Pharmacology Biochemistry and Behavior, Vol. 43, pp. 1143–1149, 1992

0091-3057/92 $5.00 + .00

Comparison of the Hyperhydrating Effects of Angiotensin II and Isoproterenol

MELVIN J. FREGLY,[1] NEIL E. ROWLAND AND J. ROBERT CADE

Departments of Physiology, Medicine, and Psychology,
Colleges of Medicine and Liberal Arts and Sciences
University of Florida, Gainesville, FL 32610

Received 18 May 1992

FREGLY, M. J., N. E. ROWLAND AND J. R. CADE. *Comparison of the hyperhydrating effects of angiotensin II and isoproterenol.* PHARMACOL BIOCHEM BEHAV **43**(4) 1143–1149, 1992.—Administration of a single dose of angiotensin II (AII) has been shown to induce a state of hyperhydration in rats that can last from 6–10 h depending upon the route of administration and the dose. The objective of the present study was to determine whether another dipsogenic agent, isoproterenol (ISO), a β-adrenoceptor agonist, could also induce a state of hyperhydration. The results indicate that a single SC dose of ISO can induce a hyperhydration that lasts from 4–6 h depending upon the dose administered. Administration of graded doses of either AII or ISO induced graded increases both in the time of hyperhydration and change in accumulative mean fluid exchange, (*ΔFE*, fluid exchange of treated less fluid exchange of control). These two parameters were related linearly and directly for each drug, although the slopes, but not the intercepts, of the relationship for each drug differed significantly. Because the objective of optimal hyperhydration should be to achieve the longest duration of positive fluid balance with the least amount of ingested fluid (i.e., *ΔFE*), the slopes of the two lines provide a convenient way to compare the hyperhydration induced by AII and ISO. By this criterion, it would appear that AII provides a more optimal hyperhydration than ISO.

Hyperhydration Angiotensin II Isoproterenol β-Adrenoceptor agonist Dipsogenic agents

AN earlier study from this laboratory showed that a single SC injection of angiotensin II (AII) into rats was capable of inducing a state of hyperhydration that lasted 6–10 h (2). Similar results were observed when AII was injected intracerebroventricularly. Because AII is a known dipsogenic agent, the possibility existed that other known dipsogenic agents may also exert a similar effect. This possibility was tested in the present experiment in which dose-response relationships were established between the administered doses of both AII and isoproterenol (ISO), a β-adrenoceptor agonist, and both the time of hyperhydration and change in mean fluid exchange (*ΔFE*, fluid exchange of treated group less fluid exchange of control group). The results of this study also provide a method for comparison of the hyperhydrating effects of these two different dipsogenic agents.

METHOD

Two separate experiments were carried out using male rats of the Long-Evans (Charles River Laboratories, Wilmington, MA) strain weighing 300–350 g. Each experiment was concerned with a specific dipsogenic agent (for Experiment 1, it was AII; for Experiment 2, ISO). Three studies comprised each experiment, each of which used a different dose of the dipsogenic agent.

Rats were kept three per cage in a vivarium maintained at 25 ± 2°C and illuminated from 7:00 a.m.–7:00 p.m. Purina Laboratory Chow (5001) and water were freely available until the beginning of the experiment, at which time containers for water and food were removed from each cage. All experiments began at 9:00 a.m. At the beginning of the study, each rat was weighed, treated as described in each study below, and placed into a metabolic cage. A pre-weighed bottle of water (25°C) was placed on each cage and water intakes measured gravimetrically every hour for the next 7 h. Fluid containers were infant nursing bottles with cast aluminum spouts (3). Urine output was also measured hourly during each study. No food was available during the study.

All data were analyzed by a one-way analysis of variance (ANOVA) and linear regression analysis. The confidence limit was set at the 95% level.

[1] Requests for reprints should be addressed to Dr. Melvin J. Fregly, Department of Physiology, Box 100274, University of Florida, College of Medicine, Gainesville, FL 32610.

Experiment 1: Effect of Acute Administration of AII on Fluid Exchange

Three separate studies using the same 12 rats were carried out. The protocols for the studies were the same in that one group (six rats) was administered one of three concentrations of AII while the second group (six rats) was administered the vehicle used to dissolve the AII. The doses of AII used in the three studies were 50, 75, and 150 μg/kg SC, respectively. In each study, rats were randomized. At the end of each study, rats were returned to their home cages, where food and water were freely available until the next test several days later.

Experiment 2: Effect of Acute Administration of ISO on Fluid Exchange

Three separate studies were carried out using the same 12 rats from Experiment 1. The studies were carried out identically to those described in Experiment 1 except the β-adrenoceptor agonist ISO, at three different doses (7.5, 15, and 25 μg/kg SC, respectively), was used as the dipsogenic agent.

RESULTS

Experiment 1

Study 1. Acute administration of 50μg AII/kg increased water intake significantly ($p < 0.05$) above that of the control group within the first hour after treatment (Fig. 1A) but did not affect urine output significantly (Fig. 1B). Mean fluid exchange (water intake less urine output) for each group is shown in Fig. 1C. Fluid exchange of the control group was negative throughout the study, reaching a value of about 5 ml/kg by the seventh hour. In contrast, fluid exchange of the AII-treated group was positive and significantly ($p < 0.01$) elevated for the first 3 h of the study and became increasingly negative thereafter. To assess the effect on fluid exchange due to AII, fluid exchange of the control group was subtracted from that of the treated group (delta accumulative mean fluid exchange, ΔFE). There was a highly significant negative linear relationship between ΔFE and time, with a correlation coefficient (r) of -0.97 ($p < 0.01$) (Fig. 2). The time for ΔFE to reach zero was approximately 5.7 h. ΔFE extrapolated to zero time was 6.6 ml/kg. This value represents the amount of fluid, over and above that of the control group, that would have been ingested if the response to administration of AII had been instantaneous.

Study 2. The effects of an increased dose of AII (75 μg/kg, SC) on water intake (Fig. 3A), urine output (Fig. 3B), and mean fluid exchange (Fig. 3C) were similar but greater than those described for the lower dose in Study 1. Both water intake and fluid exchange of the treated group were elevated significantly ($p < 0.01$) above those of the control group throughout the study. However, urine outputs were not significantly different. ΔFE for this dose of AII was also a negative linear function of time and had a highly significant correlation coefficient ($r = -0.986$; $p < 0.01$) (Fig. 2). The time for ΔFE to reach zero was 10.4 h, while the ΔFE at zero time was 9.5 ml/kg.

Study 3. The effects of administration of 150 μg AII/kg SC on water intake (Fig. 4A), urine output (Fig. 4B), and mean fluid exchange (Fig. 4C) were proportionately greater than those observed in the earlier studies. Water intake and fluid exchange of the treated group were again elevated significantly ($p < 0.01$) above those of the control group throughout the study. However, urine outputs were not significantly different from control. Again, ΔFE showed a negative linear relationship with time that was highly significant ($r = -0.92$; $p < 0.01$) (Fig. 2).

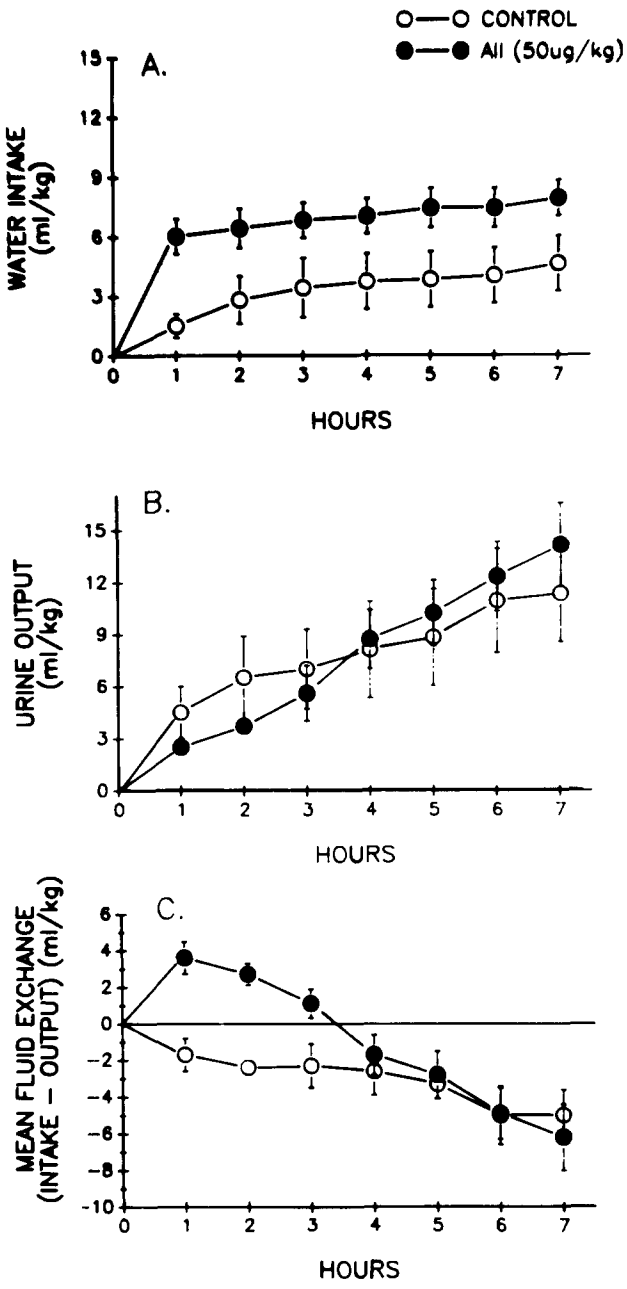

FIG. 1. Effect of administration of angiotensin II (AII) (50 μg/kg, SC) on water intake (A), urine output (B), and mean fluid exchange (C). The groups are designated. Means ± one SE are shown.

ΔFE for the three doses of AII vs. time are compared in Fig. 2. There were no significant differences in the slopes of the lines but the intercepts of any two regressions differed significantly ($p < 0.01$). Thus, there was a curvilinear dose-response relationship between ΔFE and dose of AII administered (Fig. 5A), as there was between time of hyperhydration and dose of AII administered (Fig. 5B). These figures suggest

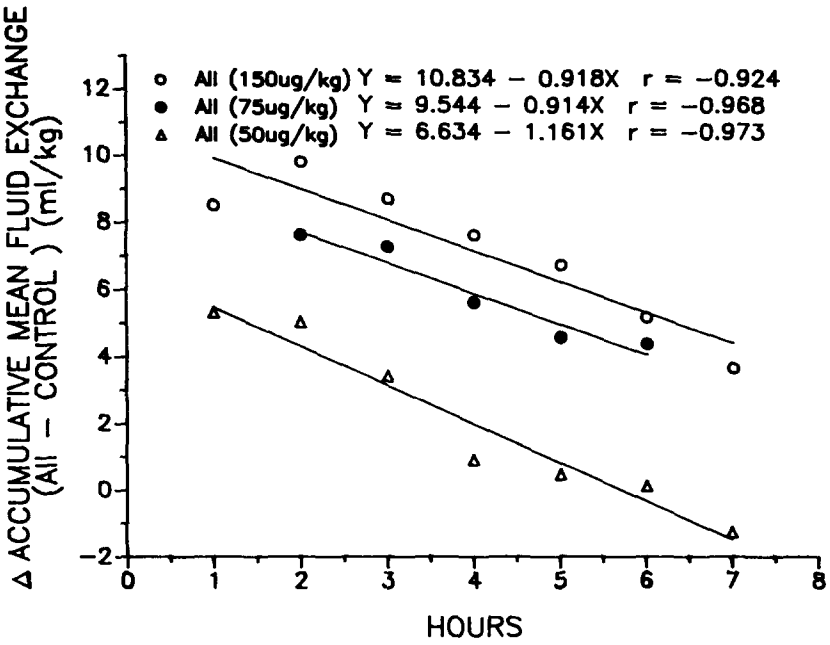

FIG. 2. Change in accumulative mean fluid exchange for each dose of angiotensin II (AII) during the course of the experiment. The groups are designated along with the equation describing the regression for that group. The correlation coefficient (r) is also given with each equation.

that near maximal effects on ΔFE and time of hyperhydration occurred when 75 μg AII/kg was administered.

Experiment 2

Study 1. Administration of the lowest dose of ISO (7.5 μg/kg, SC) increased water intake significantly ($p < 0.05$) by the first hour after administration (Fig. 6A). Approximately 75% of the total fluid ingested during the 7-h study was ingested within the first hour.

Urine output of the ISO-treated group was less than that of the control group during the first hour after treatment but exceeded that of the control group thereafter for the remainder of the study (Fig. 6B). The difference between groups, however, was not significant at any time.

Mean fluid exchange of the treated group was significantly ($p < 0.01$) increased only during the first hour after treatment and reached zero by the second hour (Fig. 6C). The control group was in negative fluid exchange throughout the study, as noted above.

Study 2. Administration of the intermediate dose of ISO (15 μg/kg, SC) resulted in significant ($p < 0.01$) increases in water intake throughout the study (Fig. 7A), urine output from 3 h onward (Fig. 7B), and fluid exchange from the first through the third hours (Fig. 7C).

Study 3. The highest dose of isoproterenol (25 μg/kg, SC) increased the intake of water significantly ($p < 0.01$) above that of the control group throughout the first 5 h of the study (Fig. 8A). However, the water intake of the control group was much greater than that observed in the previous two studies. Reasons for this difference from previous studies are not clear. The urine output of the treated group first increased significantly ($p < 0.05$) above that of the control group during the third hour of the study and remained significantly ($p < 0.01$) elevated thereafter. Urine output of the control group increased above that observed in Study 2 (Fig. 8B). Fluid exchange of the treated group was significantly ($p < 0.01$) greater than that of the control group during the first 2 h of the study (Fig. 8C).

ΔFEs vs. time for the three studies are shown in Fig. 9. For each dose of ISO administered, there is a negative linear relationship between ΔFE and time. The slopes of the three lines do not differ significantly although the intercepts do.

The relationship between ΔFE and dose of ISO administered is shown in Fig. 10A. Similarly, the relationship between time of hyperhydration and dose of ISO administered is shown in Fig. 10B. These suggest that the responses are approximately maximal at 25 μg ISO/kg SC.

Figure 11 shows the relationship between ΔFE and time of hyperhydration for both AII and ISO. The relationships are direct and linear in both cases. The slopes, but not the intercepts, differ significantly. This relationship suggests that for each drug the time of hyperhydration is directly related to the extra amount of fluid ingested initially (i.e., ΔFE at 0 time). However, the relationship between these two parameters apparently differs with different treatments. The importance of the relationship shown in Fig. 11 is that it allows a comparison of the effect of different treatments on hyperhydration where doses of drugs or other parameters cannot be equated initially.

DISCUSSION

One of the objectives of these studies was to determine whether a dipsogenic agent other than AII could induce a state of hyperhydration in rats. The results of these studies reveal that the β-adrenoceptor agonist and dipsogenic agent isoproterenol can also induce a hyperhydration. Whether other

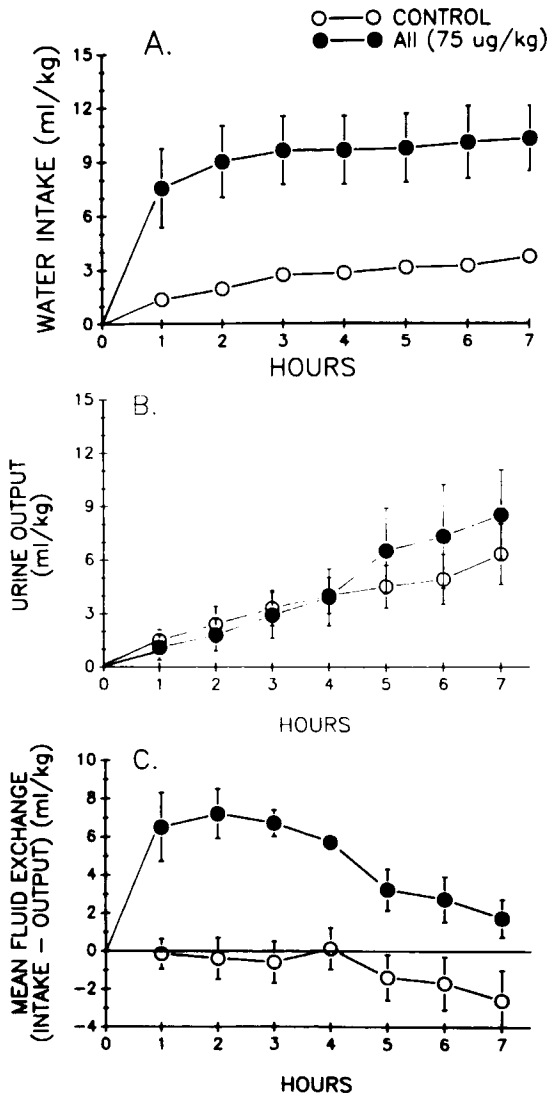

FIG. 3. Effect of administration of angiotensin II (AII) (75 μg/kg, SC) on water intake (A), urine output (B), and mean fluid exchange (C). The groups are designated. Means ± one SE are shown.

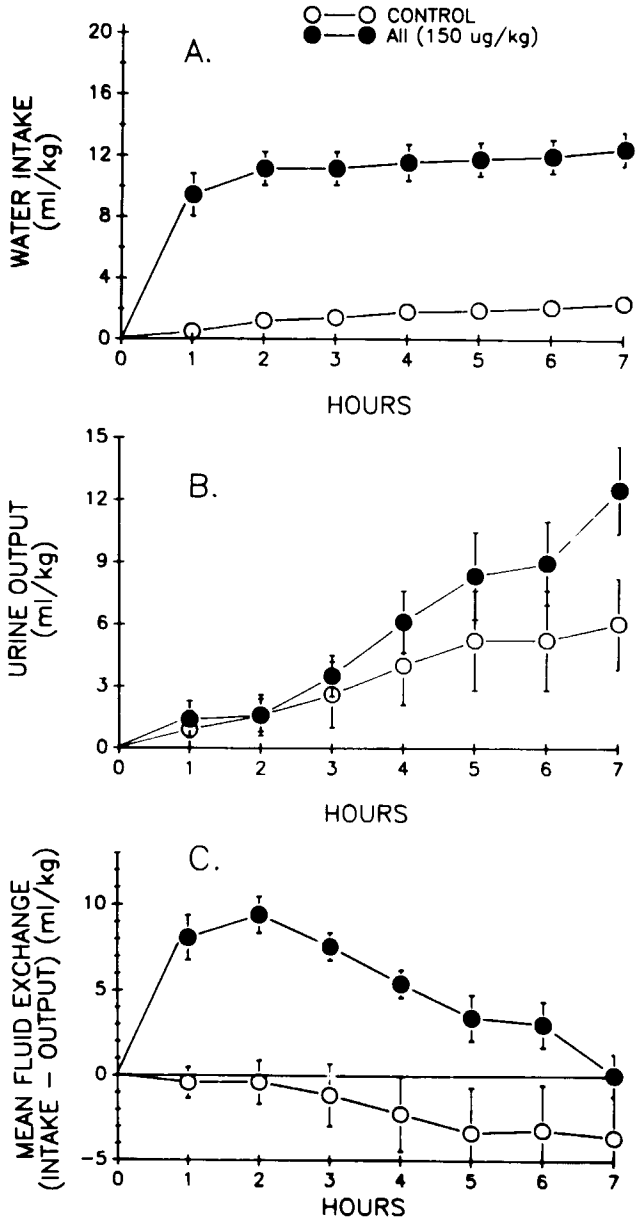

FIG. 4. Effect of administration of angiotensin II (AII) (150 μg/kg, SC) on water intake (A), urine output (B), and mean fluid exchange (C). The groups are designated. Means ± one SE are shown.

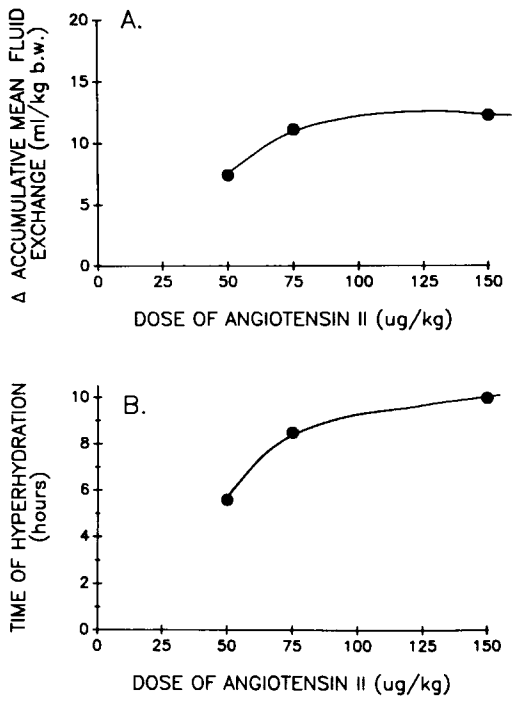

FIG. 5. (A). Change in accumulative mean fluid exchange at each dose of angiotensin II (AII) administered. (B). Time of hyperhydration for each dose of AII administered.

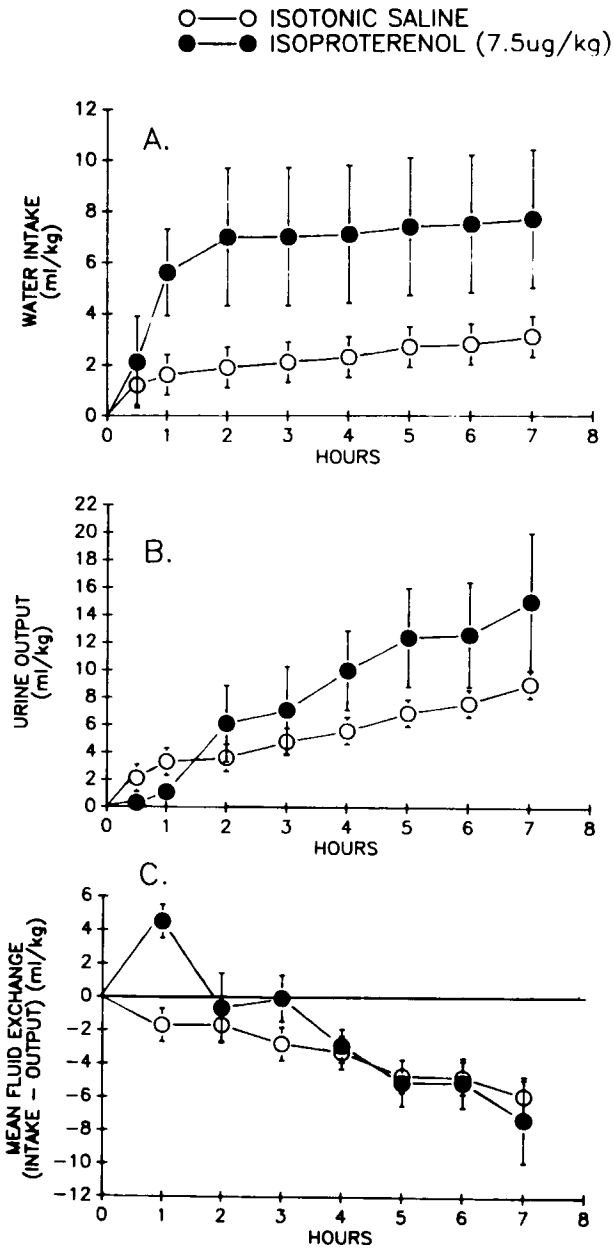

FIG. 6. Effect of administration of isoproterenol (ISO) (7.5 μg/kg, SC) on water intake (A), urine output (B), and mean fluid exchange (C). The groups are designated. Means ± one SE are shown.

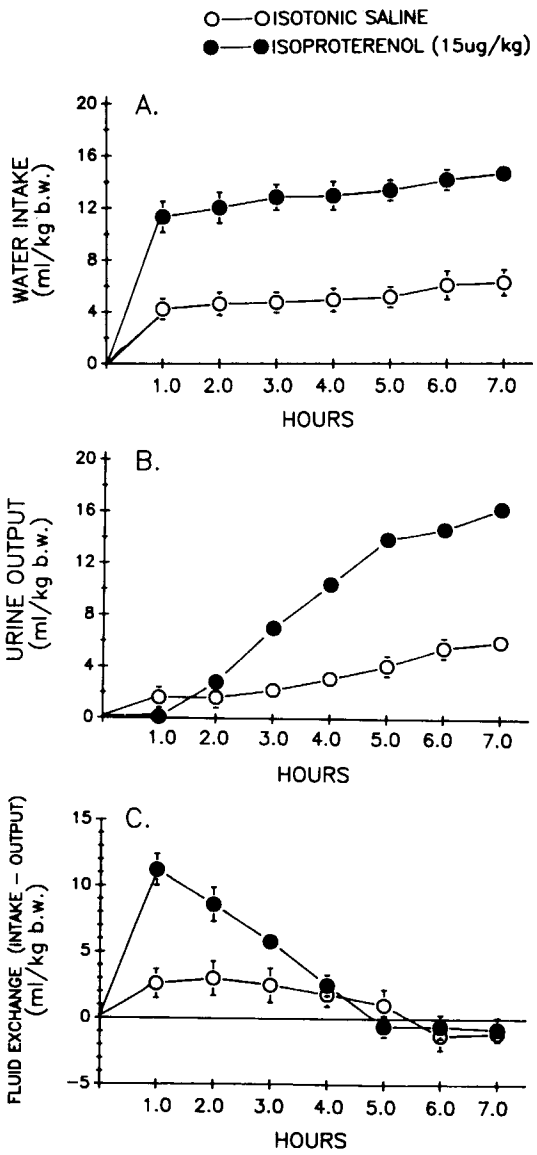

FIG. 7. Effect of administration of isoproterenol (ISO) (15 μg/kg, SC) on water intake (A), urine output (B), and mean fluid exchange (C). The groups are designated. Means ± one SE are shown. When SE bars are not shown, they fall within the symbol.

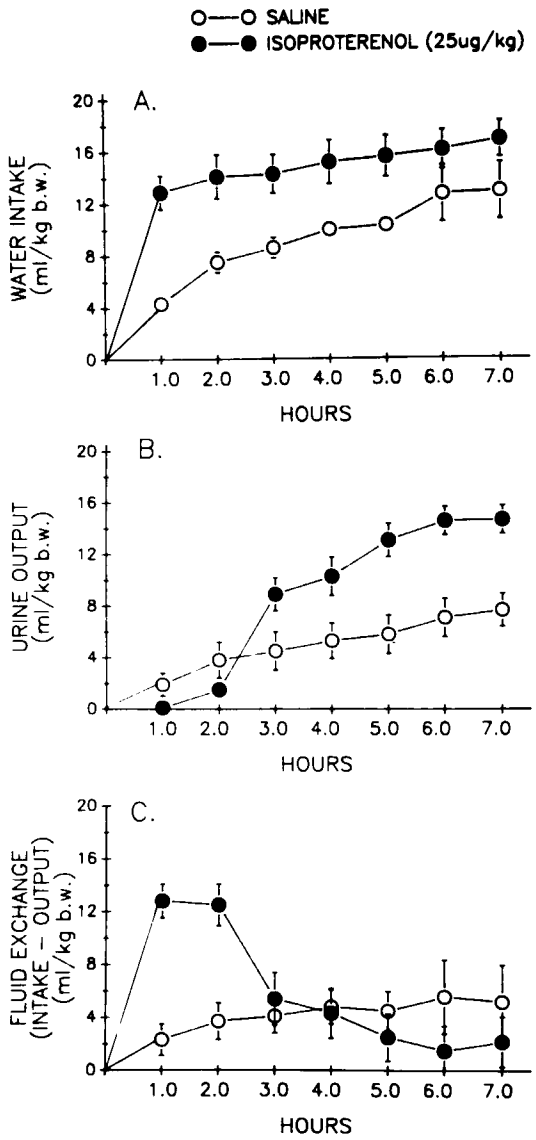

FIG. 8. Effect of administration of isoproterenol (ISO) (25 μg/kg, SC) on water intake (A), urine output (B), and mean fluid exchange (C). The groups are designated. Means ± one SE are shown. When SE bars are not shown, they fall within the symbol.

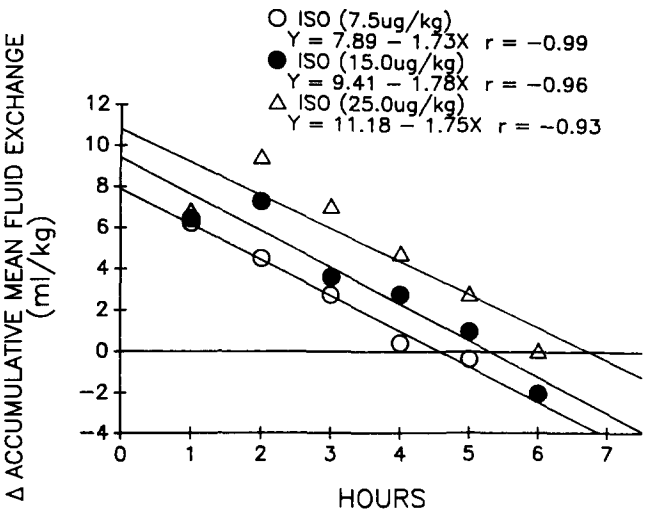

FIG. 9. Change in mean fluid exchange during the 6-h experiment for groups receiving the three doses of isoproterenol (ISO). The groups are designated. The equation describing each regression, along with its correlation coefficient (r), are given.

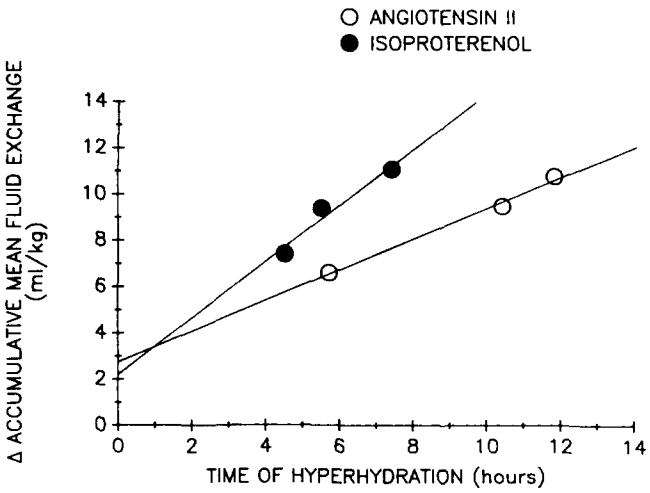

FIG. 11. Relationship between the change in accumulative mean fluid exchange and time of hyperhydration for groups treated with angiotensin II (AII) (○) and isoproterenol (ISO) (●). Equations for the relationships are given.

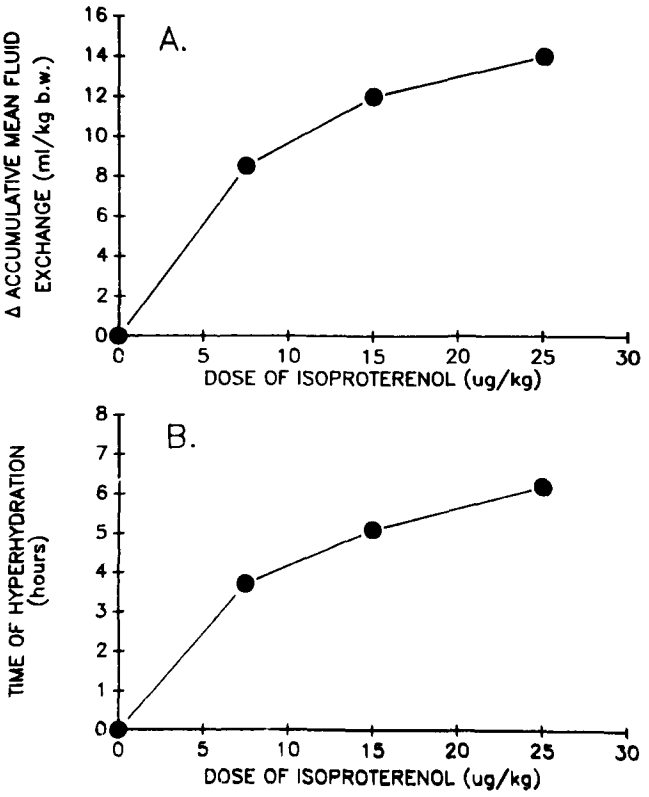

FIG. 10. Change in accumulative mean fluid exchange (A) and time of hyperhydration (B) at each dose of isoproterenol (ISO) administered.

known dipsogenic agents can do likewise will await additional experimentation.

A further aim was to establish a dose-response relationship between the dose of dipsogenic agent administered and time of hyperhydration. This was also accomplished by these studies both for AII and ISO.

An interesting additional finding was that the relationship between ΔFE and time was direct, linear, and dose related. ΔFE extrapolated to time = 0 represents the amount of fluid, above that of the control group, that would have been ingested if intake were instantaneous. Hence, a dose-response relationship would be expected. Extrapolation of the line to $\Delta FE = 0$ represents the time to fluid equilibrium or the time of hyperhydration.

The importance of the results presented here is that they provide a way to compare two different dipsogenic agents. Of necessity, different doses of AII and ISO were used here. Further, one (AII) is a pressor agent while the other is a depressor. One (ISO) increases heart rate while the other does not. Other differences exist in the physiological responses to the two drugs. Hence, equating their effects on hyperhydration on the basis of these responses would be difficult. Because both drugs increase both the time of hyperhydration and ΔFE in a direct, dose-related fashion, relating ΔFE to time of hyperhydration resulted in a positive direct linear relationship between these two parameters for each drug. Comparison of the two lines revealed a significant difference in slopes but not intercepts. Because the objective of optimal hyperhydration should be to achieve the longest duration of positive fluid balance with the least amount of ingested fluid (i.e., ΔFE), the slopes of the two lines provide a convenient way to compare the two drugs. AII can be seen (Fig. 11) to have a longer time of hyperhydration at any level of ΔFE. It is therefore the better drug for purposes of hyperhydration. This conclusion is verified by comparison of the linear regressions of ΔFE vs. time for the doses of AII with those for ISO. Each dose of AII consistently has a smaller slope than does that of ISO, indicating that with AII a smaller rate of decrease in ΔFE is occurring, thus making AII a better agent for hyperhydration.

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 (1). 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 (2).

The present results are in agreement with those reported earlier in that control rats in each study allow themselves to go into negative fluid exchange by as much as 5–8 ml/kg body weight by the end of the 7 h of daylight during which measurements were made (2). It is surprising that such a large negative fluid exchange would occur without inducing sufficient drinking to return animals to balance. This interesting observation requires additional study but is in agreement with earlier results that have shown that water intake by rats occurs almost exclusively at night (5). Further, because all of these studies were started in the morning no attempt has been made to examine whether and how the duration of hyperhydration might vary with time of day. Because other urinary parameters have a pronounced circadian rhythm (4), we would anticipate a day/night difference. However, such studies have not yet been carried out.

The results of these studies, as well as our earlier study (2), reveal that perturbation of fluid exchange resulting from either an acute peripheral or central injection of AII, or peripheral injection of ISO, extends for at least 6 h after injection, that is, well beyond the half-life of the compounds in the circulation. The duration of this perturbation should be kept in mind by those investigators who use these compounds to study the physiological regulations and responses of body fluids to their administration.

ACKNOWLEDGEMENTS

The authors thank C. Edelstein, M. Leveritt, T. Connor, and H. Clark for technical assistance. This research was supported by Grant HL39154-06 from the National Heart, Lung, and Blood Institute (Bethesda, MD).

REFERENCES

1. Fregly, M. J. Effect of exposure to cold on evaporative water loss. Am. J. Physiol. 213:1003–1008; 1967.
2. Fregly, M. J.; Wilson, K. M.; Rowland, N. E.; Cade, J. R. Hyperhydrating effect of acute administration of angiotensin II in rats. Pharmacol. Biochem. Behav. 41:183–188; 1992.
3. Lazarow, A. Methods for quantitative measurement of water intake. Meth. Med. Res. 6:254–259; 1954.
4. Moore-Ede, M. C.; Sulzman, F. M.; Fuller, C. A. The clock that times us. Cambridge, MA: Harvard University Press; 1982.
5. Rowland, N. E.; Bellush, L. L.; Fregly, M. J. Nycthemeral rhythms and sodium chloride appetite in rats. Am. J. Physiol. 249 (Reg. Integr. Comp. Physiol. 18):R375–R378; 1985.