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Induction of Hyperhydration in Rats by IP Loading With Graded Concentrations of NaCl Solution

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FREGLY, M. J., N. E. ROWLAND AND J. R. CADE. *Induction of hyperhydration in rats by IP loading with graded concentrations of NaCl solution.* PHARMACOL BIOCHEM BEHAV 45(2) 451–454, 1993.—IP loads of NaCl solution (1% of body weight) varying in concentration from 0.15–1.0 M were used to assess their ability to induce hyperhydration in rats that were allowed access to water for 6 h after loading. The hypertonic concentrations (0.25, 0.50, and 1.0 M) increased water intake in a concentration-related fashion. Only loads of 0.50 and 1.0 M NaCl solution increased urine output above that of water-loaded controls. All hypertonic concentrations increased fluid exchange (i.e., water intake less urine output) significantly. There was a direct concentration-related increase in accumulative mean fluid exchange (ΔFE, fluid exchange of NaCl-loaded group less that of control group). There was also a direct concentration-related increase in the time of hyperhydration. When related to each other, ΔFE was a direct linear function of time of hyperhydration. The slope and intercept of this relationship were compared with those found in an earlier study for angiotensin II (AngII) and isoproterenol (ISO), both potent dipsogens. Comparison revealed that slopes, but not intercepts, of the relationship between ΔFE and time of hyperhydration for any two of the three treatments differed significantly. These data suggest that a given time of hyperhydration can be achieved at a lower ΔFE with NaCl loads than with administration of either AngII or ISO. This suggests, in turn, that loading with NaCl solutions produces a more effective hyperhydration than is achieved with administration of either AngII or ISO.

Hyperhydration NaCl loads Water intake Urine output Fluid exchange

PREVIOUS studies from this laboratory have shown that acute administration of the dipsogenic agents, angiotensin II (AII) and isoproterenol (ISO), can induce a state of hyperhydration in rats (4,5). Thus, the results of these studies revealed that acute administration of either compound can induce a positive fluid exchange (i.e., water intake less urine output) that lasts for 6–10 h or many times the half-life of each compound in the circulation. These studies also provided a new technique for comparing the effectiveness of the two dipsogenic agents in the induction of hyperhydration. They revealed that, for graded doses of each compound, the change in accumulative mean fluid exchange (fluid exchange of treated group less that of control) was a direct, linear function of time of hyperhydration (4,5). Thus, the slope and intercept of this relationship for a given dipsogenic agent can be compared with those of another dipsogenic agent independent of doses of the compounds used. On the assumption that the objective of optimal hyperhydration is to achieve the longest duration of positive fluid balance with the least amount of ingested fluid [i.e., delta accumulative mean fluid exchange (ΔFE)], AII appeared to be a better hyperhydrating agent than isoproterenol (4).

Induction of drinking by administration of hypertonic NaCl solutions to rats has been known for many years (1,2,6). In addition, a number of investigators has shown that water intake is a monotonic function of the concentration of NaCl administered (1,2,6). In most cases, water intake was measured for only relatively brief periods of time, that is, from 0.5–2 h (2,6). The present study was carried out to determine the effect on fluid exchange of administration of a constant IP volume (load) of graded concentrations of NaCl solutions. On the expectation that the results would be similar to those reported for AII and ISO, an additional objective was to compare the slope and intercept of the relationship between ΔFE and time of hyperhydration with those observed previously for AII and ISO (4).

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METHOD

Thirty-six male rats of the Sprague Dawley (Harlan Industries, Indianapolis, IN) strain weighing 300–350 g were used. Rats were kept three per cage in a room maintained at 25±2°C and illuminated from 7:00 a.m.–7:00 p.m. Food (Purina Laboratory Chow, 5001) and tapwater were freely available.

Animals were divided into six equal groups. At 9:00 a.m. on the day of the experiment, all food and water containers were removed from the cages and each rat was weighed. Rats in each of the six groups received the following treatments: Group 1 served as the untreated control group; group 2 received 1% of body weight distilled water IP (warmed to 37°C prior to injection); group 3, 1% of body weight isotonic (0.15 M) saline; group 4, 0.25 M NaCl; group 5, 0.50 M NaCl; and group 6, 1.0 M NaCl. After administration of the load, each rat was placed alone in a metabolic cage and given a preweighed bottle of water (25°C). No food was available during the study. Water intakes and urine outputs were then measured hourly for 6 h. Fluid containers were infant nursing bottles with cast aluminum spouts (7).

Fluid exchange (water intake less urine output) of each group was calculated for each hour of the experiment. To assess more closely the time required for fluid exchange to return to control level, the ΔFE between treated and control groups was plotted against time after administration of the IP load. The equation of each line was calculated by linear regression to determine the goodness of fit of the data as well as the slopes and intercepts of the line. We pointed out elsewhere that the *X*-intercept of the line represents the time of hyperhydration while the *Y*-intercept represents the ΔFE (ml/kg) that would have occurred if the changes had been instantaneous (4,5).

All data in this experiment were analyzed by a repeated measures one-way analysis of variance (ANOVA). The differences between groups were compared by a Newman-Keuls posthoc analysis. Significance was set at the 95% confidence limit.

RESULTS

IP loading with NaCl solutions increased water intake in proportion to the concentration of injected solution (Fig. 1A). Output of urine was also roughly dose related (Fig. 1B), while fluid exchange was more clearly dose related than urine output (Fig. 1C).

ΔFE, which was calculated without inclusion of the volume of the load administered as part of intake, is shown in Fig. 2A. Here, it can be seen that the intercepts, but not the slopes, of the lines for each of the loading solutions administered were different and dose related. Further, the correlation coefficient of each line was significant (all $p < 0.05$). Calculation of ΔFE by addition of the load administered initially to the fluid intakes gives a similar series of straight lines that again differ in intercept but not slope (Fig. 2B). It should be noted that inclusion of the fluid load as a part of fluid intake did not change the slopes of the lines.

The times of hyperhydration and ΔFE at time = 0 for the five treated groups were the following: water, 20.6 h and 9.3 ml/kg; 0.15 M NaCl load, 22.1 and 12.2; 0.25 M NaCl load, 19.1 and 14.0; 0.50 M NaCl load, 33.7 and 15.7; and 1.00 M NaCl load, 40.6 and 21.6, respectively. There was a direct linear relationship between concentration of the NaCl load administered and both ΔFE (Fig. 3A) and time of hyperhydration (Fig. 3B). Figure 4 shows the relationship between ΔFE and time of hyperhydration. The fact that the two variables are significantly correlated indicates their interdependence. Thus, to increase the time of hyperhydration the ΔFE must also be increased.

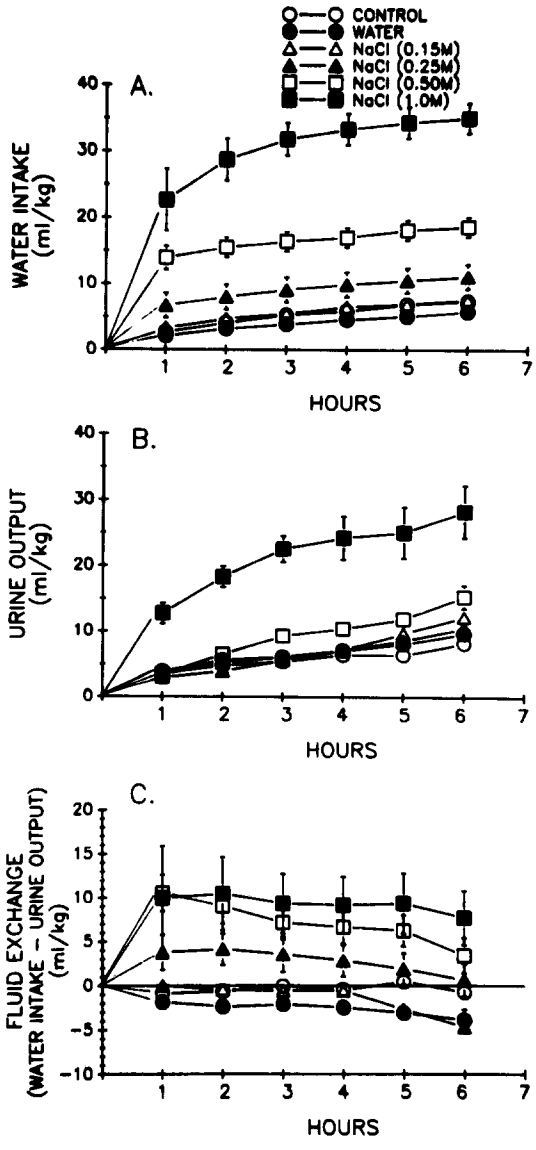

FIG. 1. Effect of an IP load (1% of body weight) of graded concentrations of NaCl solution on water intake (A), urine output (B), and fluid exchange (C) of rats during 6 h after loading. The groups are designated in the figure. Means ± SE are shown.

We pointed out elsewhere that the significance of the slope of the relationship between ΔFE and time of hyperhydration is that it provides a method for comparison of the effectiveness of two or more different methods for the induction of hyperhydration (4). Figure 5 compares loading with NaCl solutions with administration of either angiotensin II or isoproterenol with respect to hyperhydration. The data for the latter two compounds are taken from an earlier study that used male Long Evans rats (4). The results indicate that the intercepts

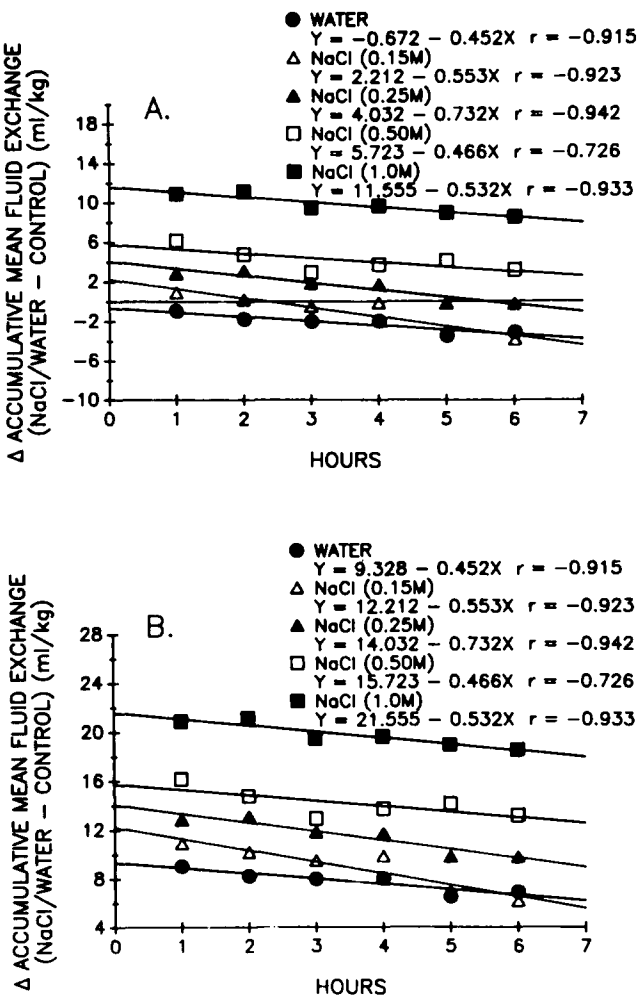

FIG. 2. The change in accumulative mean fluid exchange is shown at each hour after administration of the IP load. (A), without inclusion of the IP load; (B), with inclusion of the IP load. The groups are designated in the figure along with the regression equation describing the relationship for that group. The coefficient of correlation (r) is also shown for each regression.

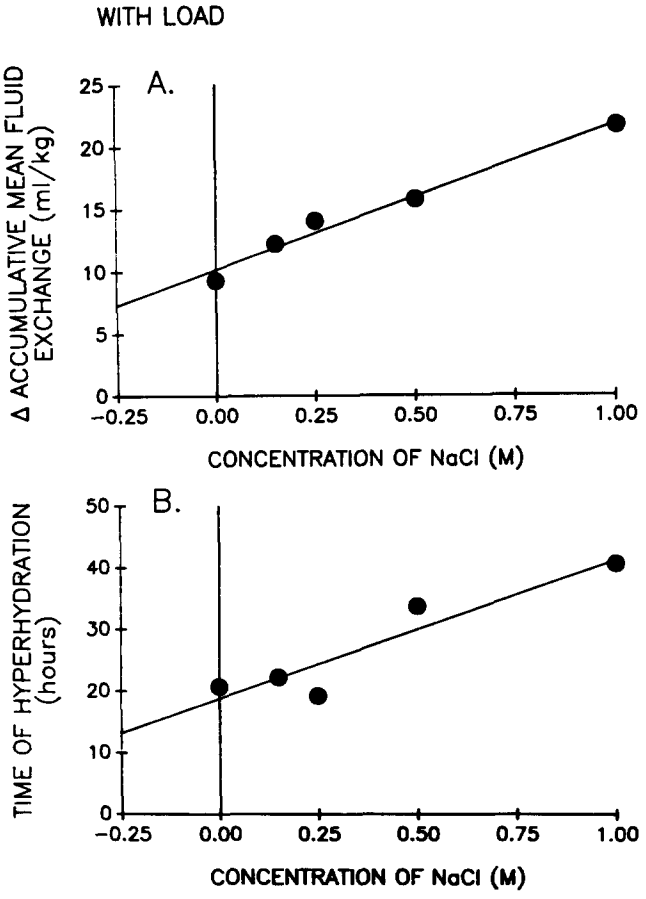

FIG. 3. Delta mean accumulative fluid exchange for each group is graphed against the concentration of the NaCl load administered (A). Time of hyperhydration for each group is also graphed against the concentration of the NaCl load administered (B).

for all three treatments are virtually the same but the slopes differ significantly ($p < 0.01$). A comparison of the effects of each treatment indicates that for a given ΔFE NaCl loading provides a greater time of hyperhydration than either administration of angiotensin II or isoproterenol.

DISCUSSION

The results of this experiment show that IP loading (1% of body weight) with graded concentrations of NaCl solutions can induce a state of hyperhydration in rats. The duration of the hyperhydration is related directly to the concentration of NaCl solution administered and thereby to the volume of water ingested voluntarily during the 6-h experiment. The contribution of the latter to the extent of hyperhydration induced is important but is limited by the output of urine induced. The difference between these two is the net fluid gain (or loss) and represents the fluid exchange (Fig. 1C). The difference between the fluid exchanges of treated and control groups (ΔFE) represents the effect of treatment. This difference decreased linearly with time. Passage of this line through zero on the X-axis represents the time of hyperhydration for that loading solution. Intersection of the line with the Y-axis repre-

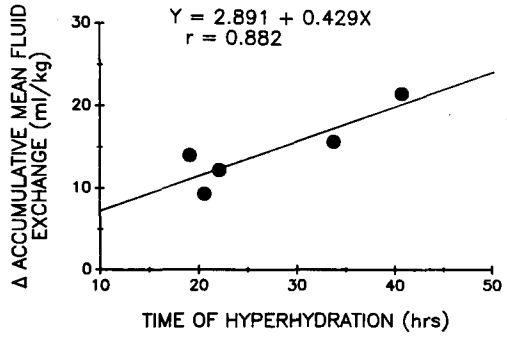

FIG. 4. Relationship between delta mean accumulative fluid exchange and time of hyperhydration. The equation describing the line and its correlation coefficient are shown.

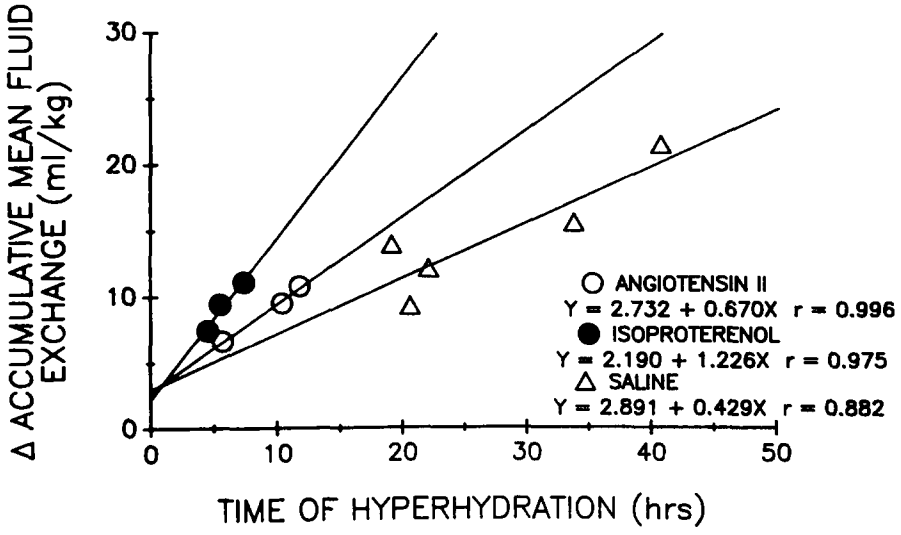

FIG. 5. Comparison of the relationships between delta mean accumulative fluid exchange and time of hyperhydration in rats treated with angiotensin II, isoproterenol, and graded concentrations of NaCl solution IP. The groups are designated in the figure. The data for angiotensin II and isoproterenol are from (4).

sents what the value of ΔFE would have been at time 0 if the responses to each loading solution could have occurred instantaneously.

It is noteworthy that both ΔFE and time of hyperhydration are related linearly and directly to the concentration of NaCl in the loading solution (Fig. 4). While it would appear obvious that the greater the initial FE the longer the time of hyperhydration, it is not obvious why different techniques for induction of hyperhydration, each of which bears similar direct linear relationships between ΔFE and time of hyperhydration, should have different slopes. The fact that they do suggests that the relationship between ΔFE and time of hyperhydration may be unique for each particular treatment. If so, this method for comparison provides a simple and useful way in which to assess the abilities of various techniques to induce a hyperhydration. The present studies provide no clue regarding mechanisms for such uniqueness. This possibility, however, remains a fertile field for further study.

These studies have not taken into account the extrarenal water losses that occur in rats. These have been measured and amount to 1.0 ml/kg body weight/h (3). Adding the extrarenal water losses to urinary losses for each group essentially cancels out its effect and does not change the time required for ΔFE to return to equilibrium (4).

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