β-Alanine Protects against Taurine and NaCl–Induced Hypernatremia in the Rat (43960)

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Abstract. **Rats drinking a combination of taurine and hypertonic saline solution rapidly develop hypernatremia, but rats drinking either solution alone do not. The mechanism by which taurine disrupts the ability to deal with a salt load is not clear. Rats housed in metabolism cages were studied. Food intake, fluid intake, plasma sodium concentration, urine output, sodium balance, visible water balance, and urine osmolality were determined over a period of 8 days. Rats drinking 0.1 *M* taurine plus 1.8% NaCl developed a mean plasma sodium concentration of 160 ± 18 m*M* by Day 6, compared with 137 ± 1.6 m*M* in water drinking controls. Ingestion of 1.8% saline alone produced only a mild, transient rise in plasma sodium (<150 m*M*), which returned to control levels by Day 8. Ingestion of neither 0.1 *M* taurine alone nor 0.1 *M* β-alanine, a taurine transport antagonist, produced any evidence of hypernatremia throughout the experiment. When β-alanine was added to the taurine + saline regimen, mean plasma sodium reached only 149 ± 16 m*M* (Day 6). Inspection of the ratio of cumulative sodium balance to cumulative water balance revealed a rapid increase until Day 2, followed by a virtual plateau thereafter in the taurine + saline group. Rats drinking saline alone showed an equally rapid rise in the ratio, but to a lower plateau level, suggesting that taurine exerts a much more pronounced disturbance of sodium balance than of water balance. The addition of β-alanine to the regimens of taurine + saline or saline alone produced ratios of cumulative sodium to cumulative water balance significantly lower than that of either regimen without β-alanine. These findings suggest that taurine induces hypernatremia by interfering with normal homeostatic control mechanisms and that β-alanine counteracts that action of taurine. The effect of β-alanine in rats drinking saline alone is consistent with a role for endogenous taurine in normal electrolyte homeostasis.** [P.S.E.B.M. 1996, Vol 211]

Ingestion of taurine (2-aminoethanesulfonic acid) in combination with hypertonic saline has been shown to induce severe hypernatremia in otherwise normal rats (1–3). While possible explanations for this have been proffered (2), the fundamental cause remains obscure.

Taurine exerts diverse regulatory actions in many tissues, particularly when conditions deviate from normal (4). As an example, oral ingestion of taurine has an antihypertensive effect in DOCA-salt rats (5), possibly through mechanisms involving the hypothalamus (6). However, in the case of the taurine + saline model of hypernatremia, taurine seems to affect the normal regulatory mechanism(s) adversely. This is of interest as it is usually assumed that the toxicity of taurine is negligible (7). Indeed, practically every action of taurine that has been described tends to maintain function in an unstable situation, a property that has been called enantiostasis (8).

This study of taurine + saline–induced hypernatremia incorporates β-alanine, a structural analog of taurine, into the protocol. β-alanine is a potent inhibitor of taurine transport systems *in vitro* (9) and sub-

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stantially increases urinary excretion of taurine *in vivo* (10). The purpose of using a taurine transport antagonist in this study was to gain additional information about the possible site and mode of action of taurine in the induction of hypernatremia.

Materials and Methods

Animals, Diet, and Drinking Regimens. Locally bred, adult (>150 g) Wistar rats of either sex were housed in metabolism cages under controlled conditions in an animal holding room and received standard (0.2% Na w/w) rat chow *ad libitum* throughout all experiments. One of the following drinking regimens also was provided *ad libitum* to each group: tap water control (C); 0.1 *M* taurine (T); 1.8% NaCl (S); 0.1 *M* taurine + 1.8% NaCl (T+S); 0.1 *M* β-alanine (β); 0.1 *M* β-alanine + 1.8% NaCl (β+S); 0.2 *M* β-alanine + 1.8% NaCl (2β+S); or 0.1 *M* β-alanine + 0.1 *M* taurine + 1.8% NaCl (β+T+S). For Group C, T, S, and T+S, $n = 12$; and for β, β+S, 2β+S, and β+T+S, $n = 6$.

Protocol. The animal holding room where metabolism cages were kept and sample collections were taken was temperature controlled with a 12:12-hr light:dark cycle. Each run comprised 12 rats, six from each of two treatment groups, which were placed into individual metabolism cages on Day −2 and given tap water *ad libitum* for 2 days to allow for stabilization prior to provision of the assigned regimen on Day 0. Measurements were made as described below beginning on Day −2, and experiments continued until final sample collections were completed on the morning of Day 8. Only data collected from Day 0 onward are reported here. This experimental duration was based on earlier findings (11) that T+S predictably produced hypernatremia midway into this period, while the associated mortality fell within acceptable limits and occurred late in the period. Measurements and sample collections were made between 0900 and 1000 hr. Body weights were determined daily and tail-tip blood samples from which plasma sodium concentration (P_{Na}) and osmolality (P_{osm}) were determined were taken on alternate days from Day −2. Food and solution consumption, urine volume (*UV*), osmolality (U_{osm}) and sodium concentration (U_{Na}) were measured daily from Day −1. Variables calculated daily using these data were: sodium balance; cumulative sodium balance (*Cum*Na); "visible" water balance (i.e., solution consumed − *UV;* and cumulative water balance (*Cum*H_2O). It should be noted that the so-called visible water balance is determined by subtracting urine volume only from total fluid volume consumed. Thus, even in the control group the result is a positive value, and, consequently, cumulative water balance increases throughout the experiment in all groups. P_{Na} and U_{Na} were determined using flame emission spectrophotometry, and P_{osm} and U_{osm} were measured using vapor pressure osmometry.

Statistical Analyses. All values reported are mean ± SEM. Statistical tests used were (12): one-way repeated measures analyses of variance (ANOVAs) for within regimens over the 8-day experimental period, with the nonparametric alternative of Friedman's repeated measures ANOVA on ranks used when the normality test failed. If the ANOVA indicated the presence of statistically significant differences, a Student-Newman-Keuls (S-N-K) test was used to compare mean values from day to day within the group. Two-way ANOVAs were used to test for significant differences among regimens over the course of the experiment, and when appropriate, were followed by S-N-K tests.

Results

Figure 1 shows that rats drinking a solution containing 0.1 *M* taurine and 1.8% NaCl (T+S) had a significantly elevated mean plasma sodium concentration (P_{Na}) on Day 2, and severe hypernatremia (>160 m*M*) by Day 6. Two of the 12 rats in this group died on Day 6, and a third died on Day 8. Rats drinking 1.8% NaCl alone (S) had a mildly elevated mean P_{Na} (147 m*M*) on Day 4, but the value returned to normal by Day 6 and remained so through Day 8. No deaths occurred in the S group. Rats ingesting solutions of either 0.1 *M* taurine alone (T) or 0.1 *M* β-alanine alone (β) had mean P_{Na} ranging from 137 to 143 m*M* over the course of the experiment. This was not significantly different from the range of 137–140 m*M* in control (C)

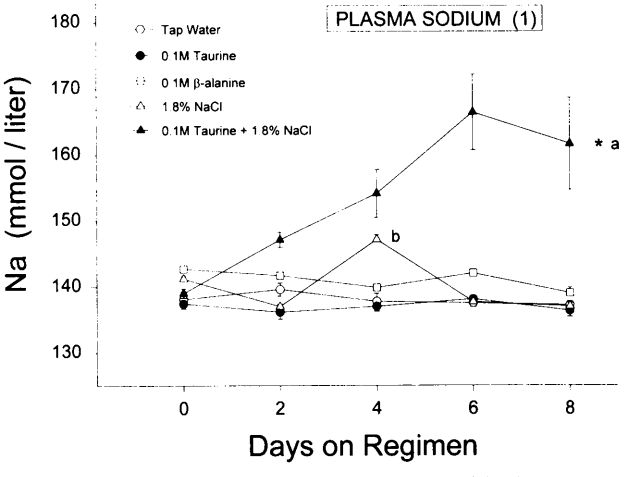

Figure 1. Plasma sodium concentration in rats drinking the regimens indicated for 8 days. All values are means ± SEM ($n = 12$ for non–β-alanine, and $n = 6$ for β-alanine regimens). *Regimen is different ($P < 0.05$, two-way ANOVA) from tap water control; [a]regimen is different from all other treatment regimens over the 8-day period; [b]value on Day 4 of this regimen is higher ($P < 0.05$, one-way ANOVA and Student-Newman-Keuls test) than values on all other days of this regimen.

rats drinking tap water. Similarly, no meaningful differences among these three groups (viz., C, T, and β) have been found in other variables reported below. Therefore, values from the C group only are shown in the ensuing figures (Fig. 2–6) to represent all "nonsaline-drinking control" groups.

As β-alanine is a structural analog of taurine, it might be expected that ingestion of 0.1 *M* β-alanine together with 1.8% NaCl (β + S) would have a similar action on P_{Na} to that of T + S. However, Fig. 2 shows that neither β + S nor 2β + S groups had P_{Na} profiles that differed from those of C. Furthermore, compared with the hypernatremia seen in the T + S regimen, β + T + S resulted in a much slower and milder rise in mean P_{Na}, reaching <150 m*M* (Fig. 2). No deaths occurred in the β + T + S group.

Cumulative sodium balance (*Cum*Na) was determined to establish this as an index which reflected the observed P_{Na} patterns (Fig. 3). Rats drinking tap water (C) showed a slight decline in *Cum*Na over the course of the experiment. Rats drinking S alone exhibited a moderate rise in *Cum*Na reaching a mean of 7.9 mmol/100 g body wt on Day 8, whereas that of those consuming T + S rose steeply to a mean of 23.7 moles/100 g on Day 8. Notably, *Cum*Na in the group drinking β + T + S reached only 5.1 mmol/100 g, and that in the β + S group did not differ from that seen in C rats.

Cumulative water balance (*Cum*H_2O), although qualitatively similar to the *Cum*Na results, showed less divergence among the groups (Fig. 4). The control group reached a mean value of 41 ml/100 g body wt on Day 8, while rats drinking T + S climbed to 88 ml/100 g over the same period. The S, β + S, and β + T + S

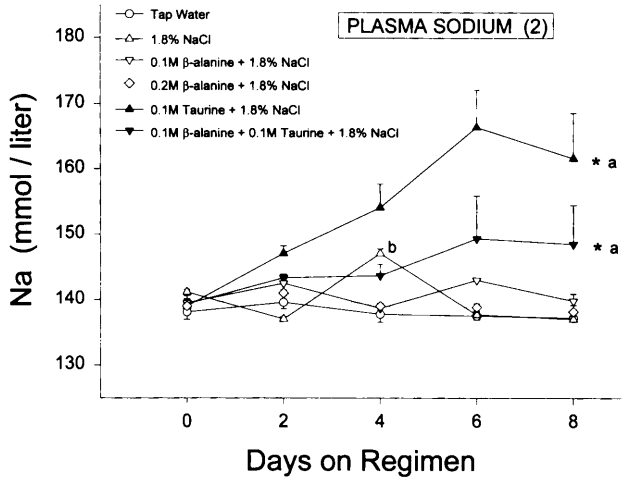

Figure 2. Plasma sodium concentration in rats drinking the regimens indicated for 8 days. All values are means ± SEM (n = 12 for non–β-alanine and n = 6 for β-alanine regimens). *Regimen is different ($P < 0.05$, two-way ANOVA) from tap water control; [a]regimen is different from all other treatment regimens over the 8-day period; [b]value on Day 4 of this regimen is higher ($P < 0.05$, one-way ANOVA and Student-Newman-Keuls test) than values on all other days of this regimen.

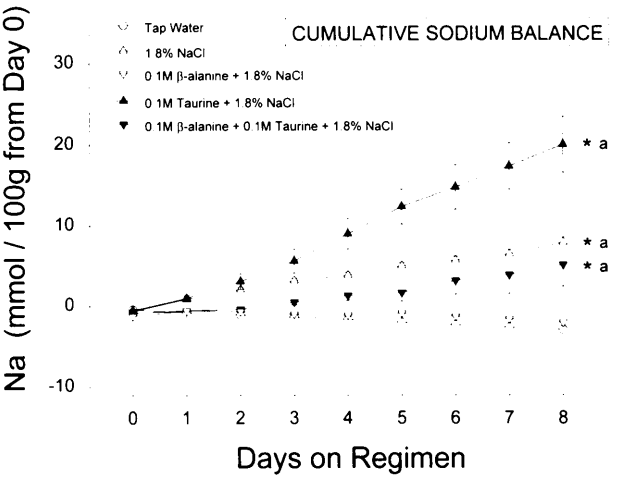

Figure 3. Cumulative sodium balance in rats drinking the regimens indicated for 8 days. Daily sodium balance was calculated as total sodium consumed minus urinary sodium excretion. All values are means ± SEM (n = 12 for non–β-alanine and n = 6 for β-alanine regimens). *Regimen is different ($P < 0.05$, two-way ANOVA) from tap water control; [a]regimen is different from all other treatment regimens over the 8-day period.

groups exhibited intermediate values with no clear distinction among the three regimens for this factor.

The daily ratios of *Cum*Na/*Cum*H_2O shown in Figure 5 emphasizes the disproportionate and rapid rise of *Cum*Na versus that of *Cum*H_2O in rats drinking S or T + S compared with their counterparts that also consumed β-alanine (i.e., β + S and β + T + S). While the ratio in all four groups rose significantly above control on the first day of the regimens, the patterns among the groups were distinct. Rats ingesting T + S exhibited the most rapid rise and stabilized at the highest plateau. β + T + S showed a markedly lower rate of rise and a significantly lower plateau level. Rats drinking S

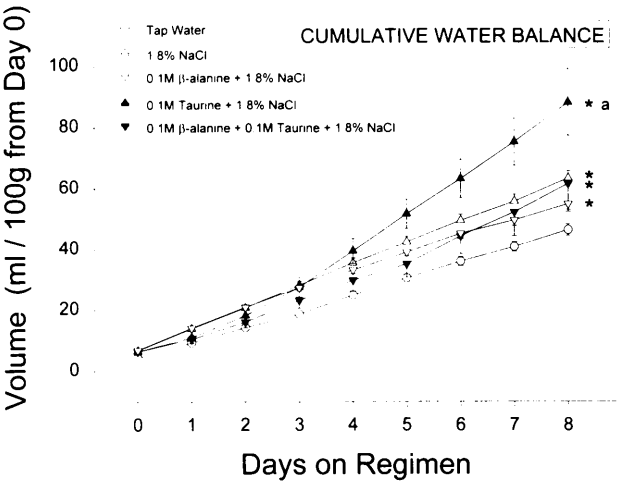

Figure 4. Cumulative water balance in rats drinking the regimens indicated for 8 days. Daily water balance was calculated as total volume consumed minus urine volume. All values are means ± SEM (n = 12 for non–β-alanine and n = 6 for β-alanine regimens). *Regimen is different ($P < 0.05$, two-way ANOVA) from tap water control; [a]regimen is different from all other treatment regimens over the 8-day period.

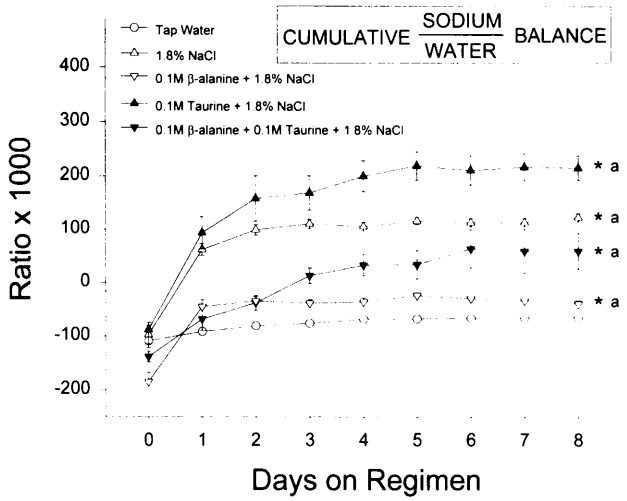

Figure 5. Cumulative sodium balance/cumulative water balance × 1000 in rats drinking the regimens indicated for 8 days. This ratio was calculated from raw data for each day of the experiment. All values are means ± SEM (n = 12 for non–β-alanine and n = 6 for β-alanine regimens). *Regimen is different ($P < 0.05$, two-way ANOVA) from tap water control; [a]regimen is different from all other treatment regimens over the 8-day period.

and β + S without taurine stabilized at levels significantly lower than those with taurine included, but again, the addition of β-alanine had a strong moderating effect. These findings indicate that rats drinking a regimen of hypertonic saline accumulate sodium out of proportion to volume, a situation that is exacerbated by the inclusion of taurine in the regimen, and mitigated by the presence of β-alanine.

Figure 6 shows that control rats maintained a urine osmolality of approximately 2000 mOsm/kg H_2O throughout the experiment. The level dropped rapidly

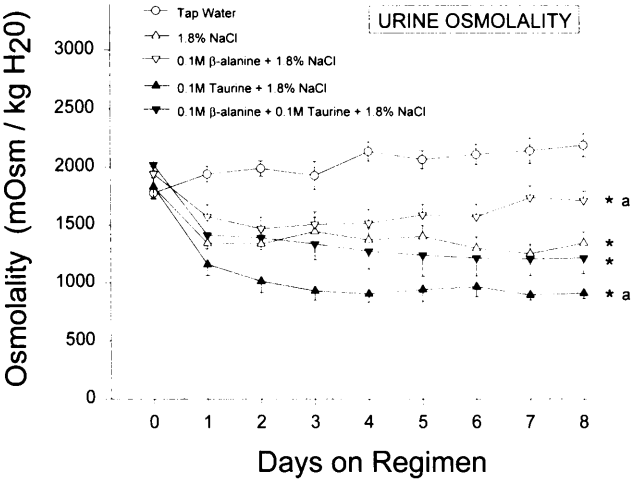

Figure 6. Urine osmolality in rats drinking the regimens indicated for 8 days. All values are means ± SEM (n = 12 for non–β-alanine and n = 6 for β-alanine regimens). *Regimen is different ($P < 0.05$, two-way ANOVA) from tap water control; [a]regimen is different from all other treatment regimens over the 8-day period.

to around 1000 mOsm/kg H_2O in rats drinking T + S, while that of the S group showed a lesser drop and stabilized at approximately 1200 to 1300 mOsm/kg H_2O. As with other factors (cf., Fig. 3 and 5), the effect of saline intake on urine concentrating ability was significantly less marked in both β + S and β + T + S groups than when β-alanine was not included.

Discussion

Taurine has many putative central regulatory actions (13) including physiological roles in hypothalamic thermoregulation (14) and blood pressure control (15), cerebral osmoregulation (16), and perhaps even as a hypothalamic neurotransmitter (17) for the regulation of neuroendocrine release (18–20). It also may act peripherally to modulate blood pressure (5) and in cellular osmoregulation (21). Several of these regulatory functions could be involved in the model of hypernatremia reported here.

It has been generally accepted that taurine acts to conserve normal function and the finding that it can cause lethal disruption of a major homeostatic mechanism was unexpected. Although untoward effects of excessive taurine administration have long been known (22), it was surprising to find that rats ingesting a combination of taurine and hypertonic saline (T + S) exhibited profound hypernatremia (mean P_{Na}: >160 mM) and subsequent death (2). The present study has confirmed this observation, and that the ingestion of taurine alone (T) has no observable effect on any of the variables measured, while hypertonic saline (S) alone evoked only mild hypernatremia (mean P_{Na}: <150 mM), which returned to normal by Day 8 of the regimen. Furthermore, unpublished data from our laboratory have shown that the hypernatremic effect of T + S is reversible. Rats with P_{Na} of 167–180 mM after drinking T + S for 3 to 6 days returned to a normal P_{Na} (140 mM or less) within 24–48 hr of recommencing a regimen of tap water. Given the Na-dependent nature of the high-affinity taurine transporter (23) and the influence of high extracellular taurine upon the low-affinity taurine uptake system, it would not be surprising if ingestion of an excess of taurine and NaCl together enhanced taurine uptake. However, it is not readily apparent why this should disrupt the handling of a salt load.

Perhaps the finding with the most important implications in the present study is the anti-hypernatremic property of β-alanine (Fig. 1 and 2). Ingestion of 0.1 M β-alanine (β) alone had no effect on P_{Na}. β-alanine (β or 2β), together with S, reduced and delayed, or eliminated, the mild hypernatremia induced by drinking S alone. And most striking, rats drinking the combina-

tion β+T+S developed significantly less severe hypernatremia (mean P_{Na}: <150 m*M*) than the T+S group, with no mortality during the duration of the experiment.

This anti-hypernatremic action of β-alanine is clearly evident from the *Cum*Na data (Fig. 3), the comparison of the ratio of *Cum*Na to *Cum*H_2O (Fig. 5), and the urine concentrating ability (Fig. 6) of the different groups. Rats drinking β+S are much closer to control levels than are rats drinking S alone; those drinking β+T+S are more like those drinking S alone than are those drinking T+S. That these distinctions are far less apparent in *Cum*H_2O (Fig. 4) suggests that β-alanine is acting more specifically upon regulation of NaCl/osmolality than upon water/volume balance.

β-alanine is known to be a potent antagonist of taurine transport *in vitro* (9), although its *in vivo* effect in the rat has been reported to be less dramatic (24). It is tempting to speculate that the action of β-alanine reported here is due to its antagonism of taurine transport systems. This might explain both its protective action against T+S–induced hypernatremia and its moderation of the mild hypernatremia associated with consumption of S alone. The latter would suggest that taurine has a physiological role in fluid and electrolyte balance, possibly at the hypothalamic level. While this fits well with evidence of other hypothalamic regulatory roles for taurine (14, 17–20), different sites of action for the involvement of taurine in the genesis of, or response to, hypernatremia cannot be ruled out. Also, the interaction of β-alanine with taurine under hypernatremia-inducing conditions requires further investigation. It has been reported recently (23) that taurine and β-alanine drastically reduce the transport of one another in an immortalized human kidney cell line, and that transport of both amino acids is increased by an increased extracellular sodium concentration. Therefore, another possible explanation of the present results is that β-alanine reduces the renal uptake of taurine in rats drinking T+S, thus providing protection against the development of hypernatremia. The nature of that protective action, however, remains unclear. Furthermore, the action of β-alanine in rats drinking β+S compared with those drinking S alone is even harder to explain, but it could indicate that β-alanine itself has some protective action against the development of hypernatremia. Whether or not such an action involves endogenous taurine remains to be seen.

Experiments looking at blood and urine taurine and β-alanine levels in rats drinking these different regimens are currently under way in an effort to clarify some of the questions arising from this study. Some involvement of vasopressin in this model of hypernatremia also seems likely, and studies directed at that possibility are in progress.

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