Natriuresis After a Water Load in Humans Under Different Sodium Body Content

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ROSAS-ARELLANO, M. P., A. GUEVARA-ROJAS, R. GUEVARA-GUZMÁN and L. P. SOLANO-FLORES. Natriuresis after a water load in humans under different sodium body content. PHYSIOL BEHAV 52(4) 647-653, 1992.—The present study was aimed at observing the diuretic and natriuretic responses after a water load (2% body weight) in four groups of young consenting volunteers submitted previously, during three days, to a hypersodic (500 mEq Na/day), hyposodic (35 mEq Na/day), and normosodic (200 mEq Na/day) diet, or treated with furosemide (Lasix, 40 mg/day). During the treatment urine was collected each day. On the fourth day, in the morning, the bladder was emptied, the water load was ingested, and the urine collected during 10 periods of 20 min each. The urinary, sodium, and chloride flows were determined. The four groups displayed diuretic curves following a similar pattern. In contrast, the natriuretic curves of the four groups were completely different; totally flat with low values for the furosemide group and a large initial natriuretic curve for the hypersodic group with a gradual decrease but maintaining high values. The results indicate that the way the organism compensates for the excess of water by means of urinary water loss is independent of the body sodium content, whereas the way in which sodium loss is accomplished is determined by its body content and is independent of the way in which the water is lost.

Sodium diet Natriuresis Diuresis Humans Water Load Homeostasis Body fluids
Renal physiology Kidney Furosemide

IN mammalian species, such as rat (12,24), dog (11), goat (1,2,4), and sheep (9), it has been observed that the injection of hypertonic NaCl solution into the third cerebral ventricle rapidly induces a marked and lasting increase in urinary sodium (Na) output. Additionally, in some of these same studies, intracerebroventricular injection of equally hypertonic solutions of other solutes, such as glucose (1,12) or NH4Cl (1), were ineffective in that regard. In other studies, the ventriculocisternal perfusion with isosmotic low-sodium solution or hyposmotic low-sodium solution produced a notable decrease of the renal Na output (25), an increase in plasmatic Na concentration, and excessive loss of water (8). Furthermore, the natriuresis induced by an intravenous hypertonic NaCl load could be blocked by lowering the cerebrospinal fluid Na concentration (8). Finally, the natriuresis observed in normal sheep after water deprivation (22) or after intravenous infusion of hypertonic saline (19) was blocked by ablation of the anterior third ventricle. All these findings, as a whole, suggest that the brain might be involved in the Na homeostatic mechanisms.

On the other hand, a sudden and prolonged antidiuresis and antinatriuresis are caused by intragastric administration of a water load equivalent to 10% of the dog's body weight in the conscious animal. If the water loading is repeated at 4-5-day intervals, the antinatriuretic response remains unchanged, but a gradual increase of the urinary volume turns the initial antidiuretic response into a progressive water diuresis after the fourth or fifth test (14,15). These data indicate that Na and water excretion can be regulated independently, and also that the corresponding mechanisms interact in harmony to maintain fluid composition under the imposed disturbances. Several authors (3,13,23) have proposed that renal excretion of Na depends on a specific mechanism that regulates body Na content. However, no experimental work has been made to study the relationship between Na and water excretion in humans submitted to body fluid disturbances.

From all the above, if a specific body Na content homeostatic mechanism existed, interacting with a body water content homeostatic mechanism, it follows then that their activity under different body Na content conditions would be reflected in the urine composition. Particularly, the Na output pattern as well as the water excretion pattern, after body fluids dilution by a water load, would depend on the previous Na content of the organism.
Hence, the aim of the present work was to determine water and Na output, plasmatic Na levels, and blood osmolality after a water loading in normal young humans submitted previously to a 3-day hyper-, hypo-, or normosodic diet, or treated with the natriuretic drug furosemide. The results indicate the existence of a homeostatic mechanism for the body Na content that seems to operate independently, and in harmony, with the homeostatic mechanism for the body water content.

**METHOD**

Experimental subjects were volunteer consenting young medical students of either sex. Their age ranged from 19 to 25 years. Men's weights were 52-80 kg and their heights, 1.63-1.84 m. Women's weights were 46-64 kg and their heights, 1.5-1.65 m. For the women, the experimental procedures were done at least 9 days after the first day of menstruation. They were also checked to present regular menstrual cycles of 28-30/4 days. All the subjects were apparently healthy, with no pathological medical report, and without present medical treatment. Medical supervision was available during all the phases of the experiment. The subjects participated in the present study as part of their Human Physiology Laboratory course; hence, all the participants were informed in the theory of the problem and were actively involved in the experimental procedure. Four groups of subjects were formed with equal numbers of men and women: the normosodic diet group (NSG; n = 16), the hyposodic diet group (HYPOSG; n = 8), the hypodietic group (HYPERSG; n = 8), and the furosemide-treated group (FURG; n = 8). The subjects were submitted to their respective diet or drug treatment during the 3 previous days to the day of the water load. The normosodic and the hypodietic diets were applied as indicated in the diet tables from the Merck Manual (5). The hypodietic diet corresponded to the normal diet but included an additional supply of salty snacks: 10 brine-soaked olives or 40 g of salty cheese during each meal, three times a day and also a package (six pieces) of brine-soaked apricots three times a day. All the subjects had a list of the type and amount of food allowed. In this way, the daily Na intake for the groups was as follows: NSG, 150-200 mEq; HYPOSG, 22-35 mEq; HYPERSG, 500 mEq. The FURG group followed the normosodic diet and was given orally a single dose of 40 mg/day of furosemide (28) (Lasix, Qulmica Hoechst de Mrxico) before breakfast during the 3 days previous to the day of the water load. The subjects immediately drank an amount of distilled water equivalent to 2% of their body weight during no more than 10 min. Every 20-min period from the start of the water loading, the bladder was totally emptied. In this way, 10 samples were collected. Three 10-ml venous blood samples were also obtained from the median cubic vein. The first sample was obtained 5 min before the start of the water load. The second sample was obtained 40 min after the start of the water load, corresponding this time to the second period of urine collection. The third sample was obtained 70 min after the start of the water load, that is, 10 min before the time of the fourth period of urine collection had been completed. Plasma samples were processed by conventional centrifugation procedures. The volume of the urine samples was measured and the urine flow (UV, ml/min) was calculated. The urine and plasma Na concentration was determined by flame photometry (Beckman Instruments, DU 2400) and the Na flow (UNaV, μmol/min) was calculated. Urine Cl concentration was determined by the Schales and Schales-Brun method (33) and the Cl flow (UClV, μmol/min) was calculated. Plasma osmolality was determined by freezing point depression with the aid of an osmometer (Os-met 2007, Precision Systems, Inc.). A two-factor split plot analysis of variance design was used, followed by a post hoc Duncan test for multiple comparisons: p < 0.05 was taken as the probability level of statistical significance. Data are presented as means.

**RESULTS**

Results are depicted in Fig. 1 and Table 1. In the four groups, no statistical intragroup nor intergroup differences were detected in the UV values during the 3 days of diet or furosemide treatment. Except for HYPERSG on the first day, the UNaV values of HYPERSG, HYPOSG, and FURG, during the 3 days of diet or furosemide treatment, were not statistically different from the UNaV values of NSG. The UNaV value of HYPERSG was significantly greater than that of HYPOSG on the first day and greater than that of HYPO and of FURG on the third day. No statistical intragroup differences were observed in UNaV values during the 3 days of diet or furosemide treatment. No statistical intragroup or intergroup differences were observed in UClV values during the 3 days of diet or furosemide treatment. Taking into account the above, the UV, UNaV, and UClV values for the third day of diet or furosemide treatment were used as controls. In the first 20-min period of urine collection, following the start of the water loading, the UV values for the four groups were not statistically different from the corresponding UV control values. In the second period, the FURG UV value was still not different from its control value. The diuretic effect due to the water load was already observed in the second period for HYPERSG, HYPOSG, and NSG. In the third period, the pattern of the UNaV curves was statistically heterogeneous among the groups during the experiment. From the first to the last period, the UNaV curve of FURG was maintained flat without differing statistically from its control value. The NSG and HYPOSG UNaV curves were statistically similar during the whole experiment. A significant natriuretic effect was observed in these two groups during the first and second periods; from the third period until the end of the experiment, the UNaV values were not different from their respective control values. The HY-
FIG. 1. Diuretic (A), natriuretic (B), and Cl excretion (C) responses after a water load in human subjects under different body Na contents, induced by a hypersodic (○), hyposodic (▲), or normosodic (●) diet, or furosemide treatment (△). The first three numbers on the abscissa, above the days label, represent the 3 days of diet or furosemide treatment previous to the day of water loading. The 0 in the abscissa represents the moment at which the bladder of the subjects was emptied and the water loading started. Black squares represent the 10 min of water loading. The numbers to the right of 0 represent the 10 successive 20-min periods of urine collection counted from 0.

PERSG group displayed a peculiar $U_{NaV}$ curve. A marked natriuresis occurred in the first period followed by a significant reduction of Na excretion during the second period, reestablishing the control value. In the third period, the natriuretic effect was observed again. From the third period on, a gradual decrease of Na excretion started to occur, and the control value was reached and maintained from the fifth period on.

In general, the FURG $U_{NaV}$ curve kept lower values with respect to the NSG $U_{NaV}$ curve, and the HYPOSG $U_{NaV}$ curve kept higher values. During the whole experiment, the HYPOSG $U_{NaV}$ values were always higher than those of FURG.

In summary, HYPOSG generally presented a natriuretic effect throughout the experiment, whereas FURG presented an antinatriuretic effect with respect to NSG.

The $U_{ClV}$ curves presented, generally, a pattern parallel to the $U_{NaV}$ curves. The FURG $U_{ClV}$ curve was practically equal to the $U_{NaV}$ curve. The NSG, HYPOSG, and HYPOSG $U_{ClV}$ curves always maintained higher values as compared to the respective $U_{NaV}$ curves. The HYPOSG $U_{ClV}$ curve presented a peculiar plateau of high Cl excretion during the third, fourth, and fifth periods. The time at which the control values of Cl excretion were reached was delayed with respect to the time at which the control values of Na excretion had been reached. Thus, the HYPOSG and NSG $U_{ClV}$ control values were reached in the fifth period, whereas those of HYPOSG were reached in the seventh period.

No intergroup nor intragroup statistical differences were observed for the plasmatic Na and Cl concentrations in the three plasma samples of the four groups. Plasmatic Na concentration range was 122.01 ± 7.83 to 156.75 ± 9.63 mmol/l.

Statistical differences were only observed in the plasma osmolality between the first (312.39 ± 4.24 mOsm/kg) and the third (296.95 ± 5.26 mOsm/kg) sample of HYPOSG and between the third sample of HYPOSG and the third sample of NSG (308.11 ± 3.26 mOsm/kg) and HYPOSG (312.19 ± 9.74 mOsm/kg).

DISCUSSION

The present study provides experimental evidences in humans that indicate the existence of a homeostatic mechanism for the body Na content, which seems to operate independently, and in harmony, with the homeostatic mechanism for the body water content.

The composition of body fluids, in a given moment, is determined by the relationship of two components: the solutes and the solvent. This composition can be altered by modifying the input or the output of each or both of these components. However, starting from a given composition, a certain new composition can be attained by different ways. Thus, the status of a body presenting a normal fluid composition can be changed into a hyperconcentrated status by addition of solute or by withdrawal of solvent. In a similar manner, a diluted status can be obtained by withdrawal of solute or by addition of solvent. In the study of body fluids physiology, different experimental models for disturbing any of the components of the body fluids have been used to observe the corresponding compensating actions of the homeostatic mechanisms. For instance, a natriuretic compensating response has been observed in hyperconcentrated animals due to dehydration (21). Urinary Na retention ensued body fluid dilution by means of rehydration in a dehydrated animal (21). A hypertonic NaCl intravenous load resulted in a compensating natriuresis (8). A severe antinatriuretic response was produced by a water load (14,15). Manipulation of Na intake by means of a controlled diet, or manipulation of Na output by
The four groups of the present study displayed UV curves for subjects, which were under different body Na content conditions. The subjects of FURG were in a severe Na deficit condition, while the acute excess of water in the organism due to disturbing water loss was independent from the semichronic circumstances, the acute excess of water in the organism due to a Na excess is able to compensate for the excess of water by means of urinary water loss.

Different body Na content conditions can be attained by different semichronic Na diets or natriuretic drug treatments, which result in a stabilization of the organism Na content to values that depend on the corresponding two groups for that day or, for that period, for either the UV, UNaV, or UqV curves. This indicates that the water balance for the four groups was available. The HYPERSG group, which was in a Na surplus condition, presented an initial marked Na loss followed by a slow and gradual decrease of Na excretion, but always presented high values with respect to the other groups. The UNaV curves of these two groups were, then, completely different and were also different from the NSG UNaV curve, which was characterized by an initial natriuresis followed by a marked decrease in the Na loss to stabilize in the control value. These facts indicate, first, that the way in which the Na loss is accomplished is determined by the previous condition of body Na content in which the organism is found at the moment of the disturbing water loading and, secondly, that the way in which Na is lost is independent from the way in which water is lost. Hence, in the regulation of body fluid composition, two different mechanisms seem to be participating. One is related to water homeostasis and the other is related to Na homeostasis. Both mechanisms seem to operate independently one from the other and in harmony, as suggested by Smith since 1957 (34).

The analysis, as a whole, of the temporal development of the UV and UNaV curves of the four groups and the relationship among them leads to conferring a physiological meaning to the different events observed in these curves. In this sense, several considerations have to be noted.

For the UV values during the 3 days of diet or drug treatment, no statistical intragroup or intergroups differences were observed. This indicates that the water balance for the four groups was similar during the 3 days of treatment, a situation that was favorable to allow the diet or drug treatment to induce the pretended changes in the body Na content. The fact that no intragroup statistical differences were observed in the UNaV values during the 3 days of diet or drug treatment for the four groups indicates that the processes responsible to attain a new body Na content condition, induced by the diet or drug treatment, were already in progress from the first day of treatment. Although the UNaV values of HYPERSG, HYPOSG, and FURG, during the 3 days of diet or drug treatment, were not statistically different from those of NSG, except for HYPERSG on the first day, on the third day statistical differences were observed between the extreme groups, i.e., HYPERSG with respect to HYPOSG and FURG, suggesting that the treatments before the water load maneuver were effective to induce different body Na content conditions, which were reflected in differences in the rate of Na excretion for that day. In contrast, for the first day of these same extreme groups, HYPERSG and FURG, no differences were found in the UNaV values, a fact that might be explained considering that both groups, that day, were excreting high Na.

### Table 1

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- • = NSG; © = HYPERSG; ▲ = HYPOSG; △ = FURG.
- Any paired set of symbols side-by-side indicates that a significant (p < 0.05) difference was found between the corresponding two groups for that day or, for that period, for either the UV, UNaV, or UqV curves.
amounts, one due to the high Na surplus and the other due to the pharmacological effect of the drug.

Since a water load is totally absorbed in a lapse of 20–30 min (27,35), and since the first sample of urine was collected 20 min after the start of the water loading maneuver, which lasted 10 min, then during the first period of urine collection, water absorption processes were already in progress. Hence, the amount of water excreted in this first period was still not significantly different from the corresponding control values of the four groups. Nevertheless, some water had already been absorbed and was available for the organism to be excreted. As the organisms of the NSG, HYPOSG, and HYPERSG subjects were in condition to lose Na, that water was used as a vehicle to excrete the excess Na. This excretion was determined by the prevailing body Na content in which the organism was at the moment of the water load. Thus, HYPERSG presented in this first period a pronounced natriuresis. Both NSG and HYPOSG presented a less marked natriuresis, and FURG maintained a low Na excretion. From the pronounced natriuresis of HYPERSG and the low Na excretion by FURG in the first period, it can be inferred that as a consequence of the diet and drug treatment, respectively, the organisms reached a new status of body Na content in which the homeostatic mechanisms were forced to function semichronically. The one, in the status of high Na surplus, was able to promptly lose Na as soon as water was available. The other, in a status of severe Na deficit, was unable to lose any more Na, no matter what the amount of water available.

During the second 20-min period of urine collection, the absorption of water had been completed and, hence, a maximal dilution of the body fluids should have been accomplished during that period. A clear and similar diuretic effect due to the water load was already observed in HYPERSG, HYPOSG, and NSG. During this period, the organism was forced to perfect the detection of the cause of the disturbance. Since a solution in a container can be diluted either by withdrawal of a certain amount of solute or by solvent addition, then two possibilities for the disturbances had to be tested as cause of the dilution. As the process of detection of the cause of the disturbance was being carried out, an apparently urgent action was taken by HY- PERSG, consisting of restraining the excretion of Na; that is, the natriuresis was stopped in such a way that the Na excretion control value was reestablished. Regarding FURG, a marked delay of water excretion was observed and, as a matter of fact, the UV value had reached its maximum, it was notably lower than in the other groups. From this period on, a gradual decrease of water excretion occurred in all the groups, with a tendency of the UV curves to behave similarly, a condition that was reached totally in the seventh period and maintained until the end of the experiment. During this fourth period, HYPERSG started a gradual decrease of the natriuretic activity until the control value was reached and maintained from the fifth period on. Both NSG and HYPOSG maintained, until the end of the experiment, the Na excretion values reached previously during the third period. As from the start of the experiment, FURG maintained a low Na excretion until the end of the experiment. Again, it is interesting to point out that the water excretion pattern was similar for all the groups during the descending phase of the UV curves and, as a matter of fact, all the Na diet groups excreted the water load during the seventh period of urine collection with no statistical differences. However, the way Na was excreted, during these same periods, depended on the body Na status. Thus, HYPERSG excreted, in general, a high Na excretion with respect to NSG, and FURG maintained a low Na excretion. These findings further support the suggestion that the activity of a Na homeostatic mechanism is not dependent on the water homeostatic mechanism. Furthermore, since the ascending phase of the FURG UV curve was delayed with respect to the other groups, it could be thought that the effector actions of the water homeostatic mechanisms for this group were subjected to the higher priority need of maintaining as low as possible the Na excretion and, as a matter of fact, at the end of the experiment.

Plasmatic Na concentrations were not statistically different among the three samples of the four groups nor were differences detected among them. This fact indicates that body fluid disturbances, under the conditions of the present study, are not reflected in the plasmatic Na levels, so it seems that the disturbances at the plasmatic level rapidly fade out, probably in a way as stated elsewhere (29) considering ... that water diffuses freely across cell membranes in response to an osmotic gradient. Thus, if extracellular osmolality rises due to loss of water or an increase in solutes, water diffuses rapidly from the intracellular to the extracellular space until osmotic equilibrium is re-established at a new, somewhat higher level. Conversely, if extracellular osmolality falls due to addition of water or loss of solute, water diffuses rapidly into cells until osmotic equilibrium is again
re-established at a lower level.” No Na plasmatic differences were found between normal rats submitted to a high NaCl diet and basal NaCl-diet animals during the 2 weeks of treatment (26). The plasmatic osmolality, in general, measured in the three samples of the four groups did not show statistical differences. Only the HYPERSG third sample osmolality was significantly lower than that of the HYPERSG first sample value, and was also lower than the third sample osmolality of NSG. Plasmatic differences were detected in the third sample, but only in the osmolality of HYPERSG and not in Na concentration. This decrease in osmolality was not due to a decrease in plasmatic Na but might have been due to another factor. For instance, the moment at which the third blood sample was obtained, although Na excretion rate of HYPERSG was already in a decreasing process, Cl excretion rate was stabilized in a plateau that lasted 30 min more; thus, other cations, besides Na, could be accompanying the excreted Cl and this action is reflected as a lowering of plasma osmolality.

Since the UNaV values for the Na diet groups kept, in general, a parallel pattern to the UClV values but always higher, different cations must be excreted besides Na. However, UClV and UNaV curves practically overlapped for FURG, a fact indicating that the organisms of these subjects, in a severe Na deficit, were re- taining as much as possible any ion that could be useful to main- tain the tonicity of body fluids. It is unlikely that Cl excretion reflects the actions of the homeostatic mechanisms for body fluid tonicity, since it has been shown that the dipsgonic effect of intracerebroventricular infusion of hypertonic NaCl is mediated mainly by the Na ion rather than the Cl ion (30).

The UV and UNaV curves of HYPOSG were parallel to the corresponding NSG curves with no statistical differences. This fact suggests that although the subjects of HYPOSG were submitted to a low Na intake, their organisms were able to retain any amount of Na, no matter how low it might have been, at least under the present conditions, so that the actions for Na retention in front of a low Na intake were very efficient to com- pensate for the low Na intake. Two notable events were observed in the two most stressed groups by the treatments; one was the restraining of Na excretion presented during the second period in the HYPERSG UNaV curve, and the other was the delay of the onset of the FURG UV curve. These facts indicate that, although the organisms of these subjects were capable to compensate for the body fluids’ disturbances, their homeostatic mechanisms required some more time to detect the cause of the disturbance to start the appropriate compensating actions: probably their homeostatic mechanisms were not working at optimal conditions.

It is interesting to point out that the organism of the subjects of HYPERSG, although capable of losing high amounts of Na with low amounts of water, as shown during the first period of urine collection after the water load, did not use this considerable amount of available water to maintain a high rate of Na excretion. The HYPERSG UNaV curve presented a gradual decrease until it was stabilized at the control value from the fifth period on. Nevertheless, during these periods, in general, the natriuretic activity of HYPERSG was higher as compared to that of NSG. This fact suggests the idea that the subjects of HYPERSG, in contrast to those of NSG, had been adapted to a different physiological operative level of Na excretion induced by the high Na intake treatment. In this way, the present study provides further information to be considered in the argumentation on understand- ing whether the body Na is regulated about a single set- point or is regulated about steady states, which vary depending on the intake of Na (6, 7, 10, 16–18, 31).

Finally, it is likely that the effector actions for Na excretion, reported in the present work, are controlled at central nervous system levels. In this regard, brain involvement in the regulation of renal sodium excretion has been clearly demonstrated (8, 20).

In conclusion, the present study has proven that the exper- imental model used to disturb body fluid composition was suc- cessful to evidence the compensating action of two different body fluids’ homeostatic mechanisms that, in harmony, operate in- dependence one from the other.

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