCA-salt rats had increased depressor response to hexamethonium bromide, a ganglion blocker. In contrast, supplementation of 1% taurine in DOCA-salt rats attenuated the development of the hypertension associated with the normalization of both the increased depressor response to ganglionic blockade and the accelerated cardiac and splenic norepinephrine turnover during either normal conditions or cold exposure. Taurine supplementation in control rats, however, had no effect on blood pressure or norepinephrine turnover during cold exposure. These results suggest that taurine supplementation suppresses sympathetic overactivity in DOCA-salt rats, thus leading to inhibition of the development of hypertension.

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KEY WORDS • taurine • deoxycorticosterone acetate–salt hypertension • norepinephrine turnover • cold exposure • sympathetic nervous system

TAURINE (2-aminoethanesulfonic acid) is abundantly present in the brain, heart, and muscle of mammals, but its possible function in normal or disease states has not been clearly defined. Taurine supplementation in the stroke-prone strain of spontaneously hypertensive rats reportedly attenuates the development of the hypertension; however, the mechanism for the antihypertensive action of taurine is unknown.

Our previous study demonstrated that dietary taurine supplementation in deoxycorticosterone acetate (DOCA)–salt rats could not only attenuate the development of the hypertension but also reduce blood pressure when taurine was given after DOCA-salt hypertension had been established. There is a good deal of evidence that increased sympathetic nervous system (SNS) activity is involved in the development of DOCA-salt hypertension in rats. The norepinephrine (NE) turnover rate, an indirect in vivo means of quantifying the activity of SNS, is increased in most peripheral sympathetically innervated organs of DOCA-salt rats, such as the heart, spleen, and intestine. Moreover, it has been reported that in vitro addition of taurine significantly attenuates the Ca\(^{2+}\)-dependent, K\(^+\)-evoked release of \([\text{H}]\)NE from cerebral cortical slices without affecting the unstimulated (spontaneous) release. These findings led to the hypothesis that taurine supplementation could attenuate the increased SNS activity in DOCA-salt rats and thus prevent the development of the hypertension, since in control rats taurine supplementation affected neither SNS activity nor blood pressure.
Therefore, we have examined 1) the effects of taurine supplementation on the depressor response to hexamethonium bromide, a ganglion blocker, and cardiac NE turnover under normal conditions in DOCA-salt rats and vehicle-injected control rats and 2) the effects of taurine supplementation on the responses of cardiac and splenic NE turnover to cold exposure, which is known to be a useful and reproducible method of studying physiological response of SNS activity in the rat.11, 12

Materials and Methods
Series 1: Effects of Taurine Supplementation on the Depressor Response to Hexamethonium Bromide and Norepinephrine Turnover Under Normal Conditions in DOCA-Salt Rats
Animal Preparation
Male Sprague-Dawley rats (Charles River Japan, Atsugi, Japan) weighing 120 to 130 g were subjected to unilateral left nephrectomy at 5 weeks of age. Following nephrectomy, 14 days was allowed for compensatory renal hypertrophy to occur before each treatment was begun. Rats were randomly divided into three groups as follows. The DOCA-salt group received weekly subcutaneous injections of 0.4 ml of a suspension containing, per milliliter of water, 25 mg 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); this group also was given 1% NaCl in tap water ad libitum. The 1% taurine-supplemented DOCA-salt group 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 received weekly injections of the vehicle suspension without DOCA and was given tap water to drink ad libitum. Throughout the study animals were housed in a room with constant temperature (23 ± 1°C) and humidity (60 ± 5%) and light from 0600 to 1800. All rats received a standard laboratory rat chow (MF; Oriental Yeast Co., Tokyo, Japan). After 2 weeks of each treatment, body weight was measured and the following experiments were performed.

Depressor Response to Hexamethonium Bromide
Blood pressure response to ganglionic blockade was used as index of peripheral SNS activity. The rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.). Tip-tapered polyethylene catheters (PE-50) were inserted into the femoral vein for drug administration and into the lower abdominal aorta through the femoral artery for blood pressure recording. The catheters were secured, tunneled subcutaneously, and externalized behind the neck. Forty-eight hours after catheter placement, mean blood pressure was recorded continuously in the conscious, freely moving rats by connecting the aortic catheter to a pressure transducer (Model TP-101T; Nihonkohden, Tokyo, Japan). After a stable mean arterial pressure was obtained (at least 30 minutes was allowed to pass after the start of recording), hexamethonium bromide (Tokyo Kasei Kogyo, Tokyo, Japan), 30 mg/kg, was infused through the venous catheter and the maximum decrease in mean arterial pressure was recorded. This dose of hexamethonium bromide has been shown to interrupt sympathetic transmission controlling the cardiovascular system in the rat.13, 14

Cardiac Norepinephrine Turnover
Turnover of NE is an indirect in vivo means of measuring SNS activity in sympathetically innervated organs of unanesthetized, unrestrained animals. After the first intraperitoneal injection of α-methyl-p-tyrosine (α-MPT; 300 mg of the methyl ester hydrochloride [Sigma] dissolved in saline per kilogram of body weight), six to eight animals from each group were killed at 4 hours. Eight animals from each group were re-injected with the same dose of α-MPT at 4 hours and killed at 8 hours, while eight animals from each group were not injected and served as the t₀ reference. Hearts were removed and quickly frozen on dry ice. The tissues were stored at −40°C until analyzed. After the tissues were homogenized in ice-cold 0.4 N perchloric acid (Wako), the homogenates were centrifuged at 40,000 g for 10 minutes. The NE was isolated with activated alumina (Woelm, West Germany), and the eluates were analyzed fluorimetrically (Model PF-500LC; Shimadzu, Kyoto, Japan) as described previously.15

Data are plotted as means ± SE for endogenous NE in each group at each time. The line representing the decline in endogenous NE with time was calculated by the method of least squares. The slope, or rate constant of decline, represents the fractional turnover rate of NE or the percentage of the pool declining per hour.4

Taurine Content in Heart
Animals were killed by decapitation without anesthesia. Hearts were removed immediately. Hearts were quickly frozen on dry ice and stored at −40°C until analyzed. Tissues were deproteinized with sulfosalicylic acid (Wako), and the deproteinized samples were analyzed on an amino acid analyzer (Model D-502; Dionex, CA, USA).

Series 2: Effects of Taurine Supplementation on the Responses of Cardiac and Splenic Norepinephrine Turnover to Cold Stress in Control and DOCA-Salt Rats
Animal Preparation
Rats were subjected to all procedures as described for the first series, except that a 1% taurine-supplemented control group was added that received injections of the vehicle suspension and was given 1% taurine in tap water ad libitum. Systolic blood pressure was measured weekly in conscious, prewarmed rats using an occlusive tail cuff and a pneumatic pulse
transducer attached to a programmed electrophysiological transducer (Model PE-300; Narco Biosystems, Houston, TX, USA). Body weight was also measured once a week. After 2 weeks of each treatment, the following experiment was performed.

Cardiac and Splenic Norepinephrine Turnover During Cold Exposure

To assess the effect of taurine supplementation on peripheral SNS activity during cold exposure (4°C, 8 hours), cardiac and splenic NE turnover was measured using the same method as described for the first series. In brief, after the first intraperitoneal injection of α-MPT (300 mg of the methyl ester hydrochloride dissolved in saline per kilogram of body weight), six to eight animals from each group were placed in a cold room at 4°C for 8 hours. These animals were re injected with the same dose of α-MPT at 4 hours and killed at 8 hours by decapitation without anesthesia, while six to eight animals from each group were not injected and served as the t0 reference. Preliminary experiments demonstrated that tissue NE content after the injection of α-MPT declines exponentially and time dependent-ly by at least 9 hours during cold exposure as well as under normal conditions. Hearts and spleens were removed immediately and quickly frozen on dry ice. Tissues were stored at −40°C until analyzed. When ready for assay, tissue samples were weighed, homogenized, and centrifuged, and NE was isolated and analyzed as described for the first series.

Statistical Analysis

Group values are expressed as means ± SE. Analysis of variance (ANOVA) and the Bonferroni method for multiple comparison were used to determine whether there were statistical differences among the DOCA-salt, 1% taurine-supplemented DOCA-salt, and normal control groups. Also, in Series 2, results were analyzed by two-way ANOVA for the differences between the control and DOCA-salt lines (F A), the effects of taurine supplementation (F B), and the differential effects of taurine supplementation on each of the two lines (interaction: F^). The Bonferroni method was used for comparisons between individual means. A p value of less than 5% was considered significant.

Table 1. Body Weight, Mean Blood Pressure, and Decrease in Mean Blood Pressure by Ganglionic Blockade in Control, DOCA-Salt, and 1% Taurine-Supplemented DOCA-Salt Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean blood pressure</th>
<th>Absolute decrease (mm Hg)</th>
<th>Percent decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (g)</td>
<td>Prehex (mm Hg)</td>
<td>Posthex (mm Hg)</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>309 ± 8</td>
<td>104 ± 3</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>DOCA-salt (n = 6)</td>
<td>301 ± 9</td>
<td>138 ± 2*</td>
<td>75 ± 2</td>
</tr>
<tr>
<td>DOCA-salt + 1% taurine (n = 7)</td>
<td>290 ± 7</td>
<td>109 ± 2†</td>
<td>81 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Prehex, Posthex = before and after hexamethonium injection.

*p < 0.01, compared with values in control group; †p < 0.01, compared with values in DOCA-salt group.

Results

Series 1

Body Weight and Blood Pressure

As shown in Table 1, body weight 2 weeks after treatment was not different among control, DOCA-salt, and 1% taurine-supplemented DOCA-salt rats. Systolic blood pressures of DOCA-salt rats at Week 2 were significantly increased compared with those of control rats. In contrast, 1% taurine supplementation inhibited the elevation of blood pressure in DOCA-salt rats.

Depressor Response to Hexamethonium Bromide

The administration of hexamethonium bromide produced a significant (p < 0.01) decrease in mean blood pressure in three groups (see Table 1). The decrease in mean blood pressure was significantly greater (p < 0.01) in DOCA-salt rats as compared with control rats. However, the depressor response to hexamethonium bromide was significantly less (p<0.01) in the taurine-supplemented DOCA-salt rats as compared with DOCA-salt rats. Thus, there was no significant difference in the decrease in mean blood pressure between control and taurine-supplemented DOCA-salt rats.

Cardiac Norepinephrine Turnover

Cardiac NE turnover was measured in the three groups of rats (Figure 1). Slope of the lines (fractional turnover rate) of cardiac NE turnover was significantly (p<0.05) increased from 2.22 ± 0.80 to 5.27 ± 1.00% per hour by DOCA-salt treatment. In contrast, 1% taurine supplementation restored the increased fractional turnover rate of cardiac NE to control values (2.25 ± 0.85% per hour; not significantly different from control) in DOCA-salt rats. The contents of endogenous NE were 562.6 ± 37.8 ng/g in control rats, 463.4 ± 34.5 ng/g in DOCA-salt rats, and 476.9 ± 33.5 ng/g in 1% taurine-supplemented DOCA-salt rats (not statistically different among the three groups).

Taurine Content in Heart

In the present study, the taurine content in hearts of DOCA-salt rats tended to be increased, but not signifi-
Cardiac norepinephrine turnover during cold exposure in control and DOCA-salt rats. In spleen, taurine supplementation had no effect on the increased NE turnover induced by cold stress in control rats, whereas the magnitude of the increase in NE turnover was reduced by taurine supplementation in the DOCA-salt rats.

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

Cardiac and Splenic Norepinephrine Turnover During Cold Exposure

Table 3 and Figure 2 show the effects of taurine supplementation on cardiac and splenic NE turnover during cold exposure in control and DOCA-salt rats. As shown in Table 3, there were no significant between-group differences in endogenous NE contents of hearts and spleens. In DOCA-salt rats the percentage of cardiac NE content remaining 8 hours after α-MPT administration and cold exposure was lower as compared with control rats (see Figure 2), while fractional turnover rate of cardiac NE was significantly higher (see Table 3). Taurine supplementation in DOCA-salt rats increased the percentage of cardiac NE content remaining 8 hours after α-MPT administration and cold exposure and moderated the increased fractional turnover rate of cardiac NE. In control rats, however, taurine supplementation affected neither the percentage of cardiac NE content nor the fractional turnover rate during cold exposure. Thus, taurine supplementation resulted in a significant attenuation of cardiac NE turnover during cold exposure in the DOCA-salt rats but no significant change in turnover during cold exposure in the control rats. In spleen, taurine supplementation had no effect on the increased NE turnover induced by cold stress in control rats, whereas the magnitude of the increase in NE turnover was reduced by taurine supplementation in the DOCA-salt rats.

Discussion

Our first relevant observation, in keeping with previous reports, is that the DOCA-salt hypertensive rats had not only an increased depressor response to a ganglion blocker but also increased NE turnover. Several lines of evidence suggest that DOCA-salt hypertensive rats have increased SNS activity. For example, vasodepression in DOCA-salt hypertensive rats reportedly is enhanced by pharmacological blockade of autonomic ganglia with chlorisondamine, and NE turnover rate is increased in most peripheral sympathetically innervated organs of DOCA-salt rats, such as heart, spleen, and intestine. Moreover, Ayitey-Smith and Varma have reported that the development of DOCA-salt hypertension was inhibited in adequately immunosympathectomized rats. De Champlain and van Ameringen showed that the combination of chemical sympathectomy with intravenous administration of 6-hydroxydopamine and bilateral adrenalectomy markedly decreased blood pressure in DOCA-salt hypertensive animals. Thus increased SNS activity seems to be essential for the development of hypertension induced by treatment with DOCA and salt. This hypothesis is supported by the present result that mean blood pressure levels after ganglionic blockade were not significantly different from control values.
Table 3. Effects of Taurine Supplementation on Cardiac and Splenic Norepinephrine Contents and Norepinephrine Turnover During Cold Exposure in Control and DOCA-Salt Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart Endogenous NE content (ng/heart)</th>
<th>Heart 8-hour NE content (ng/heart)</th>
<th>Fractional turnover rate (hr)</th>
<th>t½ (hr)</th>
<th>Spleen Endogenous NE content (ng/spleen)</th>
<th>Spleen 8-hour NE content (ng/spleen)</th>
<th>Fractional turnover rate (hr)</th>
<th>t½ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>553.7 ± 41.7</td>
<td>220.6 ± 36.1</td>
<td>12.74 ± 1.40</td>
<td>5.44</td>
<td>453.1 ± 43.0</td>
<td>261.6 ± 21.3</td>
<td>7.15 ± 1.10</td>
<td>9.69</td>
</tr>
<tr>
<td>Control + 1% taurine</td>
<td>611.0 ± 85.4</td>
<td>250.5 ± 31.3</td>
<td>12.57 ± 2.36</td>
<td>5.69</td>
<td>477.0 ± 34.9</td>
<td>283.8 ± 17.0</td>
<td>7.39 ± 1.05</td>
<td>9.38</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>516.1 ± 35.0</td>
<td>67.7 ± 12.6</td>
<td>27.41 ± 2.97</td>
<td>2.53</td>
<td>474.5 ± 44.5</td>
<td>88.4 ± 15.1</td>
<td>22.03 ± 1.81</td>
<td>3.15</td>
</tr>
<tr>
<td>DOCA-salt + 1% taurine</td>
<td>537.1 ± 43.3</td>
<td>192.3 ± 38.3</td>
<td>14.15 ± 2.29</td>
<td>4.90</td>
<td>469.4 ± 53.8</td>
<td>247.4 ± 24.7</td>
<td>8.34 ± 1.33</td>
<td>8.31</td>
</tr>
</tbody>
</table>

Values are means ± SE for groups of six to eight rats. NE = norepinephrine. 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): HEART: endogenous NE content was not significant; 8-hour NE content: F A = 6.474 (p < 0.02), F B = 12.087 (p < 0.01), F AB = 2.432 (DOCA-salt < DOCA-salt + 1% taurine = control + 1% taurine); fractional turnover rate: F A = 6.845 (p < 0.02), F B = 10.021 (p < 0.01), F AB = 6.502 (DOCA-salt > DOCA-salt + 1% taurine = control + 1% taurine; p < 0.02). SPLEEN: endogenous NE content was not significant; 8-hour NE content: F A = 21.944 (p < 0.01), F B = 29.241 (p < 0.01), F AB = 12.443 (DOCA-salt < DOCA-salt + 1% taurine = control + 1% taurine; p < 0.01), fractional turnover rate: F A = 22.500 (p < 0.01), F B = 31.165 (p < 0.01), F AB = 24.135 (DOCA-salt > DOCA-salt + 1% taurine = control + 1% taurine; p < 0.01).

Our second observation is that cardiac and splenic NE turnover during cold exposure was markedly increased in DOCA-salt rats compared with control rats (see Table 3 and Figure 2). The stimulatory effect of short-term cold exposure on SNS activity and the crucial importance of sympathetic activation in the defense of body temperature in mammals are well recognized.11 12 Several lines of evidence suggest that hyperresponsiveness of SNS activity to stressful stimuli plays an important role in the development or maintenance, or both, of hypertension. Spontaneously hypertensive rats showed higher values of plasma NE to environmental stresses such as foot shock,18 immobilization,19 and cold20 compared with normotensive Wistar-Kyoto rats. On the other hand, long-term reduction in the level of environmental stress prevents the development of hypertension in spontaneously hypertensive rats but has no effect on the blood pressure of normotensive controls.21 22 Repeated immobilization of normotensive strains of rats results in high circulating levels of catecholamines and the development of permanent elevations in blood pressure.23 Also, young patients with essential hypertension had significantly higher plasma NE levels after postural stimulation than did the age-matched controls.24 The results of these studies are consistent with those of the present study: DOCA-salt rats had increased SNS activity in response to environmental stimuli in the early stage of the hypertension.

The third observation in the present study is that 1% taurine supplementation in DOCA-salt rats effectively prevented the development of hypertension. Concomitant with the antihypertensive effect of taurine, both the increased depressor response to ganglionic blockade and the increased cardiac NE turnover were normalized by taurine supplementation. Taurine supplementation also restored to control levels the increased cardiac and splenic NE turnover during cold exposure in DOCA-salt rats. In contrast, 1% taurine supplementation in normotensive control rats affected neither blood pressure levels nor cardiac and splenic NE turnover during cold exposure. This finding suggests that the antihypertensive effect of taurine may be attributed to the suppression of SNS overactivity in DOCA-salt rats. Supporting this possibility are reports suggesting that in vitro addition of taurine significantly attenuated the Ca2+‐dependent, K+‐evoked release of [3H]NE from a variety of neuronal tissues without affecting uptake or unstimulated (spontaneous) release.9 10 Moreover, when given intravenously to rats, taurine...
caused an immediate decrease in blood pressure—an effect that disappeared after chemical denervation with guanethidine or 6-hydroxydopamine. These observations were interpreted in terms of an effect of taurine on catecholamine release. Furthermore, while in vitro addition of taurine suppressed the stress-induced release of NE from rat superior cervical ganglia, neither leucine nor methionine had any preventive effect on the NE or acetylcholine release. These findings suggest that the suppression of sympathetic overactivity and the resultant attenuation of the development of hypertension should be attributed to the specific effect of taurine on the SNS. They also support the hypothesis that the antihypertensive action of taurine can be exhibited in DOCA-salt rats with increased SNS activity, whereas in normotensive control rats with normal SNS activity, taurine supplementation can affect neither SNS activity nor blood pressure.

The results of our recent study indicate that taurine loading attenuates the augmented hypothalamic and cardiac noradrenergic activity by cold stress in the DOCA-salt hypertensive rats. The evidence presented suggests that suppression of sympathetic overactivity by taurine supplementation might be intimately related to the central noradrenergic mechanisms in DOCA-salt rats.

In the present study the taurine content in hearts of DOCA-salt rats tended to be increased as compared with that in hearts of control rats. There is some possibility that the increased SNS activity in DOCA-salt hypertensive rats might change the taurine metabolism in the heart, since it has been reported that cardiac taurine content is increased in rats exposed to environmental stress. This increase in cardiac taurine has been shown to derive from influx of exogenous taurine into the heart rather than from cardiac biosynthesis. Therefore, the increased SNS activity may be responsible for the increased taurine content in the hearts of DOCA-salt hypertensive rats. In turn, the resultant increased taurine content in the heart might exert protective action against the sympathetic overactivity. Thus, this increased taurine content might have a compensatory function against sympathetic overactivity in DOCA-salt rats.

Furthermore, taurine supplementation resulted in a further significant increase in taurine content of hearts in DOCA-salt rats (see Table 2). In respect to taurine-induced suppression of NE release from sympathetic nerve endings, it has been suggested that taurine might inhibit release of calcium from mitochondria without affecting the uptake process, thus leading to the decreased availability of intracellular calcium in nerve endings. It is well known that calcium plays an important role in the process of neurotransmitter release. Thus, the increased taurine content in hearts of taurine-supplemented DOCA-salt rats might modulate the activity of SNS, possibly by interacting with the retention mechanism for calcium within mitochondria, resulting in the normalization of SNS activity. However, the precise role of intracellular calcium movements in the taurine-induced suppression of NE release needs to be clarified.

In conclusion, taurine supplementation in DOCA-salt rats attenuated the development of the hypertension associated with the suppression of sympathetic overactivity, not only under normal conditions but also during cold exposure. However, in normotensive control rats, taurine supplementation affected neither sympathetic activity nor blood pressure. These results suggest that the suppression of sympathetic overactivity may be responsible for the antihypertensive action of taurine in DOCA-salt rats.

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