PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **181**, 411–415 (1986)

Atrial Natriuretic Factor in Taurine-Treated Normal and Cardiomyopathic Hamsters (42273)

H. DLOUHA AND M. J. MCBROOM

Department of Physiology, Faculty of Medicine, P.O. Box 24923, Kuwait University, Kuwait

Abstract. Diuretic and natriuretic activities of atrial extracts from BIO 14.6 (cardiomyopathic) and F_1B (normal) hamsters at 180 days of age were measured by rat bioassay. Both activities were lower in BIO 14.6 extracts. Because of the reported protective action of taurine in the cardiomyopathic hamster, we tested the effect of 0.1 *M* taurine drinking upon the activity of atrial extracts. Urine flow and Na^+ excretion were increased in both BIO 14.6 and F_1B; however, comparatively larger increases in BIO 14.6 taurine drinkers abolished strain differences that were observed in water drinkers. Taurine drinking BIO 14.6 hamsters exhibited an increased plasma sodium concentration. Drinking of 0.6% NaCl also produced an elevated plasma sodium concentration in BIO 14.6. Extracts from hamsters with increased salt intake had diuretic and natriuretic activities that were not different from those of water drinkers. These findings confirm that ANF activity is deficient in BIO 14.6 hamsters, and this suggests a role for taurine in its production, release, and/or activation.

The BIO 14.6 Syrian hamster strain is well known as an experimental model of hereditary cardiomyopathy (1). BIO 14.6 hamsters progressively develop congestive heart failure with cardiac dilatation and edema evident by about 200 days of age (2). Decreased levels of urinary sodium excretion and increased sodium concentration in renal tissue of these animals also have been demonstrated (3). Experiments in which BIO 14.6 and normal hamsters were parabionts provided evidence for a bloodborne humoral factor in normal hamsters that improved volume and electrolyte regulation in cardiomyopathic animals and extended their life span (1).

The discovery (4) and increased understanding of atrial natriuretic factor (ANF) brought us and others (5) to reason that a shortage of ANF might underly the observed water and electrolyte imbalances in cardiomyopathic BIO 14.6 hamsters.

It has been reported that chronic administration of taurine (2-aminoethane sulfonic acid, a natural constituent of the heart) retarded the calcium accumulation typically seen in the myopathic hearts and decreased the severity of cardiac lesions in cardiomyopathic hamsters (6–8). This protective effect of taurine was supposed to be due to its modulatory action on calcium binding within heart cells (9). Taurine also has been shown to be involved in cellular osmoregulation, as well as in modulation of Na^+,K^+-ATPase activity (10, 11).

In the present study, we measured the diuretic and natriuretic activities of atrial extracts from both normal (F_1B) and BIO 14.6 hamsters under the following conditions: (i) drinking tap water (control); (ii) drinking taurine solution (because of protective actions of taurine in BIO 14.6 animals); or (iii) drinking NaCl solution (to test the ability to respond to an increased sodium intake). Plasma sodium concentration was also measured in each group.

Materials and Methods. Cardiomyopathic (BIO 14.6) and normal (F_1B) hamsters (BIO Research Consultants, Cambridge, Mass.) were caged singly upon arrival (approximately 40 days of age) and placed on one of the drinking regimes described below. Animals were kept under automatically controlled conditions of temperature, humidity, and a 12-hr light/dark cycle; standard rodent pellets (E. Dixon & Sons, England) and drinking fluids were provided *ad libitum*. The drinking regimes employed during the period of 40–180 days of age were (i) tap water; (ii) 0.1 *M* taurine solution; and (iii) 0.6% NaCl solution. Animals were sacrificed by cervical dislocation at 180 days of age, blood samples obtained by direct cardiac puncture, and extracts of cardiac tissue prepared. Plasma sodium concentration was measured by flame photometry.

0037-9727/86 $1.50

Preparation of tissue extracts. Hearts were rapidly removed and placed in ice-cooled phosphate-buffered saline (PBS, 0.9% NaCl in sodium phosphate buffer, 10 mmole/liter, pH 7.4). Atria were dissected, thoroughly rinsed in PBS, blotted dry, and weighed. Atria from 6 to 10 hamsters were pooled for each extract preparation. Similar amounts of ventricular tissue were used for parallel experiments with ventricular extracts. The numbers of pooled extracts prepared for the different experimental groups were (i) water drinkers—F_1B, 10 atrial and 3 ventricular extracts; BIO 14.6, 7 atrial extracts; (ii) taurine drinkers—F_1B, 4, and BIO 14.6, 3 atrial extracts; and (iii) 0.6% NaCl drinkers—4 and 3 atrial extracts, respectively. Each of the pooled atrial extracts provided enough material for two or three bioassays. PBS was added to provide a tissue-to-saline ratio of 1:2 and samples were homogenized using a polytron. Homogenates were boiled for 10 min, cooled, and centrifuged at 4°C at 7000*g* for 30 min; the supernatant was deep-frozen and stored at −40°C. Protein concentration of the extract was determined using dual wavelength absorption spectrophotometry (12). No difference ($P > 0.05$) was found between protein concentrations of atrial extracts from the two strains: 4.37 ± 0.18 mg/ml in F_1B ($n = 18$) vs 4.07 ± 0.26 in BIO 14.6 ($n = 11$). Thus, comparison of the extract activities was justified.

Bioassay. Extracts from both strains of hamsters were assayed in female Wistar rats under standardized laboratory conditions. Rats were anesthetized with thiopental sodium (50 mg/kg) and rectal temperature was maintained at 37 ± 1°C during the experiment by the use of a heated operating table. The right jugular vein and the urinary bladder were cannulated with polyethylene tubing. Ringer's solution was infused at a rate of 1.2 ml/hr via the jugular cannula. After urine flow stabilized, collections were made over three consecutive 10-min control periods. A 0.2-ml aliquot of extract was then injected in approximately 5 sec via the jugular cannula; urine was subsequently collected for three additional 10-min periods. Urine volume was determined by weighing and urinary sodium concentration was measured by flame photometry.

Statistical comparisons of "before" and "after" bioassay results were made using Student's *t* test for paired data, those between water drinkers and taurine or NaCl drinkers employed an unpaired *t*, and those involving both within- and between-strain comparisons of plasma sodium levels were performed with Duncan's new multiple range test as modified by Kramer (13). In all cases, the significance level selected for rejecting the null hypothesis was $P \leq 0.05$.

Results. Baseline diuretic and natriuretic activities are shown in Table I. Atrial extracts from both hamster strains exhibited profound diuretic and natriuretic activities, with the

TABLE I. URINE FLOW AND SODIUM EXCRETION IN BIOASSAY RATS BEFORE AND AFTER SALINE, VENTRICULAR, OR ATRIAL EXTRACTS FROM NORMAL F_1B (F) AND CARDIOMYOPATHIC BIO 14.6 (B) HAMSTERS

	Urine flow ($\mu l \cdot 100\ g^{-1} \cdot 10\ min^{-1}$)		Sodium excretion ($\mu mole \cdot 100\ g^{-1} \cdot 10\ min^{-1}$)	
	Before	After	Before	After
0.9% Saline	22.43 ± 9.32 (3)	24.57 ± 2.74 (3)	2.39 ± 0.20 (3)	2.33 ± 0.77 (3)
Ventricular extract (F)	23.20 ± 4.30 (7)	30.44 ± 6.87 (7)	3.62 ± 1.34 (7)	4.35 ± 1.94 (7)
Atrial extract (F)	29.54 ± 4.11 (24)	93.71 ± 11.98* (24)	4.29 ± 0.81 (24)	14.10 ± 1.90* (24)
Atrial extract (B)	16.19 ± 2.16 (21)	54.87 ± 9.09* (21)	2.17 ± 0.43 (21)	8.80 ± 1.92* (21)

Note. Values are means ± SEM. Numbers in parentheses are numbers of bioassays performed. "Before" and "after" indicate respective values for the 10-min periods before and after saline or extract administration.

* $P < 0.001$ vs corresponding "before" value using Student's *t* test for paired data.

values in normal (F_1B) animals notably higher than those in the cardiomyopathic (BIO 14.6) strain. Neither normal saline nor ventricular extracts, in volumes equal to that of atrial extracts, displayed any significant activities.

The responses to taurine and NaCl drinking were assessed in the two hamster strains in terms of (i) activities of atrial extracts; and (ii) ability to regulate plasma sodium concentration.

Figure 1 depicts Δ (change from control level, i.e., the difference between 10-min periods before and after the administration of atrial extracts) for water and taurine drinkers in each strain. In both strains, the taurine regime produced diuretic and natriuretic activities that were markedly higher than those of water drinkers. Furthermore, the strain differences in water drinking controls were abolished by a disproportionately larger effect of taurine in BIO 14.6.

The effects of hypotonic NaCl drinking upon the activities of atrial extracts are shown in Fig. 2. The changes in diuretic and natriuretic activities caused by the increased salt intake failed to reach statistical significance within either strain; the strain differences observed in water drinkers were magnified by NaCl drinking.

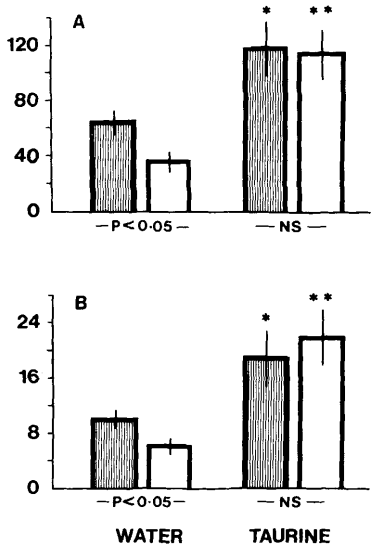

FIG. 1. Changes (Δ) in urine flow (A) and sodium excretion (B) in bioassay rats after injection of atrial extracts from 180-day-old normal F_1B (striped bars) and cardiomyopathic BIO 14.6 (open bars) hamsters drinking water (left) or 0.1 *M* taurine (right). Bars are means ± SEM. Number of bioassays: water drinkers—24 F_1B, 21 BIO 14.6; taurine drinkers—8 F_1B, 6 BIO 14.6. Asterisks indicate difference from corresponding water drinking control using unpaired Student's *t* test. $*P < 0.05$. $**P < 0.01$.

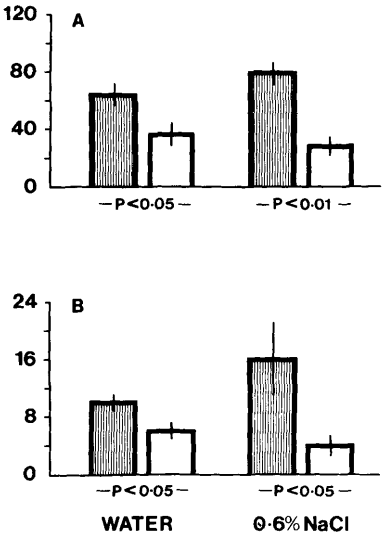

FIG. 2. Changes (Δ) in urine flow (A) and sodium excretion (B) in bioassay rats after injection of atrial extracts from 180-day-old normal F_1B (striped bars) and cardiomyopathic BIO 14.6 (open bars) hamsters drinking water (left) or 0.6% NaCl (right). Bars are means ± SEM. Number of bioassays: water drinkers—24 F_1B, 21 BIO 14.6; NaCl drinkers—9 F_1B and 6 BIO 14.6.

Plasma sodium measurements are presented in Table II. Sodium concentrations did not differ between water drinkers of the two strains; nor did altered drinking regimes change the plasma sodium concentration in F_1B. By contrast, both NaCl and taurine drinking increased plasma sodium by about 5% in BIO 14.6 hamsters.

Discussion. Evidence has been steadily accumulating that the abnormalities in the atrial peptide levels may be associated with conditions of fluid imbalance, altered vascular tone, or cardiac degeneration (14).

In the present study we found reduced diuretic and natriuretic activities of ANF in cardiomyopathic BIO 14.6 hamsters aged 180 days relative to their F_1B controls. This is in agreement with data reported by Chimoskey *et al.* (5), who speculated that this deficiency may be partially responsible for the development of edema. It was reported elsewhere that

TABLE II. PLASMA SODIUM CONCENTRATION IN NORMAL (F_1B) AND CARDIOMYOPATHIC (BIO 14.6) HAMSTERS ON DRINKING REGIMES OF TAP WATER, SALINE (NaCl), OR TAURINE SOLUTION

	Plasma Na^+ concentration (mmole/liter)	
Drinking regime	F_1B	BIO 14.6
Tap water control	145.20 ± 0.95 (9)	148.60 ± 1.96 (5)
NaCl (0.6%)	147.89 ± 0.54 (9)	157.33 ± 1.50[a] (6)
Taurine (0.1 *M*)	145.20 ± 1.46 (5)	155.75 ± 1.03[a] (5)

Note. Values are means ± SEM. Numbers in parentheses are numbers of animals. Statistical comparisons were made using Duncan's new multiple range test for unequal replications.

[a] $P < 0.01$ vs BIO 14.6 tap water control group, and $P < 0.01$ vs corresponding F_1B group.

the intravenous infusion of synthetic ANF for 7 days by a subcutaneously implanted minipump significantly reduced cardiac hypertrophy in BIO 14.6 hamsters (15).

A decreased severity of cardiac lesions and decreased accumulation of calcium was reported in hearts of cardiomyopathic BIO 14.6 hamsters drinking taurine in place of tap water (8). In our experiments long lasting drinking of taurine solution markedly increased ANF activities in both strains of hamsters. The link between ANF and taurine has not yet been studied. An osmoregulatory role has been suggested for taurine in mammalian heart (10, 16), as has a relationship to cardiac sodium homeostasis: acutely hyponatremic rats exhibited a 37% fall in heart taurine concentration (17); chronically hypernatremic mice displayed a 30% rise in heart taurine (16).

In the present experiments, the more pronounced increase of ANF activities caused by taurine drinking in BIO 14.6 hamsters served to eliminate strain differences seen in water drinking controls. The ability of taurine to abolish strain differences between cardiomyopathic and normal hamsters has been reported for other parameters: low cardiac Na^+,K^+-ATPase activity in BIO 14.6 was brought nearly to normal by taurine administration (9), as was high myocardial calcium content in BIO 14.6 (7). ANF content in atria represents the difference between its synthesis and release and does not indicate turnover rates or amounts released into the circulation. The findings presented here cannot distinguish stimulated synthesis from inhibited release of ANF in taurine drinking hamsters. Nevertheless, the profound increase in ANF activities accompanied by severe hypernatremia tempts us to associate the effect of taurine in BIO 14.6 preferentially with inhibited ANF release, even though simultaneously stimulated synthesis cannot be excluded. In F_1B hamsters drinking taurine, ANF activities increased less than in BIO 14.6 hamsters and no rise in plasma sodium level was measured; therefore, increased synthesis without inhibited release might be occurring in these animals. It is worth noting that the rate of taurine influx was found to be higher in hearts of BIO 14.6 than in normal controls (8).

Increased plasma sodium level without any increase in sodium intake in taurine drinking BIO 14.6 hamsters was a rather unexpected result. The same rise in plasma sodium in these animals was caused by hypotonic saline drinking (Table II). Drinking of hypotonic saline solution is a mild challenge to animals with *intact* homeostatic systems; they maintain normal body fluid volume and composition despite long-lasting exposure to this drinking regime. In contrast, drinking of 0.6% NaCl markedly increased plasma sodium concentration in Brattleboro rats, the special Long–Evans substrain with hereditary deficiency of antidiuretic hormone (18).

In the present study, drinking of hypotonic saline caused no significant changes in ANF activities in either strain; existing strain differences not only persisted, but were magnified with BIO 14.6 values markedly lower than F_1B. Hence, the mild increase of salt intake produced hypernatremia only in those animals with low ANF activity. This tempts us to suggest that the deficiency in hormones regulating body fluid volume and composition increases the susceptibility of animals to hypernatremia even without a severe rise in sodium intake.

In conclusion, the present data indicate a link between taurine and ANF. However, further investigations are necessary to determine which of the steps of ANF—synthesis, release, or activation—represents the target for the action of taurine. Furthermore, it remains to be

elucidated if the protective effect of taurine in cardiomyopathic hamsters involves its relationship to ANF.

This work was supported by Kuwait University Research Project MY 005. We thank Mrs. Mini Mathew for her valuable technical assistance.

1. Bajusz E, Baker JR, Nixon CW, Homburger F. Spontaneous, hereditary myocardial degeneration and congestive heart failure in the Syrian hamster. Ann NY Acad Sci **156**:105–129, 1969.
2. Bajusz E. Hereditary cardiomyopathy: A new disease model. Amer Heart J **77**:686–696, 1969.
3. Cantin M, Leone A, Bajusz E. Participation of renal electrolytes in the mechanism of edema in congestive heart failure: Studies on cardiomyopathic hamsters. In: Bajusz E, Rona G, eds. Recent Advances in Studies on Cardiac Structure and Metabolism. Baltimore, Univ Park Press, Vol 1:pp303–324, 1972.
4. de Bold AJ, Bornstein HB, Veress AT, Sonnenberg HA. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. Life Sci **28**:89–94, 1981.
5. Chimoskey JE, Spielman WS, Brandt MA, Heidemann SR. Cardiac atria of BIO 14.6 hamsters are deficient in natriuretic factor. Science (Washington, DC) **223**:820–822, 1984.
6. Welty MC, Welty JD, McBroom MJ. Effect of isoproterenol and taurine on heart calcium in normal and cardiomyopathic hamsters. J Mol Cell Cardiol **14**:353–357, 1982.
7. McBroom MJ, Welty JD. Effects of taurine on heart calcium in the cardiomyopathic hamster. J Mol Cell Cardiol **9**:853–858, 1977.
8. Azari J, Brumbaugh P, Huxtable R. Prophylaxis by taurine in the hearts of cardiomyopathic hamsters. J Mol Cell Cardiol **12**:1353–1366, 1980.
9. Welty JD, Welty MC. Effects of taurine on subcellular calcium dynamics in the normal and cardiomyopathic hamster heart. In: Schaffer SW, Baskin SI, Kocsis JJ, eds. The Effects of Taurine on Excitable Tissues. New York, Spectrum Publ, pp295–312, 1981.
10. Atlas M, Bahl JJ, Roeske W, Bressler R. In vitro osmoregulation of taurine in fetal mouse hearts. J Mol Cell Cardiol **16**:311–320, 1984.
11. Hastings DF, Welty JD, Rohani F. Taurine stimulation of isolated hamster brain Na^+,K^+-ATPase: Activation kinetics and chemical specificity. J Neurochem **44**:1764–1770, 1985.
12. Varley H, Gowenlock AH, Bell M. Practical Clinical Biochemistry. London, Heinemann Medical Books, Vol 1:pp548–549, 1980.
13. Kramer CY. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics **12**:307–310, 1956.
14. Needleman P, Adams SP, Cole BR, Currie MG, Geller DM, Michener ML, Saper CB, Schwartz D, Standaert DG. Atriopeptins as cardiac hormones. Hypertension **7**:469–482, 1985.
15. Heinel LA, Blaine EH. Atrial natriuretic factor (ANF) reduces cardiac weight of hamsters with congestive heart failure. Fed Proc **44**:1730, 1985.
16. Thurston JH, Hauhart RE, Naccarato EF. Taurine: Possible role in osmotic regulation of mammalian heart. Science (Washington, DC) **214**:1373–1374, 1981.
17. Welty JD, McBroom MJ, Appelt AW, Peterson MB, Read WO. Effect of taurine on heart and brain electrolyte imbalances. In: Huxtable R, Barbeau A, eds. Taurine. New York, Raven Press, pp155–163, 1976.
18. Dlouha H, Krecek J, Zicha J. Hypertension in Bratleboro rats and the injurious influence of salt in youth. Physiol Bohemoslov **30**:531–538, 1981.

Received September 17, 1985. P.S.E.B.M. 1986, Vol. 181.
Accepted November 13, 1985.