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EFFECT OF DIETARY TAURINE ON BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—Previous studies have shown the dietary supplementation with sulfur amino acids modified the development of hypertension in rats. In the current study, it was found that the addition of taurine (3%) to the drinking water of rats during the period of 4–14 weeks of age had little effect on blood pressure in normotensive Wistar–Kyoto rats (WKR), and slightly retarded the development of hypertension in spontaneously hypertensive rats (SHR). In the stroke-prone substrain (SHR-SP), however, there is a highly significant reduction in the development of hypertension. The endogenous content of taurine in the liver of SHR-SP is less than 50 per cent of that measured in WKR. The livers of the SHR contain an intermediate amount of taurine. The cysteic acid decarboxylase activity of liver is similar in all three strains. The tissues of animals treated chronically with taurine contain only slightly greater amounts of taurine, with small but significant increases in brain taurine being noted. It appears that genetically hypertensive rats, particularly the SHR-SP substrain, have a defect in taurine metabolism, and that this may be related to the severity of hypertension. There is no evidence to suggest that taurine plays a normal regulatory role in blood pressure maintenance.

The interaction of genetic and environmental factors in the etiology of diseases is increasingly becoming a focal point for investigations. It is believed that genetically hypertensive rats provide a model for at least some of the genetic factors that may be involved in human essential hypertension [1–3]. Spontaneous hypertensive rats (SHR), derived from the Wistar–Kyoto rat (WKR), and a stroke-prone substrain (SHR-SP) [4, 5] have been studied intensively for the past decade. Recent studies suggest that the severity of cardiovascular diseases in these rats is related to their diet. The incidence of stroke, which is over 80 per cent in the SHR-SP, can be reduced significantly when the rats are fed a diet containing a large amount of fish protein [6]. Such a diet also retarded the development of hypertension. An examination of various protein sources for rat diets indicated that there may be a correlation between methionine content and the effectiveness of the diet in reducing blood pressure in the SHR-SP [7].

Taurine, a metabolite of methionine, is distributed widely in mammalian tissue. Taurine is derived more directly from cysteic acid or cysteine sulfinic acid by the action of cysteic acid decarboxylase. The role of this enzyme in regulating tissue taurine is not known. Large amounts are present in brain, although its physiological role is obscure [8]. It has been postulated to be either a neurotransmitter or a neuromodulator [9–12]. Since numerous studies [13] have indicated that neuronal systems play a role in the pathogenesis of hypertension, we undertook an investigation of the effect of taurine administration on the development of elevated blood pressure in genetically hypertensive rats.

In the current studies, the effects of long-term administration of taurine on the development of hypertension and tissue taurine content were determined. The activity of cysteic acid decarboxylase was also determined.

MATERIALS AND METHODS

Male SHR, stroke-prone SHR and Wistar–Kyoto rats used in this study were obtained from the Animal Production Section of the National Institutes of Health.

In the taurine administration experiment, the animals of each strain were separated into two groups of six to eight animals each. One group received tap water to drink and the other group received water containing 3% taurine. The experiments were initiated when the animals were 32 days of age and continued until they were 107-days-old. Rats received the standard NIH rat chow diet. All animals were fasted and given only tap water to drink 24 hr before death.

Animals were anesthetized with sodium pentobarbitol (50 mg/kg), killed by heart puncture, and brain, heart, liver, skeletal muscle and aorta were removed. Blood was collected in a syringe containing a small amount of CPD† solution and then was centrifuged at 3000 rev/min for 10 min to obtain plasma. The brain was separated further into four regions (telencephalon, cerebellum, diencephalon and lower brainstem) by the method of Glowinski and Iversen [14]. Each dissected tissue was frozen on dry ice and stored at −20° until assayed.

Tissues were homogenized in 3 vol. of 1% picric acid solution. The homogenate was centrifuged at

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† CPD solution contains sodium citrate-2 H_2O, 28.8 g; citric acid-H_2O, 3.2 g; $NaHPO_4$-H_2O, 2.18 g; and Dextrane, 25.0 g made to 1000 ml with distilled water.

11,000 *g* for 20 min and the supernatant fraction was applied to an ion-exchange column containing AG 1-X4 resin (0.5 × 5 cm) to remove the picric acid. The effluent was evaporated to dryness and the residue was redissolved in 0.25 N citric acid, pH 2.1. Taurine was measured by using a Beckman 120 C amino acid analyzer. Cysteic acid decarboxylase activity was measured according to the method of Mathur *et al.* [15], with a slight modification. The animals were killed by decapitation. The brain and liver were removed and were homogenized in 3 vol. of 0.067 M Sorensen's phosphate buffer, pH 6.8, containing 9.4×10^{-5} M PLP, 10^{-3} M dithiothreitol and 0.2% Triton X-100. The liver homogenate was centrifuged at 11,000 *g* for 20 min and the supernatant fraction was used for the enzyme assay. Enzyme activity was similar in both the homogenate and supernatant fractions.

The reaction mixture contained 80 μl of the homogenate or the supernatant fraction and 0.5 mM [^{14}C-U]cysteic acid (0.5 μCi radioactivity), and the final volume was made 100 μl. After incubation for 30 min at 37°, the reaction was stopped by adding 20 μl of 40% trichloroacetic acid (TCA) solution and the formed $^{14}CO_2$ was collected in 100 μl NCS solubilizer (Amersham–Searle, Arlington Heights, IL) by further incubation at 37° for 60 min.

Cysteic acid[^{14}C-U] was prepared by oxidation of cystine[^{14}C-U] [16]. Cystine[^{14}C-U] was purchased from the New England Nuclear Co., Boston, MA. Protein was measured by the method of Lowry *et al.* [17]. Systolic blood pressure was measured repeatedly from 32 to 107 days of age by an indirect tail-pulse-pickup method [18]. All chemicals used were analytical reagent grade.

RESULTS

The effects of taurine on blood pressure in Wistar–Kyoto rats, SHR and stroke-prone SHR are shown in Fig. 1. There were no differences in growth rate between control and experimental rats. Water intake and taurine consumption were measured during the first week of the experiment. There were no differences in taurine intake between the groups, and the mean value was 0.74 g/day/rat. The depressor effect of taurine was apparent about 30 days after the start of the experiment. The effect was marginal in SHR but clear in stroke-prone SHR. After 72 days of administration, the blood pressure in SHR-SP was reduced to about 180 mm Hg and the difference in blood pressure between control and experimental groups was about 30 mm Hg. In the SHR, blood pressure appeared to be lower in the taurine group than in the control group but the difference was significant only at 36 and 71 days after the beginning of the experiment. While the SHR-SP normally have significantly higher blood pressure than the SHR, taurine appeared to equalize blood pressure in these two strains. Taurine had no effect on the blood pressure of the WKR.

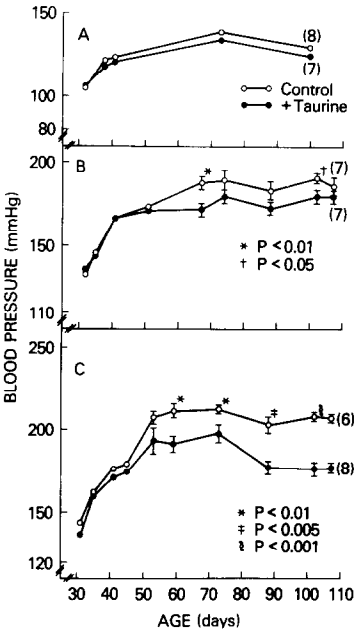

Fig. 1. Effect of taurine administration on blood pressure in WKR, SHR and SHR-SP. (A) WKR, (B) SHR and (C) SHR-SP.

Table 1. Taurine content of brain, heart, liver, skeletal muscle and aorta*

	Heart	Liver	Muscle	Aorta
		(μmoles/g)		
WKR	27.39 ± 1.19 (5)	7.77 ± 1.13 (9)	14.53 ± 0.69 (5)	0.516 ± 0.04 (4)
SHR	26.13 ± 1.32 (5)	5.11 ± 0.95 (9)	13.31 ± 0.22 (5)	0.714 ± 0.077 (4)
SHR-SP	29.82 ± 0.54 (5)	3.44 ± 0.92† (9)	12.55 ± 1.13 (5)	0.621 ± 0.105 (4)

	Brain			
	Telencephalon	Cerebellum	Diencephalon	Lower brainstem
		(μmoles/g)		
WKR	4.75 ± 0.10 (4)	4.45 ± 0.30 (4)	2.32 ± 0.05 (4)	1.68 ± 0.03 (4)
SHR	4.83 ± 0.15 (4)	4.45 ± 0.17 (4)	2.39 ± 0.03 (4)	1.63 ± 0.07 (4)
SHR-SP	4.90 ± 0.14 (4)	4.72 ± 0.05 (4)	2.29 ± 0.05 (4)	1.54 ± 0.09 (4)

* The amount was measured as described in Materials and Methods. Aortas from three animals were used to measure the taurine amount. Animals were 3-month-old rats. The blood pressure in WKR, SHR and SHR-SP was 129 ± 3, 189 ± 2 and 227 ± 6 respectively. Values are expressed as the mean ± S.E. The number of animals is given in parentheses.

† Significant difference between SKR and SHR-SP ($P < 0.01$).

Table 2. Serum concentration of taurine in genetically hypertensive rats*

Treatment	WKR (μmoles/ml)	SHR (μmoles/ml)	SHR-SP (μmoles/ml)
Tap water	0.170 ± 0.034 (8)	0.138 ± 0.013 (7)	0.094 ± 0.011† (6)
Taurine	0.184 ± 0.028 (7)	0.138 ± 0.010‡ (7)	0.112 + 0.008§ (8)

* These animals were those reported in Fig. 1. The values are expressed as the mean ± S.E.M. The number of animals is given in parentheses.
† Significant difference between control WKR and SHR-SP ($P < 0.025$).
‡ Significant difference between WKR and SHR-SP given taurine ($P < 0.025$).
§ Significant difference between SHR and SHR-SP given taurine ($P < 0.05$).

Table 3. Taurine content of SHR-SP brain and liver after taurine administration

Treatment	Liver (μmoles/g)	Brain			
		Telencephalon	Cerebellum	Diencephalon	Lower brainstem
		(μmoles/g)			
Tap water	3.12 ± 1.01 (4)	4.85 ± 0.09 (6)	3.98 ± 0.12 (6)	2.11 ± 0.17 (6)	1.23 ± 0.03 (6)
Taurine	4.34 ± 0.73 (8)	5.22 ± 0.07 (8)	4.34 ± 0.16* (8)	2.64 ± 0.11† (8)	1.64 ± 0.06‡ (9)

Values are expressed as the mean ± S.E. The number of animals is given in parentheses.
* Significant difference ($P < 0.01$).
† Significant difference ($P < 0.025$).
‡ Significant difference ($P < 0.001$).

An examination of the taurine content of various tissues of untreated rats revealed a significant reduction of taurine in the liver of the SHR-SP. As seen in Table 1, heart and skeletal muscle have the highest concentration of taurine, but no strain differences are apparent. Also, the regional content of brain is similar in all three strains. It is interesting that the greatest apparent reduction in taurine concentration in liver occurs in the SHR-SP, which also show the largest blood pressure response to oral administration.

The serum concentration of taurine was determined in the groups of rats used in the chronic administration experiment (Table 2). The serum concentration pattern is similar to that of liver, with the SHR-SP having significantly less circulating taurine then the WKR. The SHR have an intermediate content. It is clear also that taurine does not accumulate and that animals which have received taurine in their drinking water for 72 days have serum concentrations similar to controls.

The tissue content of taurine in SHR-SP that had received taurine showed very little accumulation (Table 3), although certain brain regions had a significant increase. Little is known about the factors that regulate taurine levels in tissue; however, it was of interest to examine the cysteic acid decarboxylase activity in liver and brain of genetically hypertensive rats. As seen in Table 4, there are no apparent differences in the activity of this enzyme in liver among any of these three strains.

Table 4. Cysteic acid decarboxylase activity in rat brain and liver*

	Brain	Liver
	(cpm/30 min/mg protein)	
WKR	1575 ± 96 (9)	22,599 ± 1753 (10)
SHR	1496 ± 118 (9)	25,672 ± 1480 (10)
SHR-SP	1445 ± 118 (9)	23,724 ± 2536 (10)

* Enzyme activity was measured as described in Material and Methods. Animals were 3-month-old rats. The blood pressure in WKR, SHR and SHR-SP was 127 ± 3, 178 ± 3 and 196 ± 4 respectively. Values are expressed as the mean ± S.E. The number of animals is given in parentheses.

DISCUSSION

Factors which regulate the level of taurine in tissues, as well as the physiological role of this compound, are unknown. While one would suspect that any influence of taurine on blood pressure would occur via an effect on neuronal systems, it appears that the most significant differences in taurine metabolism occur in the liver. In this regard it is of interest to note that, while small increases in taurine were noted in all tissues, the only statistically significant increases in tissue taurine content after chronic feeding occurred in the brain. It has been reported [19] that taurine turns over very slowly in brain, with a half-life of 24 days. The rather slow onset of the depressor effect and the small increment in brain levels after chronic treatment suggest the central nervous system (CNS) as a possible site of action. It has yet to be determined whether taurine is effective in reducing blood pressure in older animals that have established hypertension. These studies possibly have a relation to human essential hypertension since two groups have reported that taurine can lower blood pressure in patients with essential hypertension [20–22].

The observation that the activity of cysteic acid decarboxylase is similar in the liver of WKR and SHR-SP, even though the latter strain has considerably less taurine in the liver, suggests that this enzyme may not be rate limiting for the synthesis of

taurine. It is also possible that taurine is synthesized by an alternative pathway or that it is metabolized more quickly in the SHR-SP. Since the turnover of taurine is more rapid in liver ($T_{1/2} < 1$ day) than in brain, it is not too surprising that 1 day after removing a diet containing taurine there were no differences in the taurine content of liver.

In contrast to the findings reported above, Huxtable and Bressler [23] reported that taurine content of heart was increased significantly in the SHR. The reason for this difference is not known.

The current studies provide an example of the potential interaction of genetic and environmental factors in the etiology of a disease. It is interesting that the SHR have only a slightly lower taurine content of the liver and respond only moderately to taurine treatment. The SHR-SP, a substrain of the SHR, have genetic information for a more severe form of hypertension. These animals have a lower liver content of taurine and respond more dramatically to taurine in the diet. The blood pressure in SHR-SP treated with taurine does not fall below similarly treated SHR, suggesting that the altered gene(s) in the stroke-prone strain may be related to taurine metabolism.

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