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Renal excretory responses of taurine-depleted rats to hypotonic and hypertonic saline infusion

M. S. Mozaffari[1], **B. K. Warren**[1], **J. Azuma**[2], and **S. W. Schaffer**[3]

[1] Department of Oral Biology, Medical College of Georgia School of Dentistry, Augusta, Georgia, U.S.A
[2] Department of Clinical Evaluation of Medicines and Therapeutics, Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan
[3] Department of Pharmacology, College of Medicine, University of South Alabama, Mobile, Alabama, U.S.A.

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Summary. Male Wistar-Kyoto rats were given either tap water (control) or 3% β-alanine (taurine-depleted) for three weeks. To prepare for the kidney function studies, the animals were then implanted with femoral vessels and bladder catheters. Two days after surgery, each rat was given an intravenous infusion of saline at the rate of 50 µl/min and urine samples were collected at specific time intervals. An isotonic saline solution (0.9% NaCl) was infused for determination of baseline parameters and was followed by the infusion of a hypotonic saline solution (0.45% NaCl). Two days later, the infusion protocol was repeated in the same animals; however, a hypertonic saline solution (1.8% NaCl) was substituted for the hypotonic saline solution. Renal excretion of fluid and sodium increased in the control, but not taurine-depleted, rats during the hypotonic saline infusion. Interestingly, diuretic and natriuretic responses were similar between the groups during hypertonic saline infusion. The results suggest that taurine-depletion in rats affects renal excretory responses to a hypotonic, but not a hypertonic, saline solution.

Keywords: Amino acids – Taurine – Rat – Natriuresis – Hypotonic saline – Hypertonic saline

Introduction

Taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid in mammalian cells. A number of physiological roles have been attributed to taurine, one of the most important being osmoregulation. Exposure of a variety of cells to a hypotonic solution results in cellular extrusion of taurine, a process which contributes to regulatory volume decrease. Conversely, exposure of cells to hypertonic media results in cellular uptake of taurine, an

important mechanism in regulatory volume increase (Burg 1995; Galietta et al., 1997; Huang et al., 1996; Fugelli et al., 1995; Trachtman et al., 1995; Moran et al., 1994; Uchida 1991; Nakanishi et al., 1994; McManus et al., 1995).

Renal tubular cells normally experience large changes in tonicity. The intracellular accumulation of organic osmolytes, i.e., taurine, by renal tubular cells serves as an adaptive mechanism to cope with an increase in interstitial osmolality; the high interstitial osmolality is established by the counter current multiplier system and is essential for the kidney to concentrate urine. Therefore, one might expect organic osmolytes, such as taurine, to contribute to the counter current mechanism of urinary concentration. Moreover, taurine is invariably linked to regulatory volume changes because of its role as an osmolyte. Since major changes in taurine flux occur in response to altered osmotic conditions, one would expect taurine movement to also influence fluid and sodium excretion by the kidney. Yet, despite the acceptance of taurine as an important osmoregulator in the kidney, information regarding the effects of endogenous taurine stores on fluid and electrolyte homeostasis is sparse. Therefore, the objective of this study was to determine whether a reduction in endogenous taurine stores would differentially affect renal excretory responses to an intravenous infusion of a hypertonic vs. a hypotonic saline solution.

Materials and methods

Seven-week old male Wistar-Kyoto (WKY) rats were obtained from Harlan laboratories (Indianapolis, Indiana). All rats were maintained 2 per cage at constant humidity (60 ± 5%), temperature (24 ± 1°C), and light cycle (0600-1,800 hr.). Two days after arrival, the animals were randomly assigned to two groups: the taurine-depleted group (n = 5) received drinking water containing 3% β-alanine and the control group (n = 6) received only tap water. β-Alanine inhibits cellular uptake of taurine (Jones et al., 1990) and its inclusion in the drinking fluid reduces endogenous taurine stores in the kidney (Mozaffari et al., 1997). Food and drinking fluid were available ad libitum throughout the study.

Two days prior to examining the natriuretic and diuretic responses to a saline infusion, each rat was instrumented, under ether anesthesia, with femoral arterial and venous catheters (PE-10 fused with PE-50), and a bladder catheter (Mozaffari et al., 1997). On the day of the experiment, each rat was placed in an environmental conditioning unit (Braintree, Braintree, MA); all rats had been conditioned to the units for 4 hours per day for 5 days prior to testing. After flushing the arterial and venous catheters with 0.3 ml of isotonic saline containing 5 U/ml of heparin, the recording of mean arterial pressure and heart rate was initiated. The animals were given an intravenous infusion of isotonic saline at the rate of 50 μl/min. A baseline urine sample (30 minutes) was obtained prior to the intravenous infusion of a hypotonic saline solution (0.45% NaCl) at the rate of 50 μl/min. After initiation of the hypotonic saline infusion, urine samples were collected at 15 minute intervals for 45 minutes. Two days later, the infusion protocol was repeated in the same animals; however, a hypertonic saline solution (1.8% NaCl) was substituted for the hypotonic saline solution. Urine samples were collected at 30 minute intervals and urine volume determined. Urinary concentration of sodium and potassium were measured with a flame photometer and used to calculate sodium and potassium excretion rates, respectively.

All data were analyzed by the analysis of variance (ANOVA), with Newman-Keuls test (significance of criteria of $p < 0.05$) employed to determine the source of significant effects. Data are reported as means ± SEM.

Table 1. Body weight, mean arterial pressure and heart rate of experimental groups

	Initial body weight (g)	Final body weight (g)	Mean arterial pressure (mm Hg)	Heart rate (beats/min)
Control	142 ± 2	222 ± 3	121 ± 2	343 ± 12
Taurine-depleted	142 ± 1	209 ± 3*	129 ± 3	323 ± 4

*$p < 0.05$ compared to the control group.

Results

Body weight was similar between the groups prior to β-alanine treatment. After three weeks of drug therapy, taurine-depleted rats weighed slightly less (~13 grams) than the control animals (Table 1). Baseline mean arterial pressure was slightly higher and heart rate slightly lower in the taurine-depleted rats (Table 1). Intravenous administration of either a hypotonic or hypertonic saline solution did not affect mean arterial pressure or heart rate in either group (data not shown).

Baseline renal excretion of fluid, but not sodium, was lower in the taurine-depleted compared to the control rats (Figs. 1A–B, Time 0). Intravenous infusion of a hypotonic saline solution did not affect renal excretion of fluid or sodium in the taurine-depleted rats (Figs. 1A–B). However, control rats manifested an increase in both fluid and sodium excretion following infusion of a hypotonic saline solution. The increase in sodium excretion (Fig. 1D) was greater than the increase in fluid excretion (Fig. 1C). Baseline potassium excretion was lower in the taurine-depleted compared to the control rats (0.5 ± 0.1 vs. 1.0 ± 0.2 μEq/min/100 g BW). Renal excretion of potassium was not affected by infusion of a hypotonic saline solution in either the control or taurine-depleted rats.

In contrast to the differential renal responses to a hypotonic saline solution, intravenous administration of a hypertonic saline solution resulted in similar increases in fluid and sodium excretion in control and taurine-depleted rats (Figs. 2A–B). However, the increase in sodium excretion was greater than fluid excretion indicating urinary excretion of a salt-laden urine in both groups. Baseline potassium excretion was lower in the taurine-depleted than the control rats (0.4 ± 0.1 vs. 0.8 ± 0.1 μEq/min/100 g BW). Potassium excretion increased to a similar extent in both groups during hypertonic saline infusion (~50%).

Discussion

The most important observation of this study is that chemically-induced, taurine depletion interferes with the natriuretic and diuretic responses of the kidney to a hypotonic saline solution. By contrast, the response to a hypertonic saline solution is unaffected by taurine depletion.

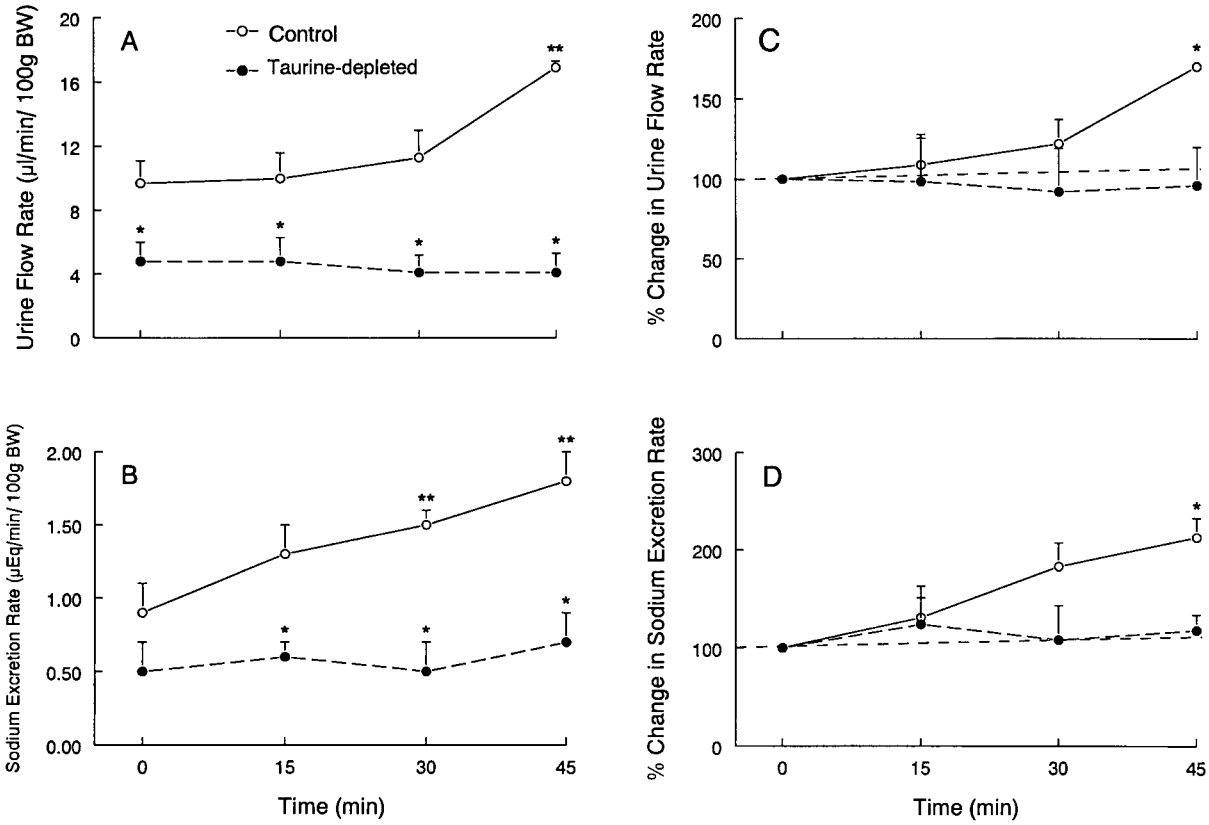

Fig. 1. Control and taurine-depleted rats were given isotonic saline solution (0.9% NaCl) for determination of baseline (Time 0) fluid (**A**) and sodium (**B**) excretion prior to administration of hypotonic saline solution (0.45% NaCl) for 45 minutes. Further, diuretic (**C**) and natriuretic (**D**) responses to hypotonic saline infusion are expressed as the percent increase in fluid and sodium excretion over baseline values for each group. Intravenous infusion of hypotonic saline solution resulted in an increase in fluid and sodium excretion in the control, but not taurine-depleted, rats. Data are average ± SEM of 6 control and 5 taurine-depleted rats. *$p < 0.05$ compared to the other group at the same time point, **$p < 0.05$ compared to time 0 in the same group

Hypertonicity and hypotonicity cause very different alterations in the cellular movement of sodium and taurine. This is not surprising because both sodium and taurine serve as important osmolytes. Hyposmotic stress rapidly initiates a signal transduction pathway culminating in the activation of several transporters responsible for the extrusion of organic and inorganic osmolytes from the cell. Thus, osmotic swelling causes the selective reduction in the intracellular concentrations of potassium, chloride and a host of organic osmolytes, of which taurine is a dominant player (McManus et al., 1995; Pasantes-Morales et al., 1990). Hyperosmotic stress also leads to the activation of several channels and transporters, however, the pathways involved are very different from those activated following a hyposmotic stimulus. Following exposure to hyperosmotic medium, the cell initially shrinks. Several trans-

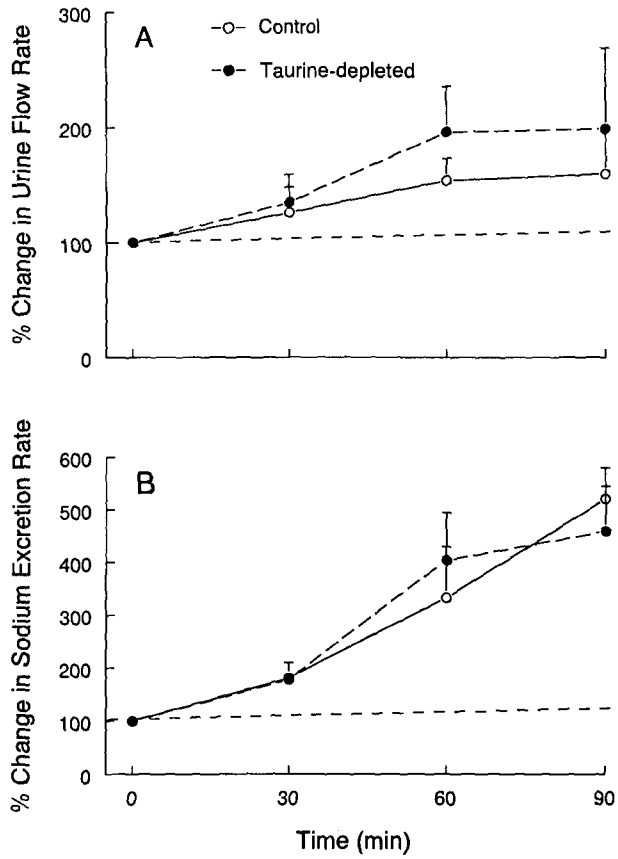

Fig. 2. Control and taurine-depleted rats were given isotonic saline solution (0.9% NaCl) for determination of baseline (Time 0) fluid (**A**) and sodium (**B**) excretion prior to administration of hypertonic saline solution (1.8% NaCl) for 90 minutes. Diuretic and natriuretic responses to hypertonic saline infusion are expressed as the percent increase in fluid and sodium excretion over baseline values for each group. Data are average ± SEM of 6 control and 5 taurine-depleted rats

port proteins are activated to restore the osmotic balance of the cell via a two-phase process. In most cells, the rapid phase is triggered by the activation of the $Na^+K^+2Cl^-$ cotransporter and electroneutral exchangers, such as the Na^+-H^+ and HCO^-_3-CL^- exchangers (McManus et al., 1995; Pasantes-Morales et al., 1990). This is followed by a slower phase of volume regulation in which there is a slow accumulation of several organic osmolytes, including taurine. Thus, hyperosmotic stress is associated with cellular accumulation of both taurine and sodium, while hyposmotic stress has the opposite effect.

In this study, we examined the effect of taurine depletion on the rate of natriuresis and diuresis minutes after administration of either a hypotonic or hypertonic saline solution. Since taurine depletion was achieved by feeding the animal large amounts of the taurine transport inhibitor, β-alanine, we originally thought that any observed effect could be related to either the actions of β-alanine or the loss of taurine from the cell. The latter is more

consistent with our data since a differential effect was observed only during hypotonic saline infusion, a condition which would be expected to promote cellular efflux of taurine and produce taurinuria with attendant natriuresis.

We originally hypothesized that taurine depletion could affect renal function through several mechanisms. First, changes in renal taurine levels might disrupt the counter current multiplier mechanism. Recent reports demonstrate a heterogeneous distribution of taurine in the kidney and suggest that the intracellular taurine concentration increases along the corticomedullary axis (Amiry-Moghaddam et al., 1994; Trachtman et al., 1993). This results in preferential localization of taurine to the inner medullary regions of the kidney, where the extracellular milieu can become extremely hypertonic. The high tonicity of the medullary interstitium is maintained by the counter current multiplier system and is essential for the ability of the kidney to concentrate urine. Therefore, intracellular accumulation of taurine in renal tubule cells serves to maintain intracellular osmolality and cell volume in the setting of the high extracellular ionic gradient of the medullary interstitium. Taurine depletion is associated with reduced baseline rates of sodium and fluid excretion, with the latter effect being more prominent. However, our data show that differences in fluid and sodium excretion between the control and taurine-depleted rats become more apparent during a hypotonic saline infusion despite the requirement to eliminate more sodium following a hypertonic saline infusion. Thus, taurine depletion does not appear to interfere with the urine concentrating ability of the kidney.

Second, taurine is an active osmolyte and therefore a redistribution of taurine from the intracellular compartment to the blood would favor elevated rates of taurine excretion, thereby influencing renal function. Consistent with this notion, Shaffer et al. (1979) found that a few days after onset of β-alanine treatment, the extrusion of taurine from most cells is accelerated, leading to enhanced rates of taurine and water elimination from the animal. However, more prolonged β-alanine exposure results in a reduction in urinary taurine output as well as fluid excretion. In this regard, it appears that a hypotonic saline infusion induces a condition similar to short-term β-alanine exposure, i.e, taurine is rapidly lost from cellular compartments, presumably elevating plasma taurine levels and promoting urinary taurine, fluid, and sodium output (Fig. 1). Since very little taurine is available for release in the chronic β-alanine treated animal, a hypotonic saline infusion would be expected to have little effect. This same mechanism would not be functional following hypertonic saline infusion because cellular efflux of taurine is not expected under this condition. This may explain the observation that taurine depletion has no influence on urinary fluid and sodium excretion in response to a hypertonic saline infusion (Fig. 2).

Another plausible explanation for the effects of taurine depletion on renal function is based on the demonstration that rat posterior pituitary contains high concentration of taurine which can be released in response to hyposmotic stimulation (Miyata et al., 1997). Based on these observations, Miyata et al. (1997) suggested a plausible scenario for central nervous system regulation of body fluid homeostasis by taurine. The authors proposed that taurine, under

basal conditions, regulates the release of neurohypophysial hormones, i.e., arginine vasopressin. Moreover, hyposmotic-induced taurine release further suppresses axon terminal depolarization culminating in a reduction in arginine vasopressin release, thereby facilitating renal fluid excretion. Our observation of significant fluid excretion in the control, but not taurine-depleted, rat during hypotonic saline infusion is in general agreement with the hypothesis that decreased arginine vasopressin release, secondary to hyposmotic-induced taurine release by the posterior pituitary, mediates renal fluid excretion (Miyata et al., 1997). These effects of taurine should be inefficient in the chronic β-alanine-treated rat, which contains reduced taurine stores. Although pituitary taurine content was not measured in this study, β-alanine is a general taurine depleting agent reducing taurine stores in prominent brain regions such as cortex, cerebellum and thalamus (Shaffer 1979). Thus one would predict that less taurine would be released from the pituitary gland of the β-alanine-treated rat following hyposmotic stress resulting in an inadequate suppression of arginine vasopressin release. The net effect would be enhanced fluid retention. Moreover, it is reasonable to suggest that even under basal conditions a dysregulation of arginine vasopressin release in the β-alanine-treated rat may underlie the reduction in baseline fluid excretion. Nonetheless, one can not rule out the possibility that the postulated central effects are due to β-alanine rather than taurine depletion.

In conclusion, intravenous infusion of a hypotonic, but not a hypertonic saline solution, unmasks a deficit in renal excretion of fluid and sodium in taurine-depleted rats, thereby providing further evidence in support of the hypothesis that taurine affects renal function. Although the underlying mechanism(s) for this finding remains to be determined, it is plausible to suggest that hypotonic saline-induced taurinuria, combined with reduced arginine vasopressin release, constitute the most likely effector mechanisms. The relative contribution of these mechanisms in this process is the subject of an ongoing investigation in our laboratory.

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Authors' address: Dr. Mahmood S. Mozaffari, Department of Oral Biology/Pharmacology, Medical College of Georgia School of Dentistry, Augusta, GA 30912-1128, U.S.A.

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