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Hyperhydrating Effect of Acute Administration of Angiotensin II in Rats[1]

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FREGLY, M. J., K. M. WILSON, N. E. ROWLAND AND J. R. CADE. *Hyperhydrating effect of acute administration of angiotensin II in rats*. PHARMACOL BIOCHEM BEHAV **41**(1) 183–188, 1992.—Water intake, urine output, and fluid exchange (water intake less urine output) were measured in rats at hourly intervals for 7 hours and at 24 hours following acute administration of angiotensin II (AII, 200 μg/kg SC). AII induced the expected abrupt increase in water intake and a more gradual increase in urine output. The change in fluid exchange (fluid exchange of the AII-treated group less fluid exchange of controls) became positive within the first hour after treatment with AII, decreased linearly with time, and reached 0 at approximately 10 to 12 hours after treatment with AII. When AII was administered intracerebroventricularly (50 ng), similar results were observed. In this case, the change in fluid exchange (ΔF) reached 0 in about 6 hours. Imposition of a water load (1% of body weight, IP) on the group receiving AII SC failed to affect the time required for ΔF to reach 0 if the water load was disregarded. However, inclusion of the load as a part of intake extended the time the rats remained in positive fluid balance beyond that of the nonloaded, AII-treated control group. In the case of the larger water load (3% of body weight, IP), ΔF returned to that of controls in about 4 to 5 hours if the water load was disregarded. However, inclusion of the load as part of intake extended the period of hyperhydration well beyond that of both the nonloaded, AII-treated group and the AII-treated group given the 1% load. The results indicate that an acute injection of AII, either peripherally or centrally, can induce a state of hyperhydration in rats that lasts for approximately 6 to 12 hours, depending on the route of administration of AII. Imposition of a water load in combination with acute administration of AII can extend the duration of hyperhydration. The results are also of interest because they provide a simple method for assessment of the duration of hyperhydration following administration of AII and other dipsogenic agents.

Angiotensin II-induced drinking Hyperhydration Fluid exchange Dipsogenic agent

FLUID balance is maintained by the integrated regulation of thirst mechanisms and water conservation (1). The mechanisms regulating fluid homeostasis are mediated by changes in either blood pressure or blood volume, plasma osmolality, plasma vasopressin concentration, the renin-angiotensin (RA) system and atrial natriuretic hormone. Water intake is induced by depletion of either extracellular (ECF) or intracellular (ICF) fluid volume (2). Most stimuli which deplete ECF induce drinking by activating the RA system (2). Thus either peripheral or central administration of angiotensin II (AII) elicits copious drinking. Within several hours, and depending upon the dose and route of administration of AII, the rat will drink a volume of water equal to about 10% of the amount normally ingested during the previous 24 hours (10). The dipsogenic response to central administration of AII is usually complete within one hour after treatment and within two hours after peripheral (SC) administration.

An objective of these experiments was to determine how long the perturbation of fluid exchange induced by acute administration of AII, both peripherally and centrally, would last and how the intake and output sides of fluid regulation adjusted during 12 to 24 hours after treatment. A further objective was to assess the effect of intraperitoneal fluid loading on the responses to administration of AII.

METHOD

General

Four separate experiments were performed using female rats of the Sprague-Dawley (Blue Spruce Farms) strain weighing 200 to 350 g. Two days prior to each experiment, all rats were placed into individual stainless steel metabolic cages to allow for adjustment to the new environment. The temperature of the vivarium was maintained at 25 ± 2°C and illuminated from 7 a.m. to 7 p.m. Food (Purina Laboratory Chow No. 5001) and tap water were freely available. However, at the beginning of each experiment, food and water were removed from the cages.

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Experiment 1: Effect of Acute Administration of Angiotensin II on Fluid Exchange at Intervals up to 24 Hours After Treatment

Study 1: Peripheral administration. In this study, 16 rats were divided into two equal groups and either 200 μg AII/kg (Sigma Chemical Co., St. Louis, MO, No. A9525) or the vehicle used to dissolve AII (isotonic saline, 1 ml/kg) was injected subcutaneously (SC). Immediately after the injection, each rat was given a preweighed bottle of water. Water intake was then measured hourly for the next 7 hours and at 24 hours thereafter. At 7 hours after injection, a container of food was returned to each cage in this and succeeding studies.

Study 2: Intracerebroventricular administration. This study was similar to Study 1 except for the site of administration of AII. One week prior to the experiment, 12 naive female rats weighing 200–250 g were implanted with a lateral cerebroventricular cannula (6). On the day of the experiment, the 12 rats were divided randomly into two equal groups and given a cerebroventricular (ICV) injection of either 50 ng AII or its vehicle (sterile isotonic saline, 5 μl). Water intakes and urine outputs were measured at the times described in the first study except that the last measurement of water intake was made at 7 instead of 24 hours after treatment with AII.

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

Experiment 2: Effect of an Intraperitoneal Load of Water on Fluid Exchange in Rats Administered Angiotensin II Acutely

Two studies were carried out. They used the same rats, but were separated in time by two weeks. The two studies were identical except that a 1% water load was administered intraperitoneally (IP) in the first study while a 3% load was administered in the second study.

Twenty-four female rats weighing 300–350 g were used. They were divided equally into four groups. The first group was administered a load of distilled water warmed to 37°C (1.0 or 3.0 ml/100 g body weight in Studies 1 and 2, respectively), and immediately thereafter injected SC with isotonic saline (1 ml/kg). The second group also received water IP, and immediately thereafter was administered AII (200 μg/kg, SC). The third group was sham-injected IP, and received 200 μg AII/kg, SC. The fourth group was also sham-injected IP, but received the isotonic saline vehicle SC. Water intake and urine output of each rat were measured hourly for the first 7 hours and at 12 hours after administration of AII.

All data in these two experiments were analyzed by a repeated measures two-way ANOVA. The differences between groups were compared by a Newman-Keuls post hoc analysis. Significance was set at the 95% confidence limit.

RESULTS

Experiment 1

Study 1. Subcutaneous (SC) administration of 200 μg AII/kg of body weight induced the expected robust increase in water intake (Fig. 1A). The treated rats drank almost all of their water intake within the first hour. They drank very little water in the next 6 hours, but drank again during hours 7–24, which included the 12 hours of darkness. Control rats drank very little during hours 0–7. The differences in water intakes between the two groups were significant ($p<0.01$) during the first 7 hours of the experiment but were not significant thereafter (Fig. 1A).

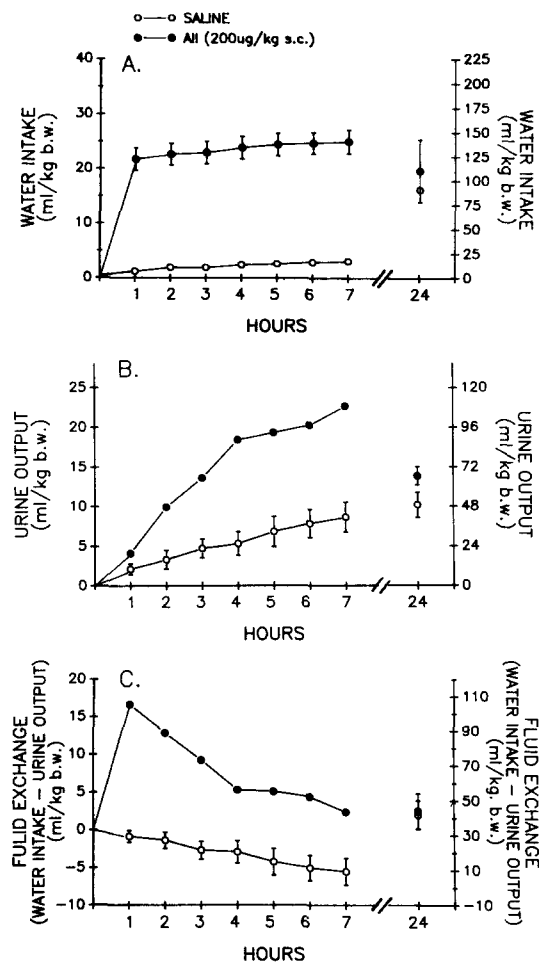

FIG. 1. The effect of acute peripheral administration of either angiotensin II (200 μg/kg, SC) or saline (1 ml/kg, SC) on water intake (A), urine output (B) and fluid exchange (water intake − urine output) (C) of rats at intervals after treatment. The two groups are designated in the figure. One standard error is set off at each mean. When no standard error is shown, it falls within the symbol. Please note the change of scale of the ordinate on the right side of each panel. Water intakes, urine outputs and fluid exchanges are accumulative during the course of the study.

Cumulative urine output of the AII-treated group was increased significantly ($p<0.05$) above that of the control group during the 7 hours postinjection (Fig. 1B). Thereafter, outputs of treated and control groups did not differ. Fluid exchange (i.e., water intake less urine output) of the treated group remained positive and significantly ($p<0.05$) greater than that of the control group for the first 7 hours postinjection. During this time, fluid exchange of the untreated control group became increasingly negative since urine output exceeded water intake.

There were no significant differences between groups in either their water intake, urine output, or fluid exchange at 24 hours after injection. Thus a period greater than 7 hours, but less than 24 hours, after injection is required for fluid exchange to return to the level of controls after a single subcutaneous (SC) injection of AII. To assess this more closely, the difference in accumulative mean fluid exchange between AII-treated and con-

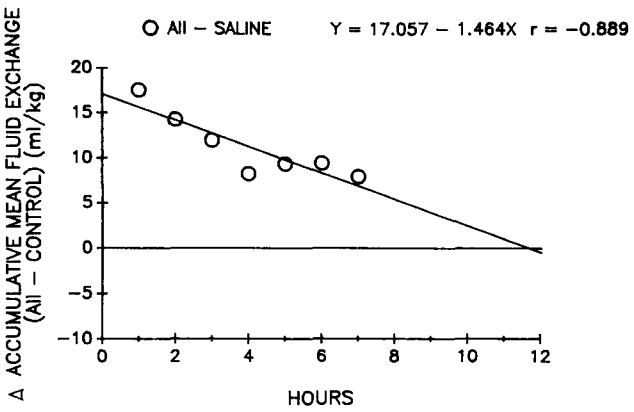

FIG. 2. The difference in accumulative mean fluid exchange (angiotensin II-treated less control) during the first 7 hours of the study shown in Fig. 1. The equation of the regression line is shown along with the correlation coefficient. The intersection of the regression line with the horizontal line drawn at 0 represents the time required to reach the level of the control group in terms of fluid exchange.

trol groups was plotted against time after injection of AII (Fig. 2). A linear regression analysis of the data revealed a highly significant ($p<0.01$) negative linear correlation ($r=-.889$) between the difference in accumulative mean fluid exchange and time after administration of AII. Extrapolation of the line to 0 suggests that about 12 hours are required for fluid exchange to return to the level of the control group after a single subcutaneous injection of AII. The time for the difference in accumulative mean fluid exchange to reach 0 should be considered only approximate because of the inherent error related to extrapolation of the line.

Study 2. The effects of IVT administration of AII on water intake, urine output, and fluid exchange were similar to those observed for peripheral administration of AII. Water intakes of the AII-treated group were increased significantly ($p<0.01$) above those of the control group throughout the experiment (Fig. 3A). Further, urine output of the AII-treated group was also elevated significantly ($p<0.01$) above that of the control group beginning at 2 hours after treatment (Fig. 3B). Fluid exchange of the AII-treated group decreased rapidly to reach a balance point at about 5 hours postinjection (Fig. 3C). The control group was in negative fluid exchange during the last 4 hours of the experiment.

To assess more closely the time for fluid exchange to return to control, the difference in fluid exchange between AII-treated and control groups was plotted against time after administration of AII (Fig. 4). There was again a significant ($p<0.01$) negative linear correlation ($r=-.931$) between the two parameters. Extrapolation of the line to 0 suggests that approximately 7 hours are required for fluid exchange to return to the level of the control group after a single intraventricular injection of AII.

Experiment 2

Study 1. To assess the effect of a prior intraperitoneal water load on the time for fluid exchange to return to control level after a single SC injection of AII, rats in this study were given a load of water amounting to 1% of body weight. Of the 4 groups in this experiment, two were administered either saline or AII without a water load while the remaining two received the same treatment, but with the water load. The results from the two groups that received no water load were similar to those

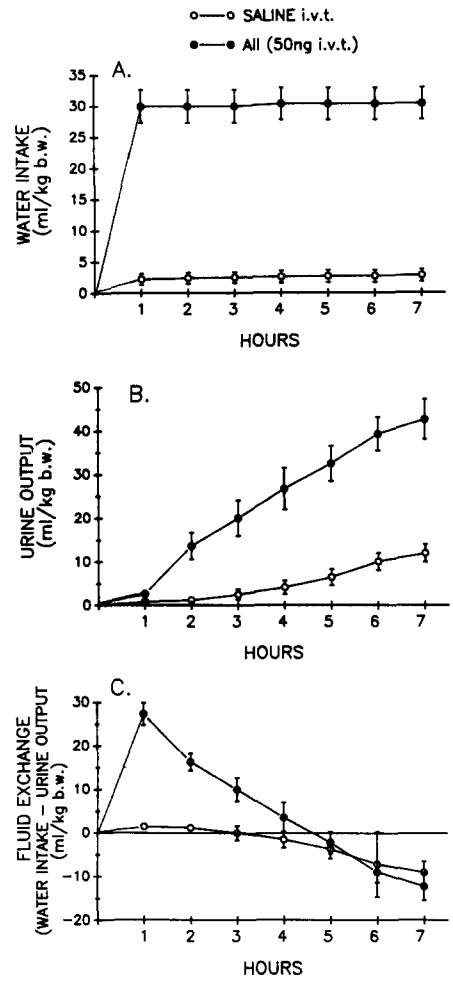

FIG. 3. The effect of acute intracerebroventricular administration of either angiotensin II (10 ng/kg) or saline on fluid intake (A), urine output (B), and fluid exchange (C) of rats at intervals after treatment. The two groups are designated in the figure. One standard error is set off at each mean. When no standard error is shown, it falls within the symbol. Water intakes, urine outputs, and fluid exchanges are accumulative during the course of the study.

of Experiment 1, shown in Fig. 1. A comparison of the nonloaded and loaded control groups indicates that a 1% water load failed to affect any of the parameters measured (Fig. 5). Untreated control rats in this study, as was the case in Experiment 1, Study 1, were in negative fluid exchange (urine output exceeded water intake) to about the same extent during the first 7 hours of both experiments. Further, water intakes (Fig. 5A), urine outputs (Fig. 5B), and fluid exchanges (Fig. 5C) of the nonloaded, AII-treated group during the first 7 hours of this study were similar to those of counterparts in Experiment 1 (Fig. 1). If the administered load is not taken into account, the results suggest that the water-loaded groups had similar water intakes, urine outputs, and fluid exchanges (Fig. 5D, E, F) to those not loaded with water, whether or not they were treated with AII. However, if the load (10 ml/kg) is included in the data shown in Fig. 5F, neither group would have come into fluid exchange balance at 7 hours postinjection. However, by 12 hours, there was no difference in fluid exchange between the two loaded

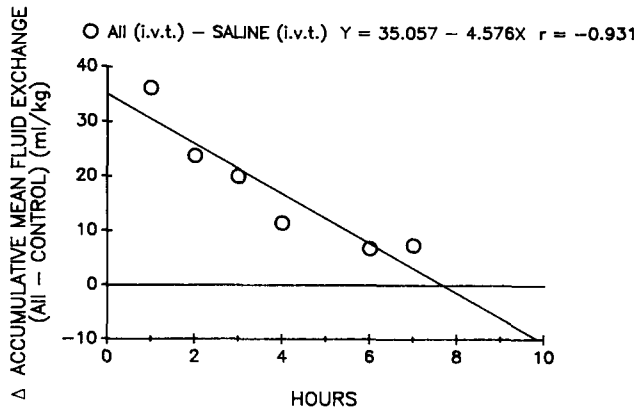

FIG. 4. The difference in mean accumulative fluid exchange (angiotensin II-treated less control) during the first 7 hours of the study shown in Fig. 3. The equation of the regression line is shown along with the correlation coefficient. The intersection of the regression line with the horizontal line drawn at 0 represents the time required to reach the level of the control group in terms of fluid exchange.

groups whether or not the load is included (Fig. 5F).

The difference in accumulative fluid exchange (AII-treated less control) is shown in Fig. 6 for loaded and nonloaded groups. The results indicate that the difference in fluid exchange for both AII-treated groups (loaded and nonloaded) reached 0 at about 10 hours. These times are similar to those shown in Figs. 2 and 4 (about 12 and 8 hours, respectively). If the injected load is considered as part of intake, approximately 15 hours would have been required for the loaded, AII-treated group to reach the level of the control group.

Study 2. Water intake, urine output, and fluid exchange of the two nonloaded groups in this experiment were similar to those of counterparts in Experiment 1, Study 1 (Fig. 7A–C).

In contrast, when the rats were given both a 3% water load and AII, water intake and urine output were not different from the group given only the water load (Fig. 7D, E). Fluid exchange (Fig. 7F) was also not different between the two groups, but was considerably more negative for both than for the groups that were not loaded (Fig. 7A, B, C). The greater negative fluid exchange occurred because of the large increase in urine output resulting from the water load. By 12 hours after administration of AII, urine output, water intake and fluid exchanges of treated

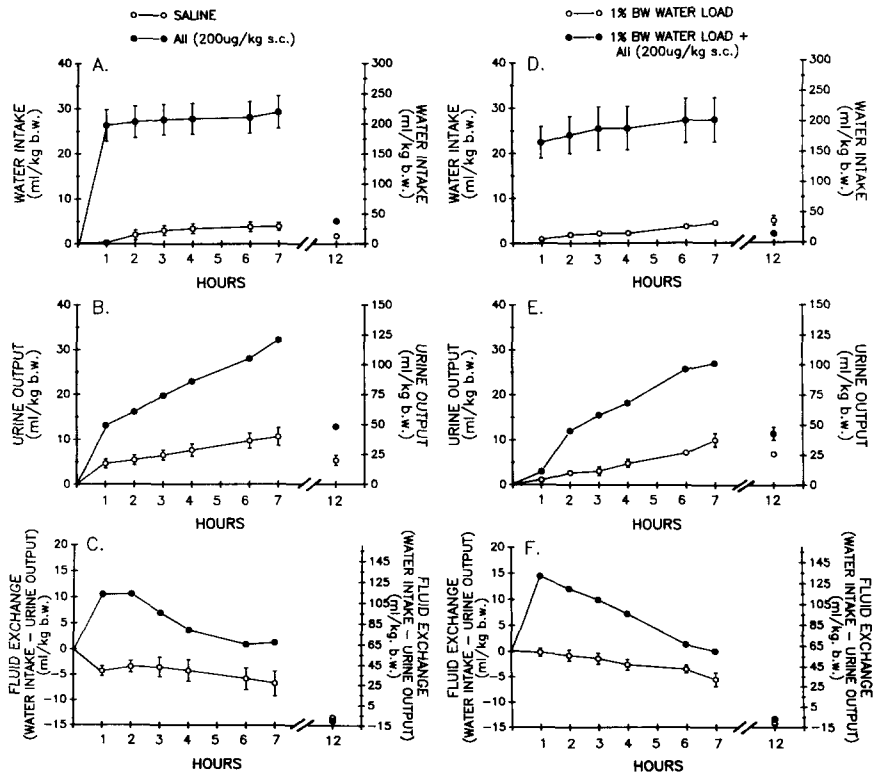

FIG. 5. The effect of intraperitoneal loading with 1% b.wt. of water on water intake (A, D), urine output (B, E) and fluid exchange (C, F) of angiotensin II-treated (200 μg/kg, SC) and control (1 ml saline/kg, SC) nonloaded (left panels) and loaded (right panels) rats at intervals after treatment. The groups are identified in the figure. One standard error is set off at each mean. When no standard error is shown, it falls within the symbol. Please note the change in scale of the ordinate shown on the right side of each set of panels. Water intakes, urine outputs and fluid exchanges are accumulative during the course of the study.

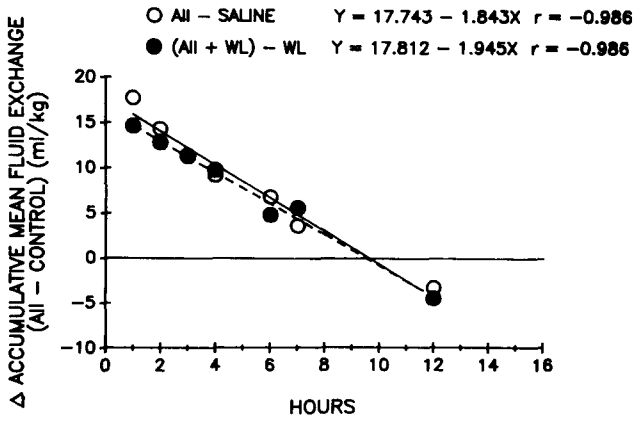

FIG. 6. The difference in mean accumulative fluid exchange during the course of the study shown in Fig. 5. Groups are designated in the figure. The equations for each regression are shown in the figure along with their correlation coefficients. Other details are the same as described in Fig. 4.

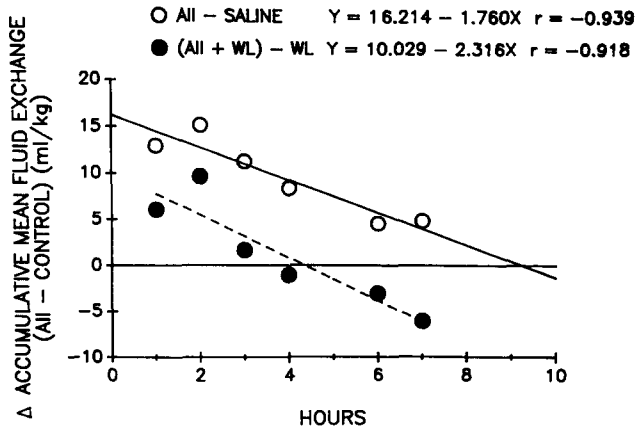

FIG. 8. The difference in mean accumulative fluid exchange during the course of the study shown in Fig. 7. Other details are the same as described in Fig. 4.

and control groups did not differ significantly. Thus between 7 and 12 hours are required for fluid exchange to return to control level following a 3% IP load of water and a single injection of AII.

The fluid exchanges of the two loaded groups in Fig. 7F must be interpreted with caution since the load (30 ml/kg) is not included in the data. If it had been, fluid exchange of the AII-treated group would have returned approximately to that of the control group in 7 hours, while the water-loaded control group would have been in positive fluid exchange by about 10 ml/kg at 7 hours. However, by 12 hours postinjection, there was no difference in fluid exchange of treated and control groups, with

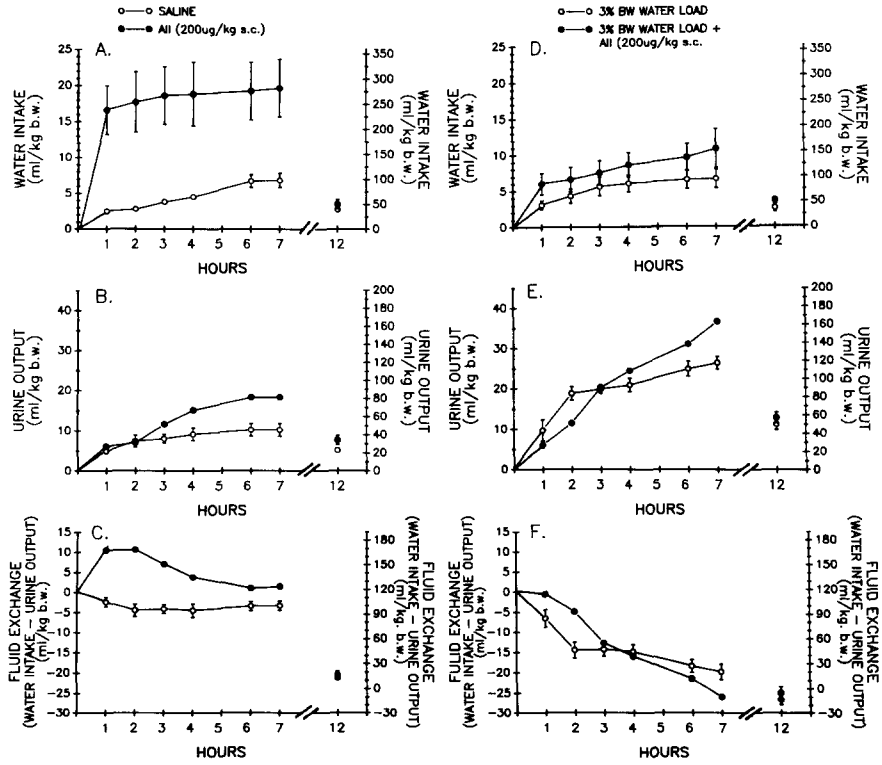

FIG. 7. The effect of intraperitoneal loading with 3% b.wt. of water on water intake (A, D), urine output (B, E), and fluid exchange (C, F) of angiotensin-treated (200 μg/kg, SC) and control (1 ml saline/kg, SC) nonloaded (left panels) and loaded (right panels) rats at intervals after treatment. Other details are the same as those described in Fig. 5.

or without the inclusion of the water load.

Again, the change in accumulative mean fluid exchange was plotted against hours after administration of AII for the nonloaded and loaded groups (Fig. 8). If the load of 3% of body weight is not included as part of intake, the plot indicates that fluid exchange of the treated group reached that of the control group in about 4 hours as compared with about 9 hours for the nonloaded group. The time required to reach the level of controls is about the same for the latter group and its counterpart in Fig. 2 (i.e., 9 versus 11 hours). If the load of 3% of body weight is included as a part of intake and a linear decrease is assumed, control level would have been reached in about 17 hours.

DISCUSSION

The results of these studies reveal that the perturbation in fluid exchange resulting from either an acute peripheral or central injection of AII extends for at least 6 to 8 hours after the injection, well beyond the half-life of the compound in the circulation. This indicates that this amount of time, at least, should be allowed between sequential administrations of AII when dipsogenic responsiveness is being tested in rats.

The results presented here also provide a simple method for assessment of the duration of hyperhydration following administration of AII. The method is applicable to an assessment of the duration of hyperhydration induced by other dipsogenic agents as well and is currently being used for such studies in this laboratory.

The present results verify that intraperitoneal administration of a water load equal to 3%, but not 1%, of body weight can inhibit the dipsogenic response to acute administration of AII (5). While drinking to AII has usually been considered to be independent of hydrational state, it is clear that this assumption is not entirely correct. We advanced the hypothesis earlier (4,5) that the movement of electrolytes into the peritoneal fluid following loading with water reduces the osmolality of the plasma which, in turn, mediates a reduction in the dipsogenic drive. Other types of experimentally induced drinking were also inhibited by either intragastric or intraperitoneal loading with water (4). In contrast, loading with similar volumes of isotonic saline exerted much less inhibitory effect on all types of drinking tested.

The effect of water loading on the time for the difference in accumulative mean fluid exchange (AII-treated less control) to reach 0 depends on whether the administered load is included or excluded from the calculation. Inclusion of the load as a part of intake extended the time the rats remained in positive fluid balance for both the 1% and 3% intraperitoneal loads, with the larger load extending positive balance for a longer period. Exclusion of the load as a part of intake had no effect on the time for the difference in accumulative mean fluid exchange to reach 0 for the group administered a 1% load and shortened the time to reach 0 for the group administered a 3% load (Fig. 8).

It is also important to draw attention to the fact that 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/hour (3). Adding the extrarenal water losses to urinary losses in the data from both studies in Experiment 1 (Figs. 1B and 3B) did not change the time required to return to equilibrium (i.e., 12 and 6 hours for Studies A and B, respectively).

It is especially interesting that control rats in each study were in negative fluid exchange by as much as 5 ml/kg of body weight by the end of the 7 hours of daylight during which measurements were made. This is in agreement with the results of others who have shown that water intake by rats occurs almost exclusively at night (9).

Studies on hyperhydration have assumed increasing importance for man in situations in which vascular volume must be maintained in order to support an increase in cardiac output, vascular perfusion of the skin, and increased heat loss via sweating (7). Such situations include strenuous exercise and subsistence in a desert environment. Recent studies of Riedesel and his associates have shown that oral administration of glycerol to man may satisfy the above requirements (8). Glycerol is distributed within the vascular compartment where it acts osmotically to maintain plasma volume which thereby maintains rate of sweating and body temperature during exercise. Glycerol also maintains plasma volume by reducing urine output. The present studies indicate a different strategy for hyperhydrating rats consisting of increasing fluid intake above the level of urine output such that fluid exchange does not return to equilibrium for 10 to 12 hours after acute administration of AII. While we do not advocate administration of AII for purposes of hyperhydration in humans, the results are of more than academic interest in that the approach used here may prove useful in the study of hyperhydration in humans.

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REFERENCES

1. Andersson, B. Thirst and brain control of water balance. Am. Sci. 59:408–415; 1971.
2. Fitzsimons, J. The physiology of thirst and sodium appetite. Cambridge: Cambridge University Press; 1979:1–526.
3. Fregly, M. J. Effect of exposure to cold on evaporative loss from rats. Am. J. Physiol. 213:1003–1008; 1967.
4. Fregly, M. J.; Greenleaf, J. E.; Rowland, N. E. Effect of intraperitoneal and intragastric loading with water and isosmotic solutions of saline and glucose on water intake of dehydrated rats. Brain Res. Bull. 16:415–420; 1986.
5. Fregly, M. J.; Rowland, N. E. Effect of intragastric and intraperitoneal water and saline loads on the pharmacologic induction of drinking in rats. Brain Res. Bull. 16:407–414; 1986.
6. Fregly, M. J.; Rowland, N. E. Do peripheral and cerebroventricular injections of angiotensin II act at the same site? Studies on additivity of drinking. Brain Res. Bull. 16:249–257; 1986.
7. Nadel, E. R.; Fortney, S. M.; Wenger, C. G. Effect of hydration state on circulatory and thermal regulations. J. Appl. Physiol. 49: 715–721; 1980.
8. Riedesel, M. L.; Allen, D. Y.; Peake, G. T.; Al-Qattan, K. Hyperhydration with glycerol solutions. J. Appl. Physiol. 63:2262–2268; 1987.
9. Rowland, N. E.; Bellush, L. L.; Fregly, M. J. Nycthemeral rhythms and sodium chloride appetite in rats. Am. J. Physiol. 249(Regul. Integr. Comp. Physiol. 18):R375–R378; 1985.
10. Wilson, K. M.; Sumners, C.; Fregly, M. J. Effects of increased circulating angiotensin II (AII) on fluid exchange and binding of AII in the brain. Brain Res. Bull. 20:493–501; 1988.