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Effects of high salt diets and taurine on the development of hypertension in the stroke-prone spontaneously hypertensive rat

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Summary. Taurine is present in high concentrations in mammalian tissues and has been implicated in cardiovascular control mechanisms. The aim of the present study was to evaluate the ability of taurine to attenuate salt-induced elevations in blood pressure and markers of damage to the kidney and cardiovascular system in stroke prone spontaneously hypertensive rats (SPSHR). Male SPSHR (6 weeks old) were placed on high salt diets that contained 1% (w/w) NaCl added to their normal chow for 84 days and then were switched to 3% added NaCl for the remaining 63 days of the study. SPSHR was given 1.5% taurine in the drinking water (n = 8), a taurine free diet (n = 8) or normal chow (n = 8). A final control group (n = 6) was not given high salt diets. High salt diets caused an acceleration in the development of hypertension in all groups. Taurine supplementation reduced ventricular hypertrophy and decreased urinary excretion of protein and creatinine. The taurine free diet did not alter serum or urinary excretion of taurine, but did result in elevated urinary nitrogen excretion, increased serum cholesterol levels, and impaired performance in a spatial learning task. Alterations in dietary taurine intake did not alter urinary or serum electrolytes (Na^+, K^+), but taurine supplementation did attenuate a rise in serum calcium seen with the high salt diets. Urinary excretion (μg/24 h) of epinephrine and dopamine was significantly reduced in SPSHR given 1% NaCl in the diet, but this effect was not seen in SPSHR on taurine free or supplemented diets. Taurine supplementation showed cardioprotective and renoprotective effects in SPSHR given high salt diets.

Keywords: Amino acids – Taurine – Hypertension – Stroke prone spontaneously hypertensive rats – Catecholamines – Salt-induced hypertension

Introduction

Taurine (2-aminoethane sulfonic acid) is an amino acid that has been implicated in a number of important cardiovascular functions which include:

osmoregulation (Huxtable, 1992), electrolyte balance (Meldrum et al., 1994), serum cholesterol regulation (Murakami et al., 1996a, b) and blood pressure regulation (Trachtman et al., 1989). A reduced renal excretion of taurine has been associated with diminished dietary intake of taurine and an increased risk for ischemic heart disease in man (Yamori et al., 1996). Increased dietary intake of taurine has been shown to reduce blood pressure in a number of experimental rodent models of hypertension (Nara et al., 1978; Sato et al., 1987; Yamamoto et al., 1985; Ideishi et al., 1994). Many of these rodent models of hypertension are associated with increased sensitivity to salt-induced exacerbation of hypertension. The exact mechanisms whereby taurine modulates cardiovascular function are not totally understood, but may involve intracellular regulation of calcium (Ristori and Verdetti, 1991; Huxtable, 1992), renal mechanisms (Inoue et al., 1988; Mozaffari et al., 1997) or modulation of neurohumoral mechanisms (Sato et al., 1987; Abe et al., 1987; Ideishi et al., 1994; Zhu et al., 1998). Taurine may also be involved in long-term structural adaptation to high blood pressure via its ability regulate cytokine-mediated growth signals (Studer et al., 1997).

The present study examined how high salt diets and dietary taurine manipulations would influence the development of hypertension in SPSHR. Elevated dietary intake of salt was expected to produce a sustained augmentation of blood pressure in the SPSHR. It was hypothesized that taurine would attenuate the rise in blood pressure and reduce markers of cardiac and renal damage. This study also examined the role of catecholamines in salt-induced hypertension in SPSHRs.

Materials and methods

Animals

Breeding stock (3 males and 5 females) of stroke prone spontaneously hypertensive rats (SHRSP/A3N) were obtained from the Genetic Resource Section of the National Institues of Health (Bethesda, MD). The pups (F1 generation) from the breeding stock were bred to generate a sufficient number of male SPSHR (F2 generation) for use in this study. The pups were weaned at 25 days of age and group housed according to sex. Six-week old male SPSHR were assigned to 4 different dietary conditions. One group (N = 6) was given Purina 5001 powdered rat chow (0.4% NaCl) and tap water to drink (normal salt controls-control/NS). The other 3 groups (N = 8 per group) were given powdered chow containing an additional 1% (w/w) sodium chloride for 84 days. The sodium chloride in the diet was increased to 3% (w/w) for an additional 63 days to produce a sustained increase in blood pressure and an acceleration in the progression of pathophysiological changes. These groups will be referred to as the high salt (HS) groups. One HS group was given Purina 5001 powdered diet and tap water to drink while the other group got the same diet but 1.5% taurine (w/v) added to the drinking water. The final HS group got tap water to drink and a modified Purina diet (5729C-M) that had soybean meal substituted for animal protein to create a taurine free diet. The food was available in glass feeding jars and drinking fluids were also available *ad libitum*. Food and fluid intake was measured weekly for the duration of the study. Animal studies were conducted under approved guidelines and were reviewed by an institutional animal use committee.

Basic experimental design

Blood pressure was measured biweekly using the tail cuff method as previously described (Dawson and Wallace, 1990). The rats were housed in hanging metal cages except for 3-day periods when metabolic studies were performed. The first 24 hours was used to acclimate the rats to the metabolic cages and measurement procedures. The second 24-hour period was used for the collection of data on food and water intake and urine output. The third 24-hour period was used to collect additional measures of food and water intake and a 24-hour urine sample for catecholamine analysis. The urine tubes for catecholamine collections contained 250 μl of 1% (w/v) EDTA in 5 N HCl. The first metabolic study was conducted 55 days after the start of the 1% NaCl diets and the second metabolic study was performed after 50 days on the 3% NaCl diets (days 133–135 of the study). Activity measures were performed using an automated photocell device (Omnitect Electronics, Inc. Digiscan Monitor) on days 77–78 of the study. The rats on the 1% NaCl diets were also tested (study days 33–36) for cognitive function using the Morris Water Maze (Morris, 1981). The rats were sacrificed by decapitation after 147 days on the diets. Serum samples were collected and the ventricles were removed and weighed to index cardiac hypertrophy. The striatum, kidney, femoral artery, adrenals, spleen and ventricle were collected for taurine determination. Tissue taurine content was assayed by HPLC as previously described (Dawson and Wallace, 1992). Figure 1 summarizes the overall experimental design.

Urine analysis and serum assays

Sodium and potassium were determined in urine and serum using an ion sensitive electrode (Nova Analyzer) as previously described (Meldrum et al., 1994). Total serum calcium was measured using a spectrophotometric technique (Stanbio Laboratory, San Antonio, TX). Urine samples were acidified and assayed for total calcium as described for serum. Urea nitrogen and creatinine were also determined in urine using commercially available kits (Stanbio Laboratory, San Antonio, TX). Serum creatinine values were also determined as indicated above for the urine samples. Glucose in serum and urine were assayed by a colormetric procedure (Stanbio Laboratory, San Antonio, TX). Total serum cholesterol was measured using an assay kit (Stanbio Laboratory, San Antonio, TX). Total serum and urinary protein were measured using the method of Bradford (1976). Serum amino acids were assayed by HPLC as previously described (Patterson et al., 1995). Urinary catecholamines (norepinephrine, epinephrine and dopamine) were extracted and assayed by high performance liquid chromagraphy with electrochemical detection (HPLC-EC) as previously described (Kontur et al., 1985; Dawson and Wallace, 1990). Urine was deproteinized with 9 volumes of methanol and centrifuged in a micofuge for 2 minutes. The urinary amino acids in the methanolic supernatant (glycine, alanine and taurine) were measured by HPLC-EC after precolumn derivatization with OPA as previously described (Dawson et al., 1999).

Behavioral measures

The SPSHR exhibits alterations in locomotor activity and cognitive function and has been suggested as an animal model of vascular dementia (Minami et al., 1997). Therefore, behavioral measures were used to assess potential CNS pathophysiology associated with elevated blood pressure and to determine if dietary taurine supplementation or restriction would alter these measures. Locomotor activity was measured after 77–78 days on the diets using an automated photocell device (Omnitech Electronics Inc.). The rats were tested in their colony room by transferring them to a standard plastic tub cage and measuring locomotor activity for 3 consecutive 5 minute blocks. The groups were ran in a counterbalanced fashion by alternating rats from each of the 4 dietary conditions. Testing was conducted from 10 am until 3 pm for 2 consecutive days. Prolonged hypertension can result in cognitive impairment (Starr and Whalley, 1992). Learning and

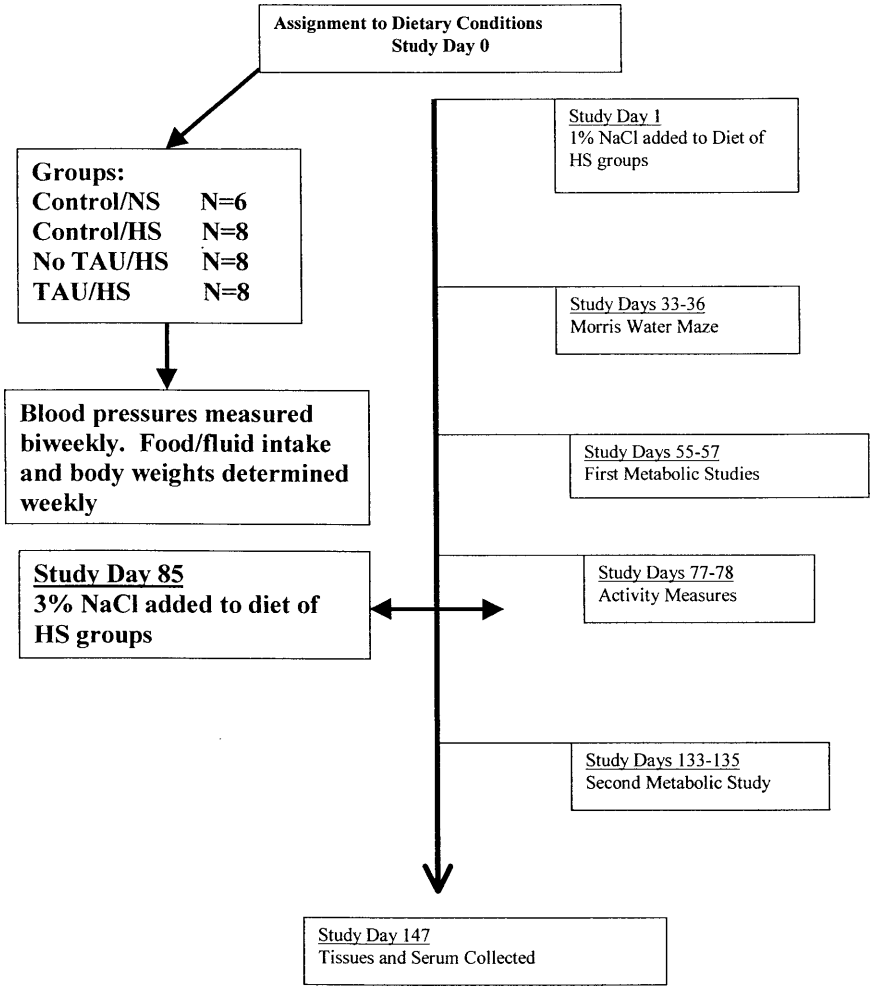

Fig. 1. An overview of the experimental design and timeline for specific experiments in this study

memory function was assessed in SPSHR on the high salt diets using the Morris water maze (Morris, 1981). The Morris water maze is a spatial learning task that requires rats to swim and locate a platform 2 cm below the surface of water made opaque with crayola powder paint. Briefly, the rats were given 8 training trials (60 sec/trials) per day for 3 consecutive days in the water maze. A probe trial was performed on the 4th day with the platform removed and latency to cross the platform area, number of platform crossings, and time spent in quadrants were recorded.

Statistical analysis

Blood pressure and body weight data were subjected to repeated measures analysis of variance (ANOVA). The blood pressure curves for the entire study were integrated and transformed into area under the curve (AUC) measures for each group. The biochemical and other data were analyzed by ANOVA to test for the effects of the various diets. Planned comparisons of individual group mean differences were performed using the Newman-Keuls test. When appropriate, nonparametric statistics (Kruskal-Wallis/Dunn's

Multiple Comparison test) were used. The statistical analyses were performed using GraphPad Prism Version 2.0 software (San Diego, CA).

Results

Body weights and measures of food and fluid intake

Three SPSHR (2 no taurine/HS and 1 control/HS) died after 30 days on the 1% NaCl supplemented diet. There was no further mortality in any of the groups for the duration of the study. There were no differences in body weights (g) among the groups after 83 days on the 1% NaCl diet (Table 1). Likewise, the body weights at the end of the study (63 days on the 3% NaCl diet) were not different among the groups (Table 1). Thus, the high salt diets or dietary manipulation of taurine intake did not appear to alter growth or body weights. Food and fluid intake measures taken during the metabolic studies are presented in Table 1. When compared to rats on the normal chow diets, food intake was significantly lower in the rats on the 1% added salt diet that contained no taurine (Table 1). The rats on the HS diets (3% NaCl) that consumed either the no taurine or taurine supplemented diets ate significantly less than the rats on the normal chow diets. Taurine supplemented water intake was not different from the other 2 groups on the high salt diets. Fluid intake was elevated 1.4–1.7 fold by the addition of 3% NaCl to the standard chow diet (Table 1).

Serum, urinary and tissue levels of taurine

Serum taurine concentrations are presented in Fig. 2A. Serum taurine was elevated over 2 fold by supplementation of the drinking water with 1.5%

Table 1. Food intake, fluid intake, urine output and body weights in SPSHR on high salt diets

Measures	Control/NS†	Control/HS	No taurine /HS	1.5% Taurine/HS
Food intake (g)				
1% NaCl	22.5 ± 0.88	20.0 ± 0.65	19.0 ± 0.59*	20.8 ± 0.96
3% NaCl	22.2 ± 0.52	21.0 ± 0.61	18.8 ± 0.30*	19.6 ± 0.74*
Fluid intake (ml)				
1% NaCl	37.4 ± 2.2	35.0 ± 1.0	34.9 ± 1.3	39.6 ± 2.0
3% NaCl	39.3 ± 1.6	66.5 ± 3.3*	55.5 ± 3.5*	60.9 ± 2.6*
24H Urine output (ml)				
1% NaCl	13.6 ± 1.1	13.6 ± 0.6	14.8 ± 1.2	16.2 ± 1.4
3% NaCl	16.0 ± 1.4	35.1 ± 2.8*	32.7 ± 2.8*	30.6 ± 3.2*
Body weight (g)				
1% NaCl	270 ± 9	269 ± 7	269 ± 6	278 ± 3
3% NaCl	308 ± 8	292 ± 10	296 ± 8	303 ± 3

† This group remained on normal chow diets for all measures. Body weights reported were determined after 83 days on the 1% NaCl diet and after 63 days on the 3% NaCl diet. * $p < 0.05$ versus control/NS group.

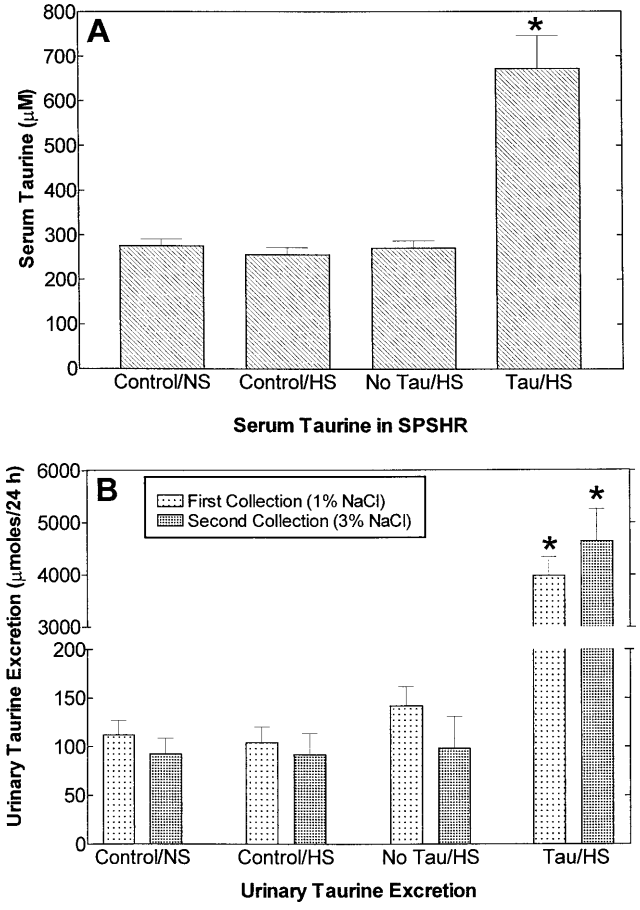

Fig. 2. The effect of high salt diets and manipulation of dietary taurine intake on serum taurine concentration (**A**) and urinary taurine excretion (**B**). Dietary supplementation with 1.5% taurine in the drinking water significantly (*$p < 0.001$) elevated serum taurine concentration (**A**) and urinary taurine elimination (**B**). The taurine free diet (*No Tau HS*) had no effect on serum or urinary taurine concentration

taurine. High salt intake did not alter serum taurine content. Serum threonine was significantly ($p < 0.01$) lower (~25%) in taurine supplemented rats than the other groups. Other amino acids measured (GLU, ASN, SER, GLN, ARG, GLY or ALA) were not altered by the high salt diet or taurine free diet (data not shown). Urinary taurine excretion in SPSHR was not affected by either the 1% (first collection) or 3% (second collection) NaCl addition to the diet (Fig. 2B). Urinary alanine and glycine excretion was also not altered by high salt diets (data not shown). Taurine supplementation caused an over 30 fold elevation in urinary taurine excretion (Fig. 2B). The taurine free diet did not induce an increase in renal reabsorption of taurine since there was no significant change in urinary elimination. Tissue levels of taurine are presented in Table 2. Long-term dietary supplementation with 1.5% taurine produced only modest changes in tissue content of taurine (Table 2). The

Table 2. Tissue taurine content in SPSHR

TISSUE	Control/NS	Control/HS	No taurine/HS	Taurine/HS
Kidney	10.39 ± 0.36	9.26 ± 0.43	10.22 ± 0.51	14.58 ± 1.41*
Spleen	10.36 ± 0.71	12.60 ± 0.47	11.33 ± 0.76	13.05 ± 1.10
Adrenal	5.52 ± 0.41	5.29 ± 0.20	5.26 ± 0.29	6.03 ± 0.53
Femoral artery	6.59 ± 0.31	6.46 ± 0.61	6.73 ± 0.68	13.77 ± 3.45
Ventricle	26.88 ± 1.30	26.00 ± 1.97	29.26 ± 1.83	32.57 ± 0.47*+
Striatum	6.09 ± 0.30	6.07 ± 0.15	6.15 ± 0.35	6.83 ± 0.27

All values expressed as μmoles/g tissue weight. *$p < 0.05$ versus control/HS. +$p < 0.05$ versus control/NS.

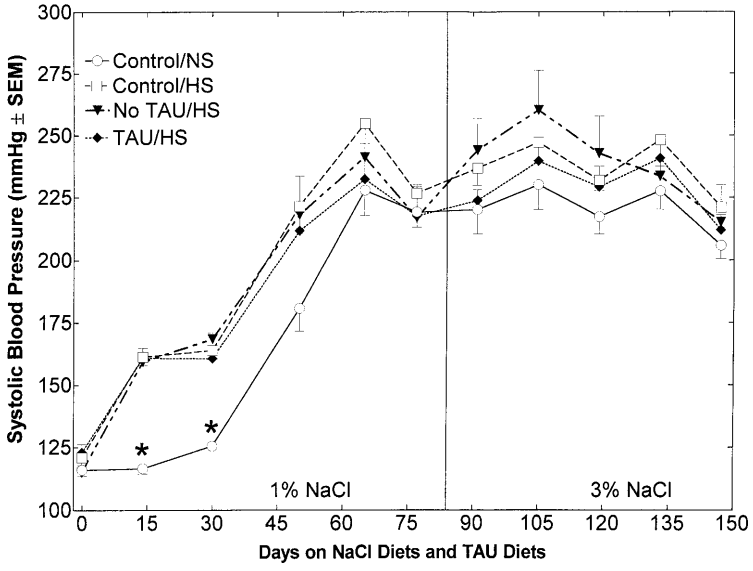

Fig. 3. Blood pressure in SPHSR given high salt diets. SPSHR given regular rat chow with no added salt had significantly (*$p < 0.05$) lower blood pressure than the SPSHR given 1% NaCl in the diet for the initial 30 days of the study. Dietary taurine supplementation or restriction had no statistical effect on blood pressure, however, mean blood pressures were lower in the taurine supplemented SPSHR for the duration of the study when compared to the other groups given high salt diets

kidney content of taurine was elevated about 37% above SPSHR on high salt diets. Taurine supplementation also elevated ventricular content of taurine. The taurine free diet or high salt diets failed to alter tissue content of taurine.

Blood pressure changes

The systolic blood pressures of the rats throughout the course of the study are presented in Fig. 3. The blood pressure of the rats on normal chow diets were significantly lower on days 15 and 30 of the study, but converged with the high

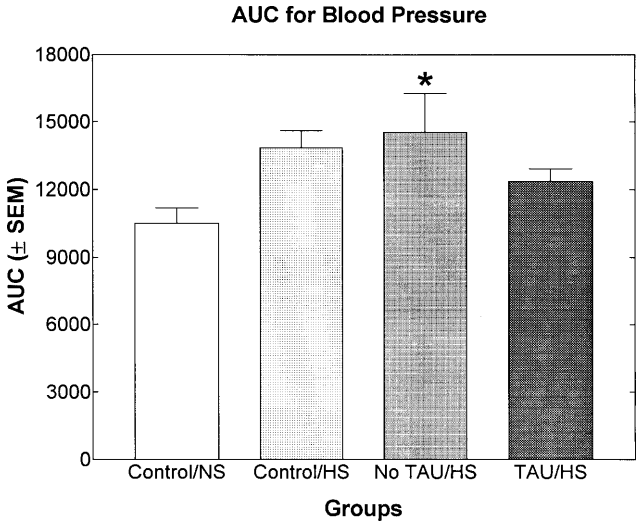

Fig. 4. The blood pressure measurements for the entire study (data from Fig. 1) were integrated into area under the curve (*AUC*) values for the individual groups. The SPSHR on the taurine free diet had a significantly (*$p < 0.05$) greater AUC value than control SPSHR given diets without added NaCl

salt diet groups at later measurements. The addition of 1% NaCl to the diet appeared to accelerate the rise in blood pressure (Fig. 3). The modification of dietary taurine intake did not significantly alter systolic blood pressure, however, the taurine supplemented group tended to have lower mean blood pressures than the control/HS and no taurine/HS groups for the duration of the study. When the systolic blood pressures for the whole study were converted to an area under the curve (AUC) measurement, the no taurine/HS group had a significantly greater AUC than the control rats given normal rat chow (Fig. 4). It is also important to note that there was no mortality in the SPSHR on normal salt diets or with taurine supplementation.

Urinary volume, creatinine excretion and catecholamines

The 24-hour urinary output of SPSHR on either the 1% or 3% NaCl diets are presented in Table 1. The modification of taurine intake or addition of 1% NaCl to the diets had no significant effect on 24 hour urine output (Table 1). The addition of 3% NaCl to the diet increased urinary volume in all 3 dietary groups significantly above that of SPSHR on normal salt diets (Table 1). Urinary creatinine excretion was significantly lower in taurine supplemented rats on high salt diets (1% or 3%) than the rats on normal salt diets (Fig. 5). Urinary catecholamine [norepinephrine (NE), epinephrine (EPI) and dopamine(DA)] excretion was measured over a 24 hour period and the data are expressed as both μg/24 hours and ng/mg creatinine. The 1% NaCl addition to the diets had no effect on urinary NE excretion (Fig. 6A), however, taurine supplementation increased NE excretion above that of SPSHR given the control HS diets (Fig. 6B). The 3% NaCl diet did not alter urinary NE

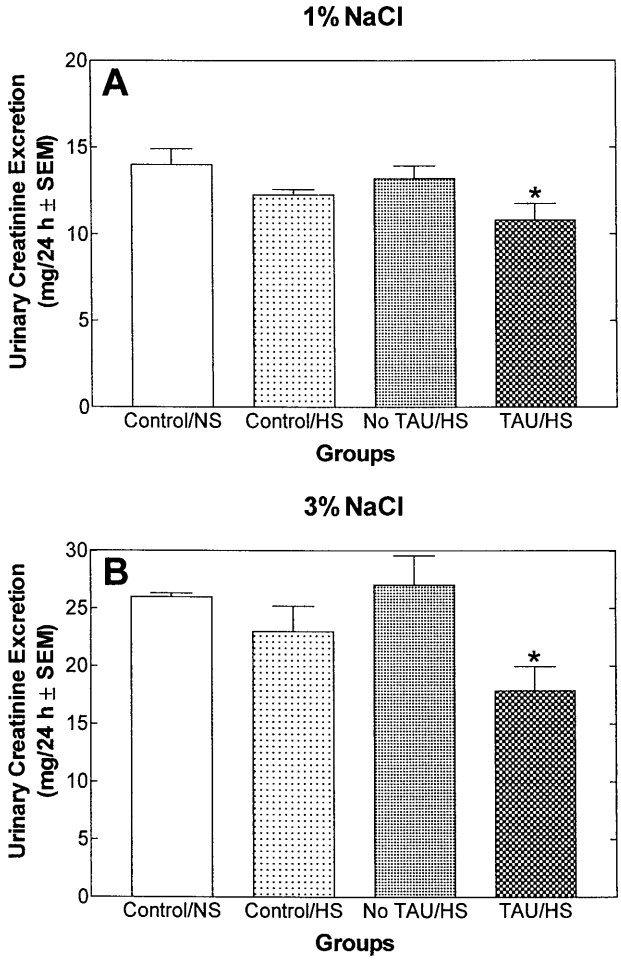

Fig. 5. Urinary creatinine excretion in SPSHR given 1% (**A**) or 3% (**B**) added NaCl in the diet. SPSHR given taurine supplementation had significantly (*$p < 0.05$) lower creatinine excretion than SPSHR on the normal salt diets

excretion (μg/24 hours), although there was a trend for NE excretion to be lower in all SPSHR on the 3% NaCl diet compared to the normal diet group (Fig. 6C). Urinary excretion of NE was higher in taurine supplemented SPSHR compared to the control/HS and no taurine/HS diet groups (Fig. 6D). EPI excretion was (μg/24 hours) significantly lower in control/HS rats on 1% NaCl when compared to control rats on normal chow diets (Fig. 7A). Taurine supplemented SPSHR had significantly elevated EPI excretion (ng/mg creatinine) compared to control SPSHR on HS diets (Fig. 7B). EPI excretion (μg/24 hours) did not differ among the groups (Fig. 7C), whereas creatinine normalized EPI excretion was higher in the taurine supplemented group relative to the SPSHR on normal diets and taurine free diets with 3% NaCl (Fig. 7D). Urinary dopamine excretion (μg/24 hours) was significantly lower in SPSHR controls on the 1% NaCl diet compared to any of the other groups (Fig. 8A). Urinary dopamine excretion normalized to creatinine was

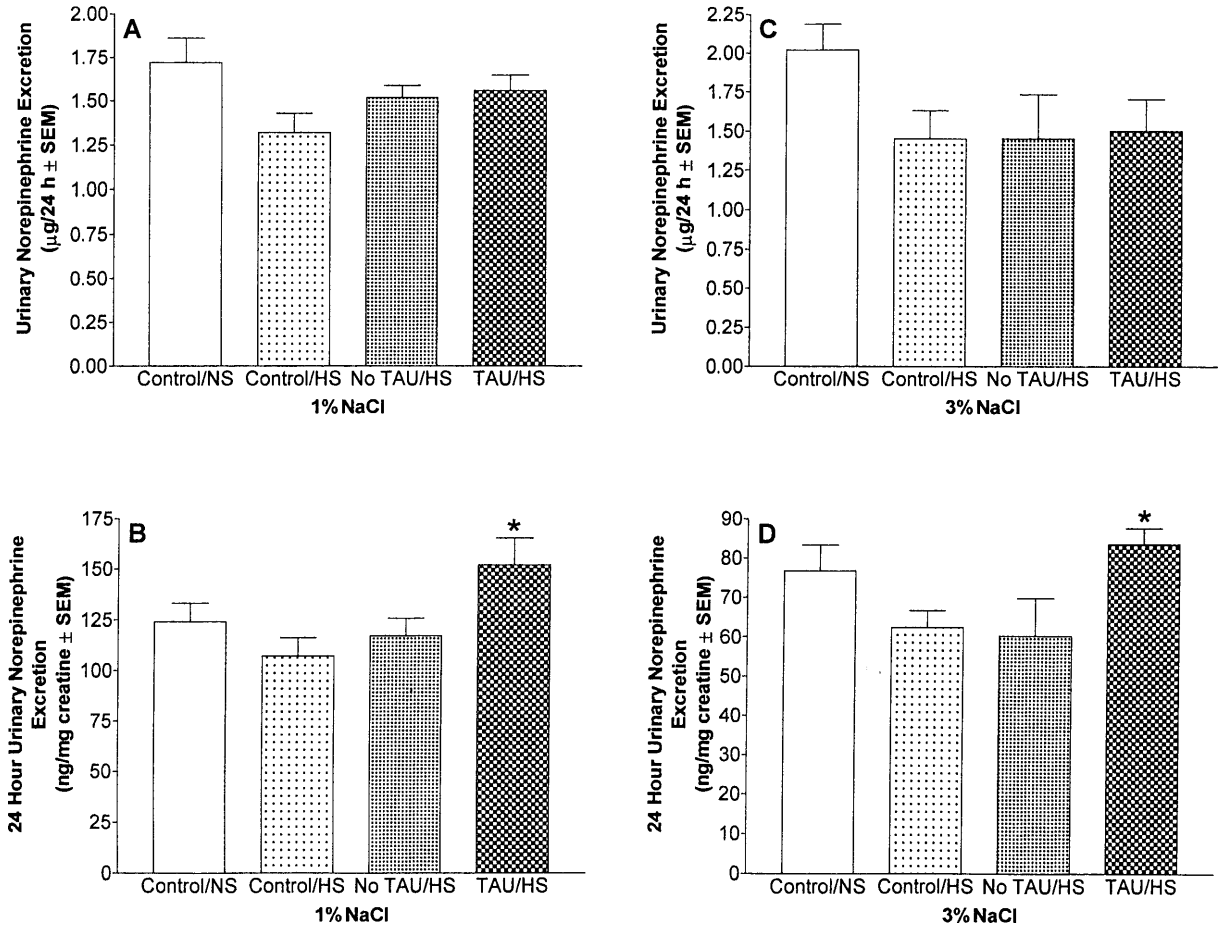

Fig. 6. Urinary norepinephrine excretion in SPSHR. Norepinephrine excretion tended to be reduced by high salt diets (**A** and **C**) when expressed as μg/24 hours, but the overall ANOVA was not significant. Urinary norepinephrine excretion normalized to creatinine excretion was significantly (*$p < 0.05$) higher in SPSHR supplemented with taurine (**B** and **D**) when compared to the other SPSHR on high salt (*HS*) diets

significantly higher in taurine supplemented rats compared to control SPSHR on the 1% NaCl diet (Fig. 8B). Dopamine excretion (μg/24 hours) was similar among groups irrespective of dietary condition when given 3% NaCl (Fig. 8C). Taurine supplementation increased creatinine normalized urinary DA excretion significantly above that of SPSHR on control/HS diets (Fig. 8D). The lower overall creatinine excretion in the taurine supplemented SPSHR most likely explains the group differences when the data were normalized by this index of renal clearance.

Urinary electrolyte, protein, glucose and nitrogen excretion

Results of the measures of urinary electrolyte excretion are presented in Table 3. As expected, urinary sodium excretion was elevated by the addition

Table 3. Effects of high salt diets and taurine on urinary electrolyte excretion

Group	Na^+ Excretion		K^+ Excretion		Ca^{++} Excretion	
Control NS diet	2.67 ± 0.16	2.65 ± 0.15	4.24 ± 0.25	4.09 ± 0.19	1.68 ± 0.33	3.69 ± 0.15
High salt diets	**1% NaCl**	**3% NaCl**	**1% NaCl**	**3% NaCl**	**1% NaCl**	**3% NaCl**
Control HS diet	4.70 ± 0.39*	10.22 ± 1.10*	3.74 ± 0.16	3.89 ± 0.34	0.86 ± 0.07	5.89 ± 0.52
No taurine diet	4.46 ± 0.34*	7.28 ± 0.67	3.96 ± 0.17	4.60 ± 0.10	1.30 ± 0.21	5.36 ± 0.38
1.5% Taurine	4.04 ± 0.46	9.87 ± 0.85*	3.73 ± 0.27	3.72 ± 0.27	2.02 ± 0.50	6.34 ± 0.92

Na^+ and K^+ excretion expressed as mmol/24 h. Calcium excretion expressed as mg/24 h. * $p < 0.05$ versus controls on normal salt diets.

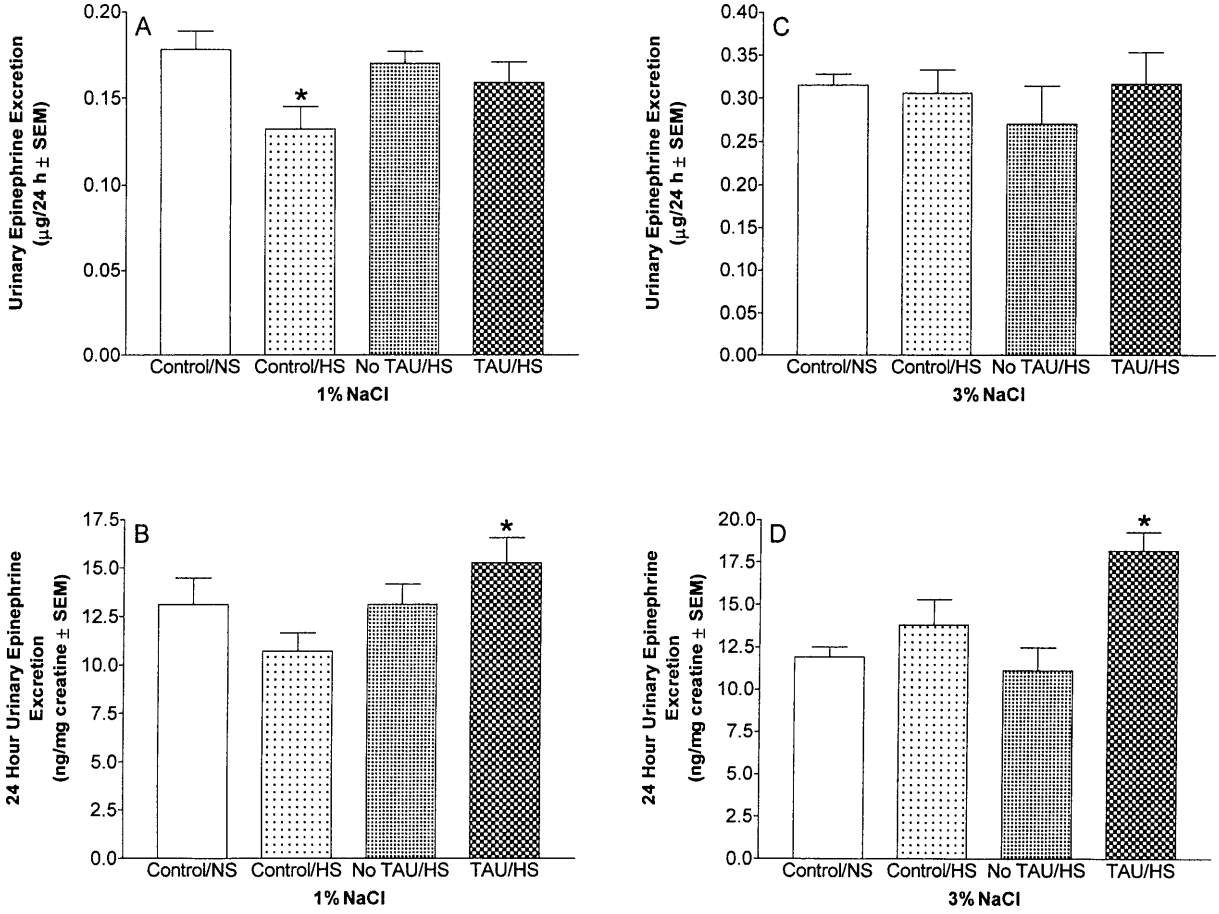

Fig. 7. Urinary epinephrine excretion in SPSHR. Epinephrine excretion (μg/24 hours) was significantly (*$p < 0.05$) lower in SPSHR given 1% NaCl addition to the regular chow diets compared to SPSHR not given added NaCl (**A**). Epinephrine excretion normalized to creatinine was significantly (*$p < 0.05$) elevated in the taurine supplemented groups when compared to the control/HS group (**B**) and to both the control/NS and no taurine HS groups (**D**)

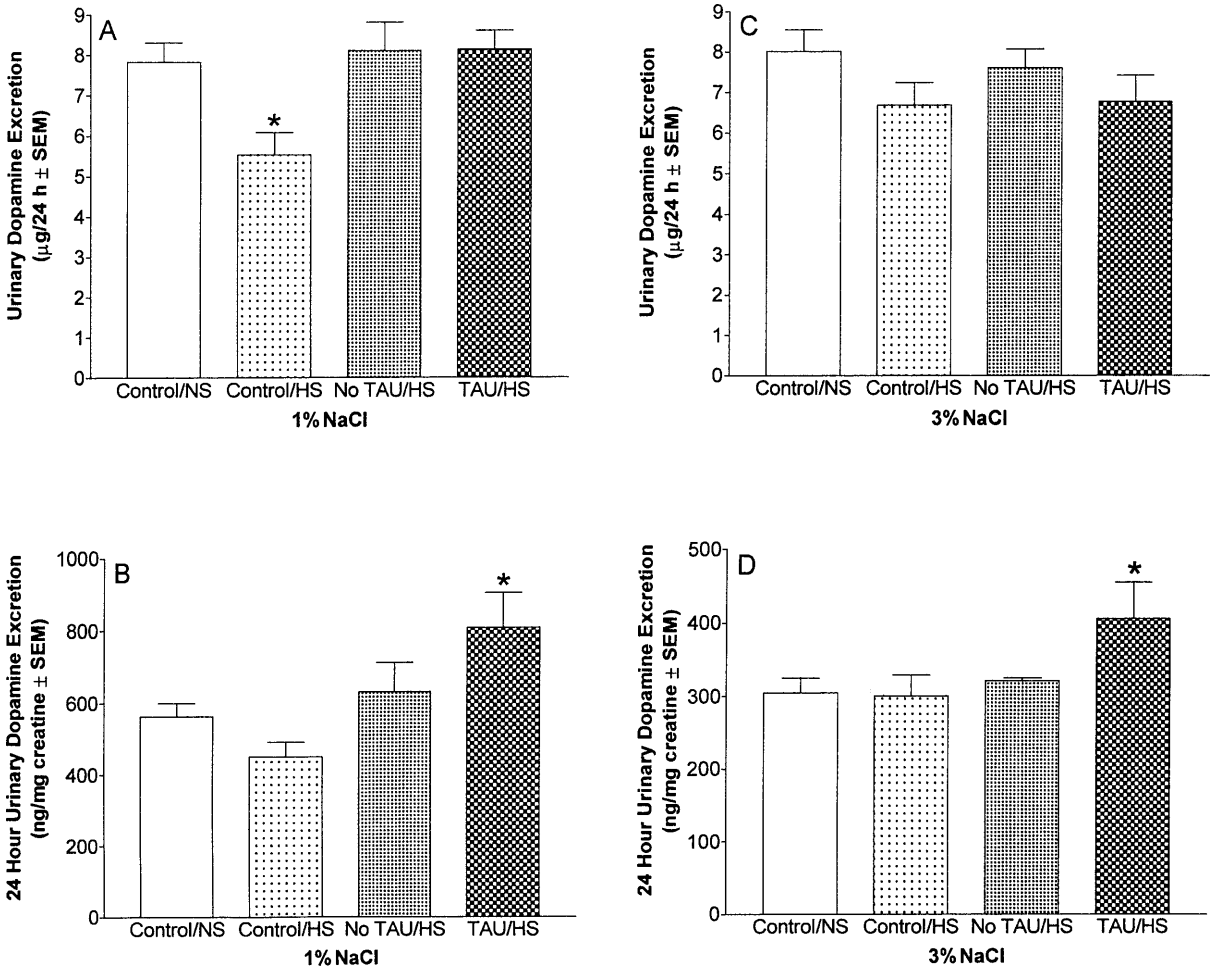

Fig. 8. Urinary dopamine excretion in SPSHR. Compared to the other groups, urinary dopamine excretion (μg/24 hours) was lower (*$p < 0.05$) in the control/HS SPSHR (**A**). Creatinine normalized dopamine excretion was higher (*$p < 0.05$) in taurine supplemented SPSHR relative to the control/HS groups (**B** and **D**)

of 1 or 3% NaCl to the diets of SPSHR. There were no significant effects of the modifications in dietary taurine on sodium excretion. Overall there were no statistically significant effects of high salt diets or alterations in dietary taurine intake on either potassium or calcium excretion into the urine. In contrast, addition of NaCl to the diets of SPSHR caused an increase in urinary protein excretion, which is an index of renal damage (Fig. 9). The increase in protein excretion was significant for the SPSHR on control/HS diets and supplementation with taurine blunted the increase in urinary protein loss (Fig. 9). The taurine free diet also appeared to attenuate urinary protein loss on the 1% NaCl diet but not on the 3% NaCl diet (Fig. 9). Urinary glucose excretion was also increased significantly by 3% NaCl diets in SPSHR on the regular chow diet (control/HS), while glucose excretion was not significantly elevated

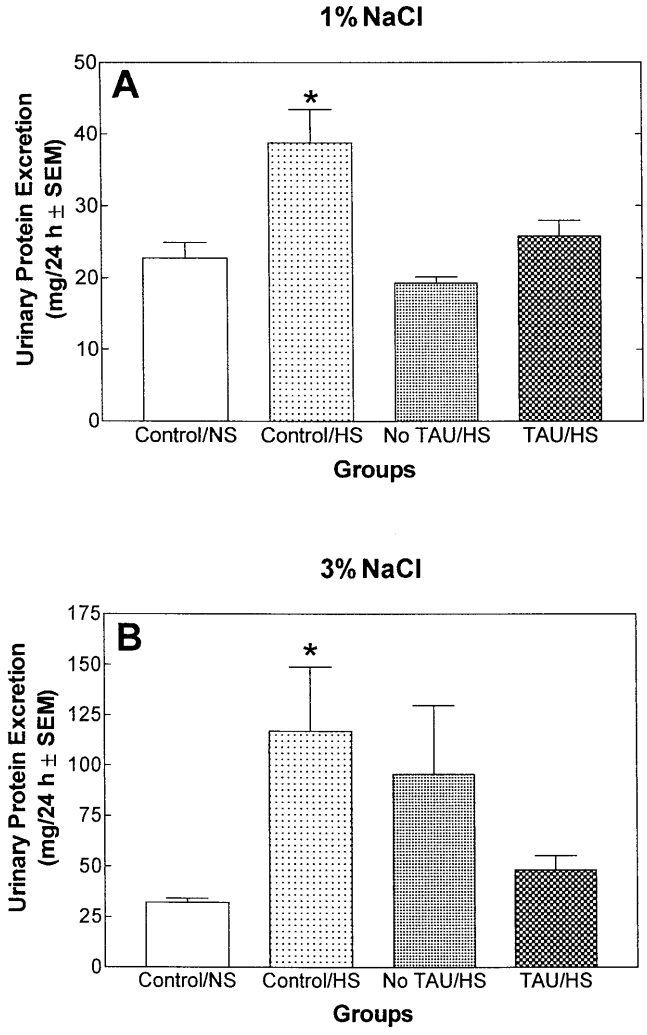

Fig. 9. Total urinary protein excretion in SPSHR. The addition of 1% NaCl to the diet resulted in a significant (*$p < 0.05$) increase in protein excretion in the urine, but this effect was blunted by the taurine free diet and by taurine supplementation (**A**). The prolonged exposure to high salt diets resulted in large increase in urinary protein excretion (note change in scale) in the SPSHR and the control/HS group showed a significant (*$p < 0.05$) increase in protein excretion relative to the SPSHR on the control diet (**B**). Taurine supplementation attenuated the increase in protein excretion (**B**)

in SPSHR on the taurine free diet or taurine supplemented diet (Fig. 10). Serum glucose was not significantly elevated in the control/HS group, but it was 10% higher than the other groups. Urinary excretion of nitrogen was significantly elevated by the combination of the 3% NaCl diet and the taurine free diet when compared to SPSHR given 1.5% taurine in the drinking water (Fig. 11B), an effect similar to that seen with creatinine excretion (Fig. 5).

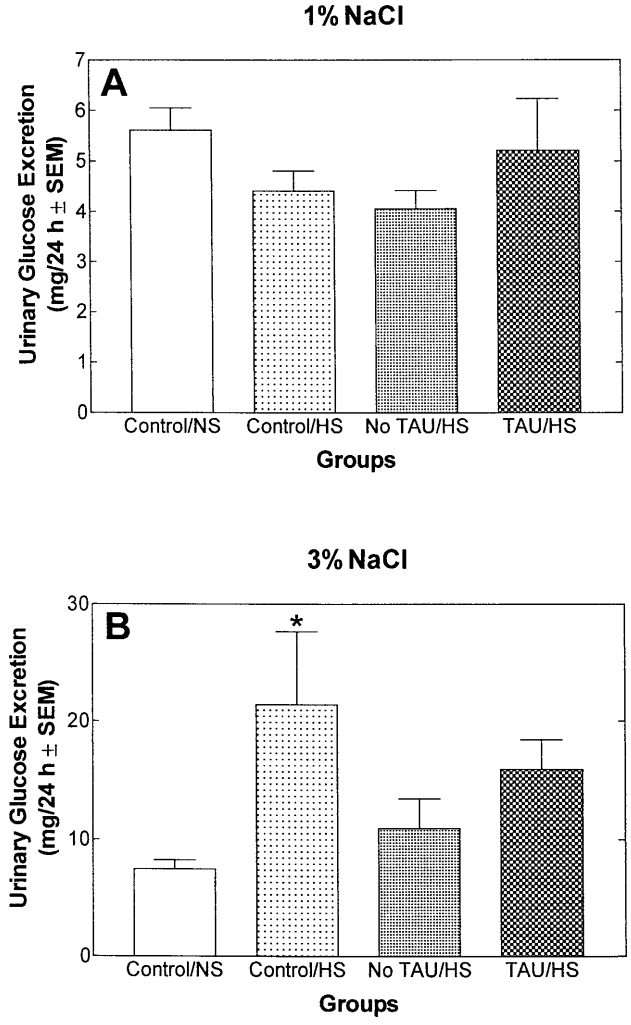

Fig. 10. Urinary glucose excretion in SPSHR. The initial metabolic study performed when the SPSHR were on the 1% NaCl diets did not reveal any differences in urinary glucose excretion related to salt intake or dietary taurine alterations (**A**). The second metabolic study performed after 135 days on the high salt diets when the SPSHR were on the 3% NaCl diet found that the control/HS group had a significant (*$p < 0.05$) elevation in glucose excretion relative to SPSHR on the diet with no added NaCl (control/NS) (**B**)

Serum measures

NaCl or dietary taurine manipulations did not have major effects on serum sodium, potassium, glucose and total protein (data not shown). Serum calcium content was not different between control SPSHR on normal diets (8.55 ± 0.70 mg/dl) and taurine supplemented SPSHR on 3% NaCl diets (8.51 ± 0.37). SPSHR on high NaCl diets had significantly higher serum calcium levels (9.94 ± 0.23) than SPSHR that received taurine supplementation. The SPSHR given the taurine free diets also had higher serum calcium (9.46 ± 0.21), but they did not differ statistically from the other groups. Serum total cholesterol

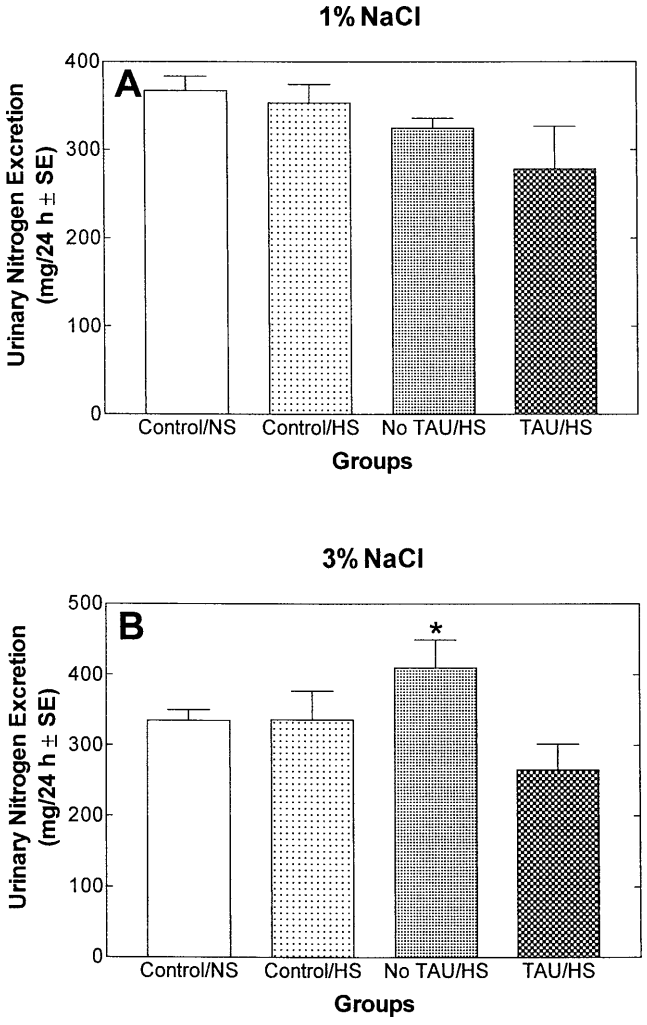

Fig. 11. Urinary nitrogen excretion in SPSHR. Urinary nitrogen excretion was not altered by 1% NaCl added to the diets or alterations in taurine intake (**A**). The prolonged treatment of SPSHR with high salt diets (3%) resulted in a significant (*$p < 0.05$) elevation in urinary nitrogen excretion in the SPSHR on the taurine free diet relative to taurine supplemented SPSHR (**B**)

was significantly elevated by the 3% NaCl diets in SPSHR on the taurine free diet but not in the other groups given the high salt diets (Fig. 12A). Despite a lower urinary excretion of creatinine in taurine supplemented SPSHR on high diets, there were no differences in serum creatinine values among the groups (data not shown).

Ventricular hypertrophy

Ventricular weights were divided by the body weight to index the degree of ventricular hypertrophy and these data are presented in Fig. 12B. Ratios of

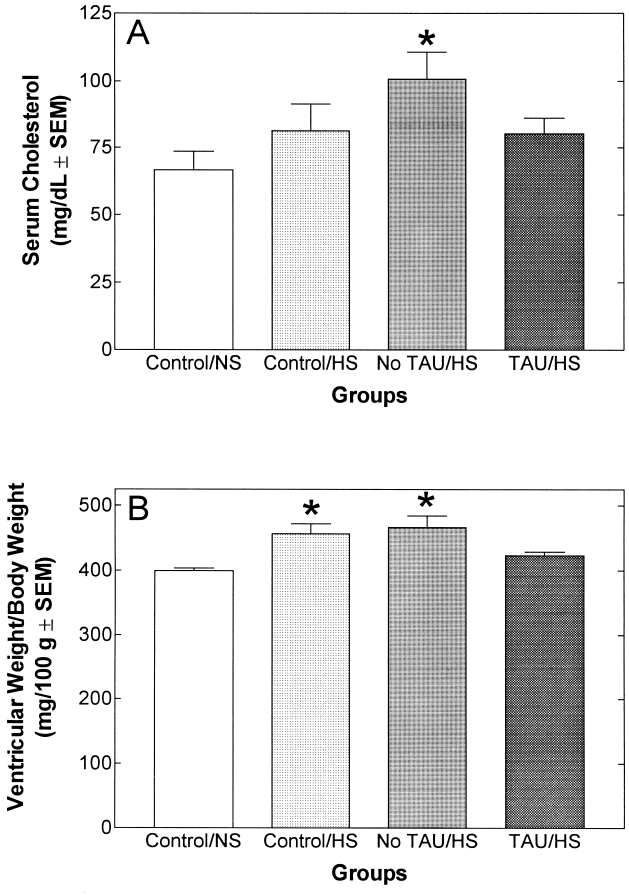

Fig. 12. Risk factors for cardiovascular disease in SPSHR related to total serum cholesterol (**A**) and cardiac hypertrophy indexed by ventricular weight normalized to body weight (**B**). Chronic intake of high salt diets combined with a taurine free diet resulted in a significant (*$p < 0.05$) elevation in total serum cholesterol in SPSHR relative to the control/NS group (**A**). The ratio of ventricular weight to body weight was significantly (*$p < 0.05$) elevated in both the control/HS and no taurine/HS groups relative to the control/NS group, while the taurine supplemented SPSHR did not show an increase

ventricular weight to body weight were significantly elevated in SPSHR by high salt diets with the exception of the taurine supplemented SPSHR (Fig. 12B).

Behavioral measures

Locomotor activity over the 15 minute period examined was not significantly altered by taurine supplementation or high salt diets (data not shown). Performance in the Morris water maze was significantly impaired in SPSHR on the taurine free diets (Fig. 13). The SPSHR consuming the taurine free diet had a longer latency to cross the target and had significantly fewer crossings of

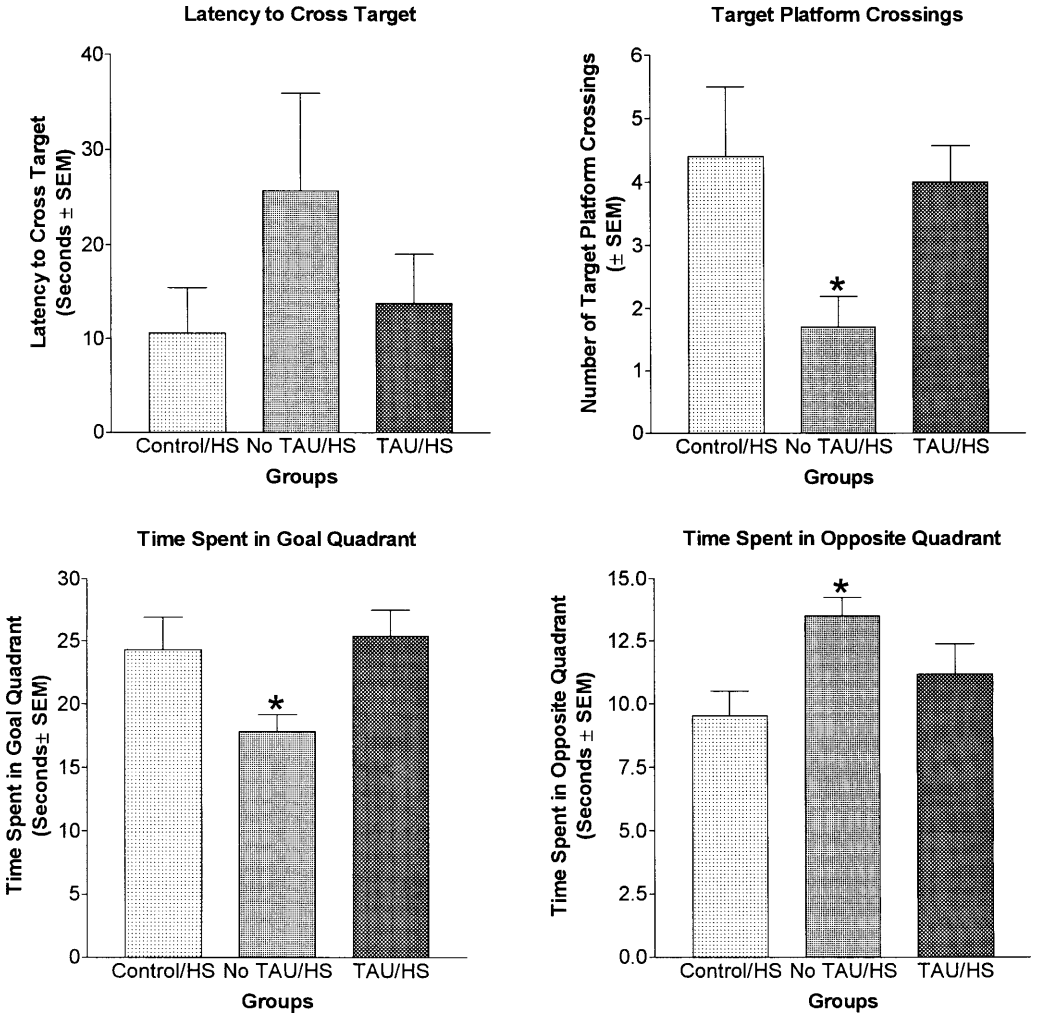

Fig. 13. Performance indicators in the Morris water maze. The SPSHR on the high salt diets were evaluated for their ability to learn a spatial memory task. The SPSHR given the taurine free diet exhibited significantly (*$p < 0.05$) impaired performance of this spatial memory task relative to the other groups. The no taurine/HS group had a slower latency to find the target and crossed over the location of the target fewer times. They also spent less time in the goal quadrant and more time in the incorrect quadrant

the target platform (Fig. 13). Likewise, the SPSHR on the taurine free diet spent significantly less time in the goal quadrant and significantly more time in the quadrant opposite to the goal quadrant (Fig. 13). The taurine supplemented SPSHR did not differ in any measure of maze performance from SPSHR on the normal/HS chow.

Discussion

In contrast to a previous study (Nara et al., 1978), dietary taurine supplementation did not statistically reduce systolic blood pressure in SPSHR

on high salt diets. Nara et al. (1978) used 3% taurine in the drinking water of SPSHR on normal diets, but a significant reduction in blood pressure was not evident until after 50 days of treatment. In our study the addition of 1% sodium chloride to the diet appeared to produce a much steeper rise in blood pressure than SPSHR on regular rat chow (Fig. 3). Thus, the higher blood pressures and lower (1.5% taurine) daily taurine intake may well account for our failure to detect a significant reduction in blood pressure. A major finding of this study was that taurine could reduce markers of cardiac hypertrophy and renal damage despite failing to produce a significant reduction in blood pressure. The taurine treated SPSHR tended to have lower blood pressures than the other groups. This is best represented in the AUC measures for the groups (Fig. 4) where dietary taurine supplementation appeared to modestly blunt the effects of the high salt diets. This was also clearly demonstrated by the ability of taurine to attenuate the effects of the high salt diets on ventricular hypertrophy. Furthermore, taurine also had a renoprotective action to blunt the effects of high salt diets to increase urinary protein loss presumably associated with glomerular damage. The renoprotective effects of taurine have previously been demonstrated in several models of hypertension (Trachtman et al., 1989; Ideishi et al., 1994) and experimental renal damage (Trachtman et al., 1992; Trachtman et al., 1993a; Venkatesan et al., 1997). Chronic intake of high salt diets also elevated glucose excretion and the magnitude of the glucose loss was not as great in SPSHR that were taurine supplemented or given taurine free diets. The high salt diets or manipulation of dietary taurine intake did not, however, alter serum glucose.

Taurine free diets did not reduce serum, urinary or tissue taurine concentrations. This is most likely due to the high capacity of rats to synthesize taurine from its amino acid precursors, methionine or cysteine. Previously, it had been suggested that SHR had a reduced capacity to synthesize taurine (Kuriyama et al., 1986). The heart, which does not have a great capacity for taurine synthesis, also maintained a normal taurine concentration even in the rats on the taurine free diet. This suggests that taurine transporters may have been upregulated in response to long-term dietary taurine deprivation. The taurine free diet had to be formulated from plant proteins since animal protein is a rich source of taurine. Studies in aged rodents have shown that diets from plant derived protein sources can reduce age-related nephropathy associated with the intake of diets based on animal protein sources (Shimokawa et al., 1993). The taurine free diet may have had protective effects as indicated by the urinary protein and glucose data. It is unclear what other specific effects may have been produced by the soybean-based diet in this study. In contrast, the taurine free diet elevated serum cholesterol relative to the other groups. This effect could be associated with the lack of intestinal taurine absorption following food intake or inadequate hepatic taurine synthesis and the resulting decreased bile acid synthesis (Murakami et al., 1996a).

Taurine has convincingly been shown to inhibit peripheral sympathetic nervous system activity in the DOCA/salt model of hypertension (Fujita and Sato, 1986; Sato et al., 1987; Inoue et al., 1988). Elevated rates of tissue

norepinephrine turnover (Sato et al., 1987; Fujita and Sato, 1988) are reduced by taurine supplementation in the DOCA/salt model of hypertension. It is less clear how taurine interacts with catecholamines in the SHR and SPSHR models of hypertension. Trachtman et al. (1989) have suggested that taurine does not reduce elevated sympathetic tone in SHR since taurine failed to reduce plasma levels of norepinephrine. Yamamoto et al. (1985) reported that basal levels of plasma catecholamines in SHR were not decreased by dietary taurine supplementation, but stress evoked elevations were blunted. Our results suggest taurine had no substantial effect on norepinephrine or epinephrine excretion and therefore did not greatly reduce sympathetic tone in SPSHR under non-stressed conditions. This may in part explain why taurine supplementation did not produce larger reductions in blood pressure as reported for other models of hypertension. We specifically focused on the role of high salt diets to exacerbate the development of hypertension in SPSHR. Urinary dopamine excretion is normally increased in normotensive rats given high salt diets (Lee, 1993), however, urinary dopamine excretion was depressed by 1% NaCl added to the diet in the SPSHR but either dietary taurine supplementation or restriction blocked this effect. In fact, taurine supplementation actually increased urinary dopamine excretion significantly when normalized to creatinine excretion. Increased renal production of dopamine is associated with natriuresis, diuresis and reductions in blood pressure (Lee, 1993). Thus the failure of SPSHR to significantly increase total renal dopamine production when given high salt diets may contribute substantially to the salt-induced increase in blood pressure. Although taurine supplementation did not appear to reduce sympathetic tone as indicated by its failure to decrease urinary norepinephrine or epinephrine excretion, taurine supplementation may have augmented renal dopaminergic mechanisms to lower blood pressure.

Taurine supplementation had no effect on serum creatinine but reduced urinary creatinine excretion and tended to lower urinary nitrogen excretion. Trachtman et al. (1989) reported that creatinine clearance was not altered in SHR given 1% taurine in the drinking water for 16 weeks. Plasma levels of urea and creatinine have been reported to be decreased by taurine supplementation in animal models of hypertension and renal disease (Yamamoto et al., 1985; Trachtman et al., 1992). It is unclear at present how dietary taurine supplementation alters the generation or elimination of nitrogenous waste products.

SHR are known to exhibit behavioral alterations which include hyperactivity (Liljequist et al., 1982) and learning impairments (Wyss et al., 1992; Gattu et al., 1997). SPSHR also show increased locomotor activity and deficits in passive avoidance learning that may be associated with vascular dementia (Minami et al., 1997). Liljequist et al. (1982) reported that 3% taurine supplementation could decrease spontaneous activity recorded over a 12 hour period during the dark phase of the circadian rhythm in rats. Taurine supplemented or taurine free diets have been reported to increase exploratory behavior in an open field test (Liljequist et al., 1982). We found no evidence that taurine supplementation (1.5%) decreased locomotor activity in SPSHR

when activity was assessed for 15 minutes during the light phase of the circadian rhythm. The taurine deficient diet did produce a significant decrement in the performance of a spatial learning task. The taurine deficient diet produced the highest blood pressures and the largest increase in the blood pressure AUC. The learning deficits in SPSHR have been associated with reduced cortical and hippocampal acetylcholine and sustained elevations in blood pressure (Togashi et al., 1996; Minami et al., 1997). The increased blood pressure in SPSHR on the taurine free diet may have been a contributing factor to their poor cognitive performance. We have previously found that aged rats with reductions in striatal taurine content show impaired performance in the Morris water maze (Dawson et al., 1999). There was, however, no evidence to suggest that the taurine free diet reduced striatal content of taurine. We, however, cannot exclude the possibility that taurine release was diminished by the taurine free diet. Further studies are required to elucidate the relationship between altered dietary taurine intake, elevated blood pressure and spatial learning performance.

Taurine has been postulated to directly or indirectly antagonize various angiotensin II-mediated effects (Abe et al., 1987; Studer et al., 1997; Takahahsi et al., 1997; Ballard-Croft et al., 1997). Taurine also can modulate intracellular calcium levels in vascular smooth muscle (Ristori and Verdetti, 1991) and cardiac muscle (Huxtable, 1992). Recently is has been shown that taurine inhibits proliferative responses and fibrotic changes in tissues induced by free radical damage (Trachtman et al., 1993b; Wang et al., 1992; Schuller-Levis et al., 1995; Studer et al., 1997). Taurine has also been shown to inhibit cardiac myocyte hypertrophy and fibroblast proliferation induced by angiotensin II treatment of primary cultures of rat heart (Takahashi et al., 1997). Thus, in addition to taurine's ability to modulate cardiovascular function via central nervous system (Bousquet et al., 1981) and peripheral mechanisms, taurine may have actions to retard some the adverse chronic effects of elevated blood pressure independent of its hypotensive effects. Taurine also has other potential beneficial effects on other risk factors for cardiovascular disease. Taurine has been shown to lower cholesterol (Murakami et al., 1996a; Murakami et al., 1996b) and have therapeutic actions in diabetic patients (Franconi et al., 1995). Therefore, the potential therapeutic effects of taurine supplementation in hypertension may go beyond it actions to modestly lower blood pressure. Our results suggest that taurine supplementation can blunt the adverse effects of high salt diets to increase cardiac hypertrophy and urinary protein excretion even in an animal model already genetically predisposed to severe hypertension. Although rats are prolific synthesizers of taurine, dietary restriction of taurine intake resulted in elevations in serum cholesterol and impaired cognitive function. Humans are more dependent on dietary sources for taurine than rodents and humans with low dietary taurine intake show reductions in blood pressure when given supplemental taurine (Yamori et al., 1996). Taurine supplementation would appear to be a safe and effective means to reduce risk factors for cardiovascular disease.

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