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Adverse Effects of Dietary and Furosemide-Induced Sodium Depletion on Thermoregulation in Rats

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ABSTRACT

In this study the diuretic furosemide was used in combination with dietary sodium (Na) restriction, to produce varying degrees of circulatory hyponatremia to quantify the effects of moderate to severe Na-depletion on heat tolerance in a validated, heat stressed rat model. Male Sprague-Dawley rats (500 g) were subjected to a Na-depletion regimen as follows: group I (control) had free access to a normal diet and tap water; group II consumed the normal diet and tap water but was treated with the diuretic furosemide (10 mg/kg/day, ip); group III had free access to a Na-free diet and deionized drinking water; group IV consumed the same Na-free diet and electrolyte-free water but was also treated with furosemide. Both the dietary and drug manipulations effected significant ($p<.05$) negative Na and water balance and hyponatremia in the experimental groups. Group IV consistently exhibited the greatest decrements. Following four days of depletion all four groups were acutely exposed to a 42°C environmental heat stress during which time rectal temperature increased. The time required for rectal temperature to reach 42.6°C was significantly ($p<.05$) decreased to 176 ± 14 and 181 ± 8 minutes in groups II and III, respectively, from a control time of 242 ± 8 min in group I; tolerance for group IV was decreased even more to 111 ± 11 minutes. It is concluded that in the sedentary rat Na deprivation and diuretic treatment can elicit a 25-50% reduction in heat tolerance which is partially due to electrolyte depletion and hypohydration. These data suggest that during environmental heat stress uncompensated negative Na balance may predispose an individual to heat illnesses.

Key words: heat tolerance, sodium balance, aldosterone, dehydration, water balance, plasma volume, rats, furosemide.

INTRODUCTION

The effects of sodium (Na) depletion on the ability to work and thermoregulate in the heat in addition to its contribution to the number and severity of heat injuries is still not well defined (1, 19, 23), and thus, requires further laboratory investigation. Na depletion is a significant problem not only for the unacclimated (20, 22) and those on Na-restricted diets, but also for trained individuals such as athletes and soldiers forced to work vigorously in hot environments (3). The signs and symptoms of Na depletion include fatigue, generalized weakness, muscle cramps, gastrointestinal disturbances, decreased blood pressure, and altered sensoria (2, 25). Clinical findings include hyponatremia, hypochloremia, and reduced urinary Na and chloride excretion. Na-depletion may therefore adversely affect physical performance.

In addition to the effects of Na-depletion on physical performance, hyponatremia may also affect thermoregulation. To date, relatively few studies have addressed the impact of Na depletion on thermoregulation alone (13, 15, 16). Technically, it is difficult to render humans and especially rodents hyponatremic by dietary Na restriction alone (15, 21, 24). For example, following chronic consumption of a Na-restricted diet, Francesconi, et al. (15, 16) reported no significant differences in plasma Na levels between a Na-restricted and a control group of immature rats. These observations were attributed to elevated mineralocorticoid activity, reduced plasma volume, and trace amounts of Na in the drinking water. However, previous work has indicated that hyponatremia can be induced acutely in experimental animals with diuretics (6, 12). It was therefore postulated that graded degrees of hyponatremia in rats could be achieved by using a combination of dietary Na restriction and diuretics. This design was therefore utilized to produce moderate to severe hyponatremia in rats

and to assess its effects on thermoregulation and clinical chemical responses in a validated rat model of severe heat stress.

METHODS

Animals. Adult male Sprague-Dawley rats (475-525 g, CD-1 strain, Charles River Breeding Laboratories, Wilmington, MA) used in this study were housed individually in wire-bottom cages and allowed to acclimate for at least one week to standard conditions: ambient temperature=22°C, fluorescent lighting on 0600-1800h, complete rodent diet and water ad libitum. Rats were then subjected to a 4d Na depletion regimen followed on the fifth day by an acute exposure to a severe environmental heat stress.

Acute Na depletion. Rats were made hyponatremic either by consumption of a Na-free diet, (Hartroft, US Biochemical Cleveland, OH, Cat. No. 21675), administration of the diuretic furosemide (Lasix®, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ), or a combination of both. It was anticipated that by varying the diet and using a diuretic one could achieve a gradation in the degree of hyponatremia. Thus, on day 1, rats were randomly assigned to one of the following four groups:

I. Normal Diet, No furosemide (control, n=17)

II. Normal Diet, Furosemide (n=20)

III. Na-free Diet, No Furosemide (n=18)

IV. Na-free Diet, Furosemide (n=21)

During this 4d experimental period, rats were housed individually in metabolic cages and allowed ad libitum access to either tap water (groups I, II) or Na-

free, deionized water (groups III, IV) and to either a normal diet (Purina rat chow #5001, Na=0.18 mEq/g, K=0.28 mEq/g) or a Na-free diet (Na=0 mEq/g, K=0.26 mEq/g). Protein and caloric content of each diet were similar.

Between 0800 and 1000h on days 1 through 4, rats in groups II and IV received an intraperitoneal injection of furosemide (10 mg/kg/day) and were immediately returned to their cages. Within 30 min prominent diuretic effects were observed. All groups of rats were monitored daily for body weight changes, food and water consumption, and urine volume and electrolyte content. Na and K concentrations were measured using standard flame photometric techniques (FLM3, Radiometer America, Cleveland, OH). Na, K and water balances were calculated from urinary output and dietary intake.

Sedentary heat stress. At approximately 0800h on the fifth day rats were passively exposed to environmental heat stress of 42°C; during this interval rats were housed individually in standard wire bottom cages without access to food or water. Just prior to heat exposure, body weight was recorded and a pre-heat stress 0.5 ml heparinized venous blood sample was withdrawn without stasis from a lateral tail vein. Rectal temperature (T_{re}) was monitored at 15 minute intervals by inserting a thermistor probe (YSI Model 423, Yellow Springs, OH, time constant = 1.4 sec) 6 cm into the colon. Temperature was displayed on a digital thermistor readout (Doric Model 450, San Diego, CA). Rats remained in the heat until their T_{re} reached a targeted value of 42.6°C since it has been demonstrated in this model that at this T_{re} there occur significant alterations in clinical indices of heat stress (15, 16, 18). T_{re} in normothermic rats is an 37.5°C. Animals were then quickly removed to a thermoneutral environment at 26°C and monitored for signs of cooling down during a 30 min recovery period. The duration of exposure required to elevate T_{re} to 42.6°C is referred to as the

heat tolerance time which is a measure of thermoregulatory capability of the animals.

Following the 30 min recovery period, a heparinized, post-heat stress blood sample was taken from anesthetized (halothane) rats via percutaneous cardiac puncture. Both pre and post heat-stress blood samples were immediately analyzed in triplicate for hemoglobin content using the cyanomethemoglobin method (Boehringer Mannheim Diagnostics, Indianapolis, IN) and for hematocrit in duplicate using the microhematocrit technique. Percent changes in plasma volume pre to post heat stress were calculated from hematocrit and hemoglobin changes by the method of Dill and Costill (8). The remainder of the blood sample was centrifuged (10,000 g, 4°C, 10 min) and the plasma was separated and stored on ice. Plasma samples were analyzed on the same day as the experiment for changes in clinical indices of heat stress and hypohydration. Total plasma protein was determined using a hand-held refractometer (AO Reichert, Buffalo, NY), and plasma osmolality by freezing point depression (Microosmometer, Precision Systems, Natick, MA). The remaining plasma was frozen and stored for measurement of circulating aldosterone levels by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA).

Statistical analyses of inter-group differences were performed by analysis of variance followed by a Bonferroni multiple t-test for all possible pairwise comparisons (BMDP7D) (9). A paired t-test was used to determine intra-group differences, i.e. comparisons between pre- and post-heat stress values. The null hypothesis was rejected at $p \leq 0.05$. In all cases, data are presented as mean $\pm$ S.E.M.

RESULTS

Acute Na depletion. The cumulative effects of the 4d Na-depletion on electrolyte and water balances are shown in Table 1. The results for groups II, III and IV have been normalized to group I, the control group. Note that all experimental groups experienced a significant negative Na, K and water balance when compared to group I. As anticipated, a graded negative Na balance was achieved with the experimental protocol. Balances of -8 ± 1, -11 ± 1 and -14 ± 1 mEq were observed in groups, II, III and IV, respectively, and were significantly different at each level. The effects on water balance appeared to mirror the effects on Na balance in that the degree of hypohydration increased with each group. The effects of the 4d depletion regimen on K balance showed a less clear trend, but was consistently affected by furosemide treatment, with balances of -13 ± 1 and -14 ± 1 mEq in groups II and IV, respectively.

Sedentary heat stress. Heat tolerance time was significantly ($p < .05$) reduced in groups I', III and IV when compared to group I (Table 2). Fluid losses due to spreading of saliva for evaporative heat loss resulted in respective body weight losses of 41 ± 2, 30 ± 2, 32 ± 2 and 19 ± 2 g, all significantly different from each other. However, the average rates of body weight losses (mg/min) were similar in all groups. The greatest rate of heat gain during the environmental heat stress was observed in group IV (.095 ± .010°C/min/Kg) and the least in group ' (.036 ± .010°C/min/Kg). The maximum rate of heat loss, calculated from the decrease in T_{re} during the 30 min recovery period, was observed in group II (.202 ± .022° C/min/Kg, $p < .01$ vs groups I, III and IV) while the T_{re} of rats in group I unexpectedly continued to rise during recovery. Percent plasma volume changes appeared to be quite variable but were the greatest, -24%, in groups III and IV. Animals in group II experienced a

change in plasma volume of only -2%. Group I experienced a typical 13% decrease in plasma volume.

Changes in plasma constituents pre- and post-heat stress are presented in Table 3. Pre-heat stress plasma Na concentration was significantly ($p < .05$) decreased in groups III and IV when compared to group I, demonstrating the efficacy of the acute Na depletion regimen to induce moderate to severe hyponatremia. As anticipated, plasma Na concentration increased in all four groups following heat exposure primarily due to loss of plasma volume as a result of enhanced salivary secretion for evaporative heat loss. Pre-heat plasma K levels were not significantly different among all groups indicating that all animals were normokalemic following the 4d depletion regimen. The effects of heat stress on plasma K concentration were diverse with no significant changes seen in groups I and III, a significant decrease in group II, and a significant increase in group IV. As a direct result of the decreased [Na^+], pre-heat plasma osmolality was significantly ($p < .05$) less in groups III and IV as compared to group I. Plasma osmolality increased to a similar post-heat stress mean (325 mOsm/Kg) in all four groups. Pre-heat hematocrits and plasma protein concentrations were not statistically different among groups I, II and III. In contrast, animals in group IV experienced severe hemoconcentration due to the 4d depletion regimen as indicated by the significant ($p < .05$) increase in pre-heat hematocrit to 49.6% as well as the significant increase in plasma protein concentration to 7.1 mg/dl. A statistically significant increase in both hematocrit and plasma protein due to heat stress was observed in all groups except III. Animals in group II experienced no change in hematocrit or plasma protein which correlates with the small percent change in plasma volume. Pre-heat stress plasma aldosterone levels were significantly elevated ($p < .05$) due

to the Na depletion regimen in groups II, III and IV as compared to group I. The greatest increase in pre-heat stress plasma aldosterone was observed in group IV which correlates well with the degree of hyponatremia in this group. Interestingly, despite markedly different pre-heat stress levels, exposure to heat induced approximately a two-fold increase in plasma aldosterone levels in all four groups.

DISCUSSION

The sodium depletion regimen used in these studies was selected because of its potential ability to create varying degrees of electrolyte depletion in rats. The experimental regimen elicited negative Na, K and water balances and a resultant hyponatremia of 125-138 mEq/L in groups II, III and IV. Along with the decrease in the plasma Na concentration there occurred reductions in plasma osmolality subsequent to loss of electrolytes. It is interesting to note that despite the significant increments in circulating aldosterone levels with increasing hyponatremia, the animals were still unable to conserve Na during this comparatively brief experimental interval. Concomitant with the hyponatremia observed in groups II, III and IV, there occurred a dehydration manifested in the tendency for pre-heat stress hematocrit and plasma protein concentrations to increase with increasing hyponatremia.

In contrast to earlier work using a chronic Na-depletion regimen (15, 16), we had no difficulty in producing circulatory hyponatremia using this 4d experimental design. It is recognized that in the previous studies chronic consumption of the low Na diet may have permitted more efficient hormonal retention of Na, reestablishment of electrolyte homeostasis in a reduced plasma

volume, significantly reduced losses of Na in urine and saliva, and batch-variability in dietary and water content of Na. It is possible that in the present study rats, especially in group III, may have been able to restore plasma electrolyte levels under more chronic conditions. In addition, older animals used in the present study may have been less able to conserve electrolytes as compared to the younger immature rats in the previous study.

It is clear from this study that negative electrolyte and water balances have detrimental effects on the ability of rats to thermoregulate in a hot environment. Animals in groups II, III and IV experienced a 27%, 25% and 54% decrement in heat tolerance time, respectively, as compared to group I. Animals in the control group (I) responded to the 42°C environmental heat stress as anticipated with significant radiant and evaporative heat loss. To maintain a normal core temperature evaporative heat loss is achieved in this non-sweating animal by salivation and a behavioral spreading of the saliva on the scrotum, foot pads and ventral body surface (11, 17). Since the rate of salivation in rats and the rate of sweating in humans and the electrolyte composition in each are regulated by similar cholinergic mechanisms the rat has been a useful model for heat stress in humans. Weight loss in the heat is therefore recorded as an indicator of body fluid losses. Heat tolerance time, body weight loss and percent body weight loss in group I is virtually identical to the results of Hubbard et al., using the same size rat, environmental heat stress, and an identical target T_{re} of 42.6° C (18). The pre-heat stress values for typical indices of heat stress for this group (Table 3) are within normal ranges (4) and the responses following heat stress, as observed in the post-heat stress blood samples, are also typical (15, 16, 18). Animals in group II, subjected to moderate hyponatremia, experienced a significant reduction in heat tolerance.

Body weight loss and percent body weight loss during heat stress were significantly less than in the control group (Table 2). Despite this apparent difference in the ability to thermoregulate, all the pre-heat stress plasma constituents, except aldosterone, were similar in groups I and II. Although, group II, unlike group I, did not experience significant increases in hematocrit, plasma Na, or plasma protein following heat stress. These data correlate with the small -2% change in plasma volume in group II. It was unusual to observe such a small plasma volume change considering the duration of time these animals spent in the heat and the relatively large body weight loss experienced. Normally, plasma volume in the heat is maintained by shifting body fluids from interstitial and intracellular compartments (10). The small plasma volume change in this group may suggest that these animals were able to maintain plasma volume despite absolute losses in body fluids by shifting fluids more rapidly than normal from the other compartments. There is a significant physiological advantage to maintaining plasma volume during heat stress since this inturn implies that cardiac output will be maintained and so will organ perfusion, especially in the cutaneous vascular bed which participates in heat dissipation. The fact that the average cooling rate in this group of rats was 0.201°C/min/kg, more than twice any other group, highly supports this contention that peripheral circulation was maintained in these animals. Since the last dose of furosemide was administered 24h prior and the half-life is less than 1h, it is not likely that the drug was present in the body at the time of heat stress and could have contributed to these effects.

This observation brings about an interesting notion of artificial or pharmacologically induced heat acclimation and the question, whether or not lower doses of furosemide could still have provided the beneficial effects seen in

plasma volume while increasing heat tolerance time. We hypothesize that the daily diuretic challenges forced the animals in group II to transiently mobilize fluids from the extravascular spaces into the plasma. This daily challenge on fluid balance over the 4 days may have been analogous to an individual experiencing daily fluid losses due to heat stress. Prior investigations inwhich furosemide was administered to humans in a single bolus or oral administration followed within hours by heat or exercise stress resulted in decrements in thermoregulatory and exercise tolerance (5, 7). Due to the design of these studies the subjects were not allowed time to rehydrate before they were subjected to heat stress which accounted for the detrimental effects. Therefore no study has been conducted to date which tests this fluid mobilization hypothesis.

Complete dietary Na restriction alone (group III) and diuretic administration alone (group II) resulted in similar heat tolerance times in each group, 176 and 181 min, respectively. Closer examination revealed that animals in group III responded to the heat stress significantly different from group II, especially during the recovery period following heat stress. For example, those animals in group III experienced a large decrease in plasma volume (-24%) and consequently had a lower rate of heat loss during recovery, 0.088°C/min/kg. The apparent inability of the animals in group III to rapidly mobilize fluids into the plasma is manifested by the significant increases in most plasma constituents following heat stress. Furthermore, it has been demonstrated that humans on a Na restricted diet experience hemoconcentration, reductions in ^{22}Na space, and reduced cardiac output (14), all of which can lead to reduced heat loss. Although relatively few patients, including severe hypertensives are placed on a complete Na free diet as were the animals in group III, these data do suggest

that patients experiencing negative electrolyte and fluid balances associated with Na restricted diets would be vulnerable to heat illnesses.

Administration of furosemide to animals on a Na free diet (group IV) resulted in excessive electrolyte and water losses over the 4d depletion regimen. These animals were severely hyponatremic, with a plasma Na concentration of 125 mEq/L, and hypoosmotic. Reductions in plasma Na level of this magnitude can result in neurological, muscular and metabolic impairments (2, 25). Prior to heat stress on day 5, the animals in group IV displayed overt signs of lethargy, poor grooming and gastrointestinal disturbances. Animals in this group were also severely dehydrated by the 4d depletion regimen as evidenced by the significant hemoconcentration with a hematocrit of 49.6% and an increase in a plasma protein concentration to 7.1 mg/dl). Thus, with dehydration there are less total body fluids available for secretion and evaporation (10). Interestingly, animals in group III and IV, despite differences in heat tolerance times, each experienced a 24% reduction in plasma volume in the heat, suggesting there exists a maximum limit of plasma volume loss at which thermoregulation can no longer occur probably due to cardiovascular instability.

In summary, dietary Na restriction and administration of the diuretic in furosemide, alone or in combination, resulted in significant decrements in heat tolerance and thermoregulation in rats. Since prior to heat stress, animals in the experimental groups were hyponatremic with hemoconcentration, but normokalemic, it is concluded that Na depletion and dehydration account for the decrements in thermoregulation observed in this study.

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals".

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Table 1

Electrolyte and Water Balances Normalized to
Group I (Control) Over the 4-Day Depletion Regimen

	Group I	Group II	Group III	Group IV
Diet	Normal	Normal	Na-free	Na-free
Furosemide	--	10 mg/kg	--	10 mg/kg
n	17	20	18	21
Na-balance (mEq)	--	-8 ± 1^{a}	-11 ± 1^{ab}	-14 ± 1^{abc}
K-balance (mEq)	--	-13 ± 1^{a}	-9 ± 1^{ab}	-14 ± 1^{ac}
Water-balance (ml)	--	-38 ± 4^{a}	-54 ± 3^{ab}	-65 ± 8^{ab}

Values are given as $X \pm$ S.E.;
a: significantly different from Group I ($p \leq 0.05$)
b: significantly different from Group II ($p \leq 0.05$), and
c: significantly different from Group III ($p \leq 0.05$).

Table 2

Effects of Na-Depletion Regimen on Heat Tolerance, Fluid Loss and Thermoregulation in Sedentary Unrestrained Rats Exposed to a 42°C Environmental Heat Stress

	Groups			
	I	II	III	IV
Heat tolerance (min)	242 ± 9	176 ± 14[a]	181 ± 8[a]	111 ± 11[abc]
Body weight loss (g)	41 ± 2	30 ± 2[a]	32 ± 2[a]	19 ± 2[abc]
% body weight loss	8.8 ± 0.4	6.4 ± 0.5[a]	6.7 ± 0.5[a]	4.2 ± 0.4[abc]
Rate of body weight loss (mg/min)	169 ± 3	170 ± 5	180 ± 10	168 ± 4
Heat gain (°C/min/Kg)	.036 ± .002	.052 ± .005[a]	.048 ± .003[a]	.095 ± .010[abc]
Heat loss (°C/min/Kg)	-.056 ± .023	.201 ± .022[a]	.088 ± 0.015[ab]	.055 ± .013[ab]
% Plasma volume change	-13 ± 2	-2 ± 2[a]	-24 ± 4[b]	-24 ± 1[ab]

Values are given as X ± S.E.;
a: significantly different from Group I ($p \leq .05$),
b: significantly different from Group II ($p \leq .05$), and
c: significantly different from Group III ($p \leq .05$).

Table 3

Changes in Plasma Constituents Measured Pre and Post Exposure
to a 42°C Environmental Heat Stress in Sedentary, Unrestrained Rats Previously Na-Depleted

	Groups			
	I	II	III	IV
Hematoctrit (%)				
Pre	45.5 ± 0.6	46.3 ± 0.7	45.7 ± 0.7	49.6 ± 0.6^{abc}
Post	48.8 ± 0.5*	47.2 ± 0.8	50.8 ± 0.8^{*b}	54.2 ± 0.8^{*abc}
Plasma Na^+ (mEq/L)				
Pre	140.2 ± 1.4	138.4 ± 1.5	129.1 ± 2.5^{ab}	125.1 ± 2.2^{ab}
Post	152.1 ± 1.3*	140.6 ± 1.7^{*a}	142.0 ± 2.1^{*a}	132.8 ± 2.0^{*abc}
Plasma K^+ (mEq/L)				
Pre	5.2 ± 0.1	5.6 ± 0.2^{*a}	5.1 ± 0.2	5.7 ± 0.2^{*abc}
Post	4.9 ± 0.2	3.9 ± 0.2^{*a}	5.2 ± 0.2^{b}	6.6 ± 0.4^{*abc}
Plasma Osmolaity (mOsm/Kg)				
Pre	304 ± 1	305 ± 2	295 ± 1^{ab}	294 ± 2^{ab}
Post	325 ± 2*	326 ± 2*	325 ± 3*	324 ± 4*
Plasma Protein (mg/dl)				
Pre	6.4 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	7.1 ± 0.2^{abc}
Post	7.0 ± 0.1*	6.5 ± 0.1^{a}	7.6 ± 0.2^{*b}	7.9 ± 0.2^{*ab}
Plasma Aldosterone (ng/dl)				
Pre	25 ± 3	79 ± 10^{a}	102 ± 20^{a}	304 ± 47^{abc}
Post	64 ± 8^{*a}	157 ± 22^{*a}	238 ± 43^{*a}	626 ± 104^{*abc}

Values are given as X ± S.E.;
*: significantly different from pre-value ($p \leq .05$),
a: significantly different from Group I ($p \leq .05$),
b: significantly different from Group II ($p \leq .05$), and
c: significantly different from Group III ($p \leq .05$).