Increased Dipsogenic Responsiveness to Angiotensin II in Rats Exposed to Cold: Rate of Loss After Return to Thermoneutral Ambient Temperature (43877)

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Abstract. **Subcutaneous administration of angiotensin II (Ang II) to rats exposed chronically to 5°C induced an increased drinking response compared with that of warm-acclimated controls. The exaggerated drinking response was also observed when graded doses of Ang II were administered into the lateral cerebroventricle (icv) of chronically cold-exposed rats. A maximal drinking response occurred in cold-treated, but not in control, rats when the lowest dose of Ang II (1.6 ng/rat) was administered icv. Thus, it is clear that the dipsogenic responsiveness to either centrally or peripherally administered Ang II is increased by chronic exposure to cold. To assess whether the increased responsiveness was retained after removal from cold, graded doses of Ang II were administered to rats removed from cold to a thermoneutral environment. The results again showed a maximal responsiveness to the lowest dose of Ang II administered (25 μg/kg, sc) to cold-treated rats that had either just been removed from cold or removed from cold 2 hr prior to treatment. Cold-exposed rats had an ED_{50} for Ang II-induced drinking that was about half that of their warm-acclimated controls. To assess how long the cold-induced increase in dipsogenic responsiveness to Ang II lasted after return to a thermoneutral temperature, rats were removed from cold for 24, 48, or 60 hr and then administered graded doses of Ang II (25, 50, or 100 μg/kg, sc). The results suggest that between 46 and 52 hr after removal from cold, the cold-induced increase in dipsogenic responsiveness to Ang II returned to the level of the controls. Hence, the physiological changes in the dipsogenic mechanism induced by exposure to cold are not immediately reversible when the rats are returned to a thermoneutral ambient temperature.** [P.S.E.B.M. 1995, Vol 209]

Rats that are exposed chronically to a cold environment may become volume depleted and/or dehydrated (1–3). Plasma osmolality and chloride concentration increase (3). Mechanisms involved in intake, as well as those involved in output, seem to be affected. Thus, daily water intake for a given daily food intake (prandial drinking) is less during exposure to cold than at a thermoneutral temperature (2). This observation is surprising because of the close coupling of daily water intake to food intake and could result in relative dehydration (4). In addition, cold-treated rats excrete more urine for a given water intake during a 24-hr period than warm-acclimated rats (2). Urinary concentrating ability after both a 24-hr dehydration and after administration of pitressin is less in cold-treated than warm-acclimated rats (5, 6). These could also result in a relative dehydration in cold-treated rats. A contrasting observation is an increased dipsogenic response to peripheral administration of angiotensin II (Ang II) to rats during exposure to cold (7). Ang II is believed to initiate drinking by interac-

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Received February 28, 1994. [P.S.E.B.M. 1995, Vol 209]
Accepted December 1, 1994.

0037-9727/95/2091-0054$10.50/0

tion with its receptors in the subfornical organ and the organum vasculosum of the lamina terminalis in the anterior portion of the third ventricle of the brain (8). Addition of Ang II to the cerebrospinal fluid bathing these areas induces drinking in the rat (8). An objective of the studies presented here was to compare the dipsogenic responsiveness of cold-treated rats to central administration of Ang II with that of warm-acclimated controls to determine whether the increased responsiveness to Ang II observed with peripheral administration is also observable with central administration.

A second objective was to determine how long after removal from cold the increased dipsogenic responsiveness to Ang II remained. To do this, it was necessary to separate the postcold (thermogenic) drinking response from that due to an increased responsiveness to Ang II. This was important to study because a striking acute drinking response occurs when rats are removed from a cold to a warm environment (thermogenic drinking) (3). Cold-treated rats may ingest as much as 10% of their daily water intake within 15 to 30 min following removal from cold (3, 9). Prior studies have shown an increase in plasma renin activity (PRA) following removal from cold to a thermoneutral temperature (9, 10). Hence, the post-cold exposure drinking response may be related both to an increase in plasma osmolality, as mentioned above, and to an increase in the release of renin from the kidneys, with an accompanying increase in production of Ang II. A further factor that may contribute is an increased dipsogenic responsiveness to Ang II. Hence, an additional objective of this study was to assess the dipsogenic responsiveness to Ang II after removal from cold to a thermoneutral environment at intervals after the thermogenic drink had subsided.

Materials and Methods

The studies described below were carried out on male Sprague-Dawley rats of the Blue Spruce Farms strain initially weighing from 250 to 300 g. They were housed individually in temperature-controlled rooms at either 26° ± 2° or 5° ± 2°C. The rooms were illuminated from 8:00 AM to 8:00 PM daily. The rats were provided with finely powdered Purina Laboratory Chow (#5001) and tap water *ad libitum*. Fluid containers consisted of infant nursing bottles with cast bronze drinking spouts (11). Food containers were spill resistant (12).

Study 1. Dipsogenic Responsiveness to Central Administration of Ang II in Cold-Treated Rats. Twelve rats were placed in air at 5°C for 12 days, while 12 controls were maintained at 26°C. On Day 12, all animals were anesthetized with 5% chloral hydrate (0.8 ml/100 g, ip). An intracerebroventricular (icv) cannula, 12 mm long and constructed of 22-gauge stainless steel tubing containing a 12-mm long stainless steel obturator, was inserted into the lateral cerebral ventricle of each rat using a Kopf stereotaxic instrument. The coordinates were 1.0 mm posterior, 1.0 mm lateral, and 5.0 mm deep with respect to bregma. Each cannula was secured to the skull with dental cement and stainless steel screws. The animals were allowed to recover fully postoperatively at 26°C prior to return to their home cages.

Between Day 20 and 25 of the study, the dipsogenic response of control and cold-treated rats to central administration of Ang II (0, 1.6, 2.5, and 10.0 ng, icv) was tested. The doses were randomized to prevent any effect of increasing dose. On the day of the test, each rat was weighed and the drug was injected into the icv cannula via PE-10 tubing attached to a 10 μl Hamilton syringe. The total volume injected was 2 μl. The solutions for injection were maintained sterile, and sterile isotonic saline was injected as a control. Immediately after injection, the rat was returned to its cage, and a preweighed bottle of water (26°C) placed in the cage. No food was available during the test. Water intake of each rat was measured gravimetrically 1 hr later.

To assess the specificity of the responses measured above, the dipsogenic cholinergic agonist, carbachol (200 ng, icv), was administered (Day 26) to both groups, and its effect on water intake was measured for 1 hr.

A final study was performed (Day 28 and 30 of cold exposure) to assess the dipsogenic response to Ang II when administered peripherally (50 and 150 μg/kg, sc). The experiment was otherwise carried out identically to that described above.

Study 2. Dipsogenic Responsiveness to Ang II in Cold-Treated Rats Removed from Cold for 2 Hr. Thirty naive rats were divided at random into three groups of 10 rats each. Two of the groups were placed in individual stainless steel cages in the cold, and a third group, also caged individually, remained at 26°C and served as warm-acclimated controls. After 3 weeks of exposure to cold, the dipsogenic responsiveness to acute administration of Ang II was assessed. At 9:00 AM, one group of cold-treated rats (postcold group) was removed to 26° ± 2°C, and allowed access to water to satiate their postcold (thermogenic) drink (1, 2). Two hours later, the second group of cold-treated rats (cold-control group) was removed from cold. At this time, food containers and water bottles were removed from the cages of the postcold, cold-, and warm-acclimated control groups. One half of the rats in each group (n = 5) received Ang II (25 μg/kg, sc; #9525; Sigma Chemical Co., St. Louis, MO), while the remaining half served as controls and received the vehicle (saline). Immediately after treatment, a preweighed water bottle was placed on the cage of each

rat, and water intakes were measured gravimetrically at 0.5, 1.0, and 2.0 hr thereafter. The two cold-treated groups were then returned to cold, and the experiment was repeated at intervals of 3–4 days with increasing doses of Ang II (50, 100, and 150 μg/kg). The animals removed from cold for 2 hr, as well as those removed from cold immediately before each test, were randomized prior to administration of Ang II.

Study 3. Dipsogenic Responsiveness to Ang II at 24, 48, and 60 hr After Removal from Cold. The rats in Study 2 above were used in this study. They were divided into five groups, each containing six rats. Four of the groups were placed in the cold and the fifth group remained at 26°C. One week after completing Study 2, the dipsogenic responsiveness to acute administration of Ang II was measured in cold-treated rats removed from cold at 60, 48, and 24 hr prior to the study, as well as in the cold- and warm-acclimated control groups. All rats were administered Ang II (25 μg/kg, sc), and water intakes were measured at 0.5, 1.0, and 2.0 hr thereafter as described above. At the end of this study, the cold-treated rats were returned to the cold, and the study was repeated with graded doses of Ang II (50 and 100 μg/kg) at 3- to 4-day intervals.

In both Study 2 and 3, the data for the dipsogenic responsiveness to Ang II were analyzed using a repeated measures, one-way ANOVA. The post-hoc Duncan's New Multiple Range test was used to test the significance of the difference between two individual means. The level of significance was set at the 95% confidence limit. Data are expressed as mean ± SEM.

Results

Study 1. The dipsogenic response to icv administration of graded doses of Ang II was increased in the cold-treated group compared with the warm-acclimated controls (Fig. 1). It is apparent in Figure 1 that the lowest dose administered to the cold-treated group increased their water intake maximally and significantly ($P < 0.01$) above that of the control group. Higher doses of Ang II did not induce a further increase in water intake in the cold-treated group. In contrast, the control group increased its water intake with increasing doses of Ang II and reached the maximal level achieved by the cold-treated group when the highest dose of Ang II was administered. There were no significant differences between the water intakes of the two groups when carbachol was administered icv. Thus, at 1 and 2 hr after treatment, accumulative water intakes of the control group were 26.1 ± 5.5 (SEM) and 26.3 ± 5.5 ml/kg body wt, respectively. At the same times, the cold-treated rats drank 17.1 ± 3.2 and 17.4 ± 3.1 ml/kg, respectively. Differences between groups at these times were not significant.

When Ang II was administered peripherally, the drinking response of the cold-treated group was increased significantly ($P < 0.05$) above that of the control group at both doses (50 and 150 μg/kg) and at all times after treatment (Fig. 2). As observed elsewhere (13), the major portion of the drinking response occurred within the first half-hour after treatment with Ang II.

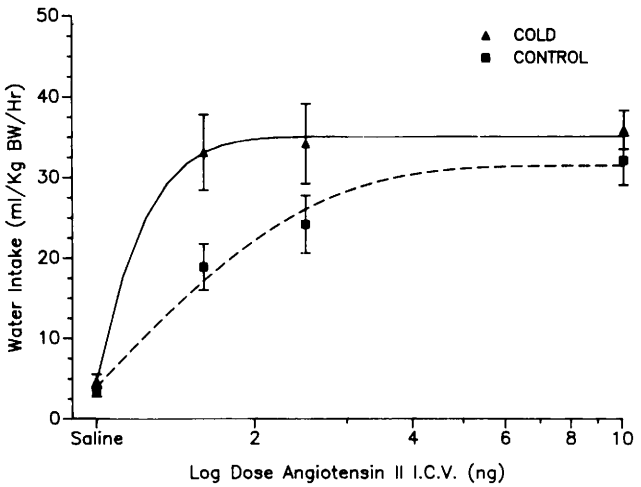

Figure 1. Effect of chronic exposure to cold on the dipsogenic responsiveness to central (icv) administration of graded doses of angiotensin II. The cold-treated and control groups are designated in the figure. Mean ± SEM are shown.

Study 2. Water intakes at 0.5, 1.0, and 2.0 hr after acute administration of Ang II (25, 50, 100, and 150 μg/kg) were greater for the group removed immediately from cold (Fig. 3, Cold AII) than for the group removed from cold 2 hr earlier (Fig. 3, Post AII) or for the warm-acclimated (Fig. 3, Warm AII) group. This was the case at all times (0.5, 1.0, and 2.0 hr) measure-

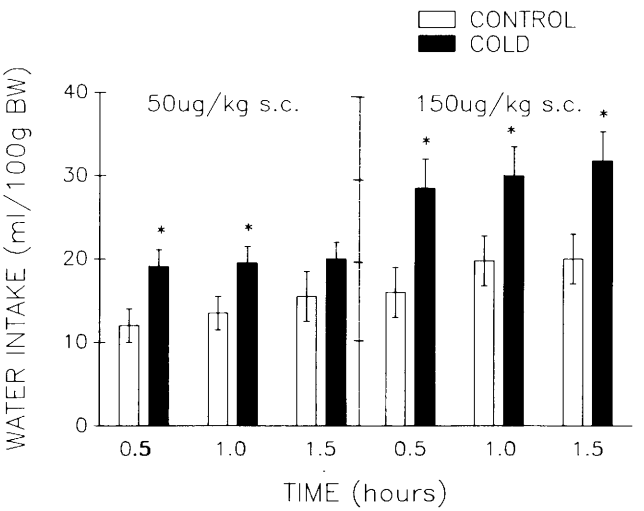

Figure 2. Effect of chronic exposure to cold on the dipsogenic responsiveness of cold-treated and control rats to peripheral (sc) administration of either 50 or 150 μg of angiotensin II/kg, sc. The treated and control groups are designated in the figure. Mean ± SEM are shown. *$P < 0.05$ compared with control group.

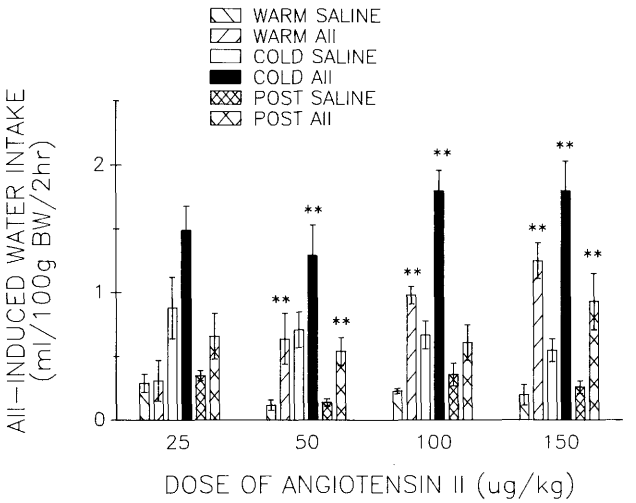

Figure 3. Dose-response relationship between water intakes of cold-treated and control groups, and graded doses of angiotensin II (μg/kg, sc). Cold-treated groups were removed either immediately prior to the study (Cold Saline and Cold AII) or 2 hr prior to the study (Post Saline or Post AII). Control rats (Warm Saline and Warm AII) were not exposed to cold. Means ± SEM are shown. The groups are designated in the figure. $^{**}P < 0.01$ compared with appropriate saline-treated control.

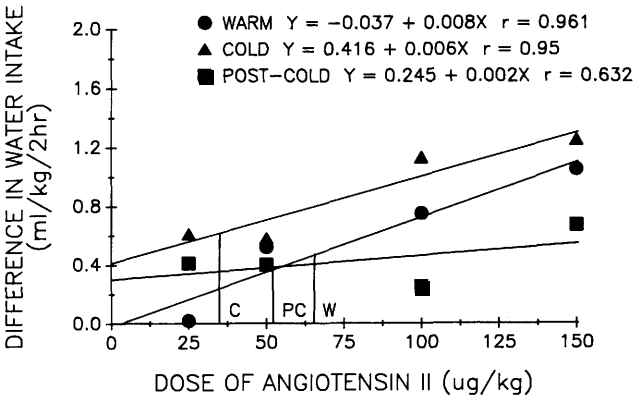

Figure 4. Linear regressions of the differences in water intake between each Ang II-treated group and its respective saline-treated control are shown in Figure 2 versus dose of Ang II administered. The groups, the regression equations, and correlation coefficients (*r*) for each equation are given in the figure. The vertical lines intersecting the *x* axis represent the ED_{50} for warm-acclimated (W), cold-acclimated (C), and post-cold (PC)-treated groups.

ments were made. Hence, only the accumulative 2-hr water intakes are shown in Figure 3. Administration of Ang II to either warm-acclimated (Fig. 3, Warm AII) or cold-treated (Fig. 3, Cold AII) groups was accompanied by a significant ($P < 0.05$) increase in water intake above that of the corresponding control group at doses of Ang II exceeding 50 μg/kg. At all doses of Ang II, there were no significant differences between the water intakes of the warm-acclimated (Fig. 3, Warm Saline) group and the group removed from cold at 2 hr prior to the study (Fig. 3, Post Saline).

The differences in water intakes between the Ang II-treated groups and their corresponding saline-treated control groups are shown in Figure 4. These differences increase with administration of increasing doses of Ang II, except for the group removed from cold 2 hr prior to treatment (Fig. 4, Post Cold). The slope of the linear regression of difference in water intake versus dose of Ang II administered to the warm-acclimated group was not different from that of the cold-acclimated group although the intercepts differed significantly ($P < 0.01$) (Fig. 4). The slope of this relationship for the postcold group was less than that of both the warm-acclimated and cold-acclimated groups, but was significantly ($P < 0.05$) less only when compared with that of the warm-acclimated group. There was no significant difference between the intercepts of the cold and post-cold-treated groups, although there was a significant ($P < 0.05$) difference between the intercepts of the warm and postcold groups.

Assuming that 150 μg Ang II/kg induces a maximal difference in water intake in each group, it can be seen in Figure 4 that the half-maximal dose (ED_{50}) of Ang II for the cold-treated group is approximately 34 μg/kg, while that of warm-acclimated controls is approximately 64 μg/kg and that of the post-cold-treated group is approximately 52 μg/kg. Thus, these comparisons show clearly that the half-maximal water intake for the cold-treated group occurs at a dose of Ang II about half that of the warm-acclimated group and 35% below that of the post-cold-treated group. Hence, chronic exposure to cold increased the dipsogenic responsiveness to Ang II.

Study 3. Water intake in response to administration of Ang II (25 μg/kg) prior to removal from cold and at intervals thereafter is shown in Figure 5. The dipsogenic responsiveness to acute administration of Ang II decreased with increasing time after removal from cold. Water intakes of the cold-treated groups

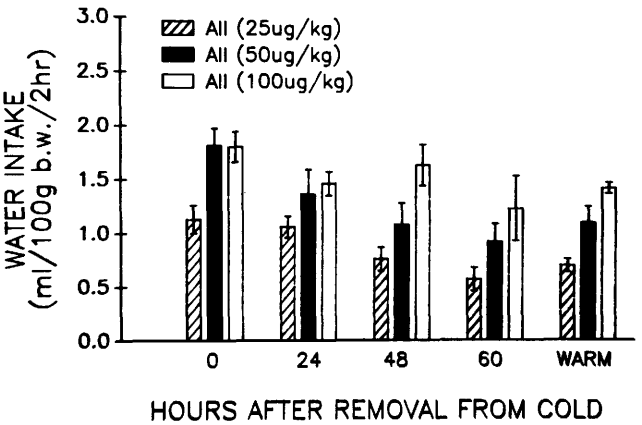

Figure 5. Water intakes before and at graded intervals after removal from cold are shown for the three doses of Ang II administered. Dipsogenic responses of warm-acclimated controls to the same doses of Ang II are shown at the right of the figure. Means ± SEM are shown. Doses are designated in the figure.

prior to removal from cold were apparently maximal when 50 μg of Ang II/kg was administered since 100 μg of Ang II/kg failed to increase water intake further. A typical dose-response relationship was observed beginning at 48 hr after removal from cold (Fig. 5).

A linear regression analysis of water intake at the various times Ang II was administered after removal from cold suggests that the increased dipsogenic responsiveness of the cold-treated rats given any of the three doses of Ang II reached that of warm-acclimated controls receiving the same dose of Ang II at some time between 46 and 52 hr after removal from cold (Fig. 6). This is calculated as the intersection of the regression lines for cold-treated groups given the three doses of Ang II shown in Figure 6 with that of their appropriate warm-acclimated control group given the same dose of Ang II and represented as a horizontal line in Figure 6. Thus, regardless of the dose of Ang II administered, the increased dipsogenic responsiveness of the cold-treated group returned to that of the warm-acclimated control group at some time between 46 and 52 hr after removal from cold.

Discussion

The increased dipsogenic responsiveness of cold-treated rats to peripheral administration of Ang II is accompanied by an increased central responsiveness as well (Fig. 1). This seems to be unique to Ang II, since it could not be shown for carbachol, another well known dipsogenic agent. The increased dipsogenic responsiveness does not disappear immediately when the rats are removed from cold to thermoneutral temperature. The dipsogenic response to administration of Ang II (25, 50, 100, and 150 μg/kg) was greater for the cold-treated group than either the control or postcold groups. It is also clear that the dipsogenic response to an injection of saline was greater in cold-treated rats (Fig. 3). This is most likely due to the postcold (thermogenic) drink, since water intake was significantly ($P < 0.01$) greater than that of similarly treated, warm-acclimated controls. The fact that the rats in the postcold group did not have a greater dipsogenic responsiveness to Ang II than controls suggests that the postcold drinking response may have been a major contributor to the dipsogenic response to Ang II at this time. However, the effect of Ang II on drinking could have been inhibited in this group either by a decrease in osmolality of the plasma due to prior ingestion of water (14, 15) or to an inhibition of the drinking response by stretch receptors in the wall of the stomach induced by prior ingestion of water (14). Other studies from this laboratory have shown that either intragastric or intraperitoneal loading (3% body wt) prior to removal from cold significantly attenuated the thermogenic drink (15). Similar loads also attenuated Ang II-induced drinking (14). Thus, the postcold (thermogenic) drinking response apparently masked the increased responsiveness to Ang II in the group removed from cold for 2 hr prior to initiation of the test. The results of additional studies carried out after the thermogenic drinking response had subsided showed that an increased responsiveness to Ang II remained for nearly 50 hr after removal from cold (Fig. 6).

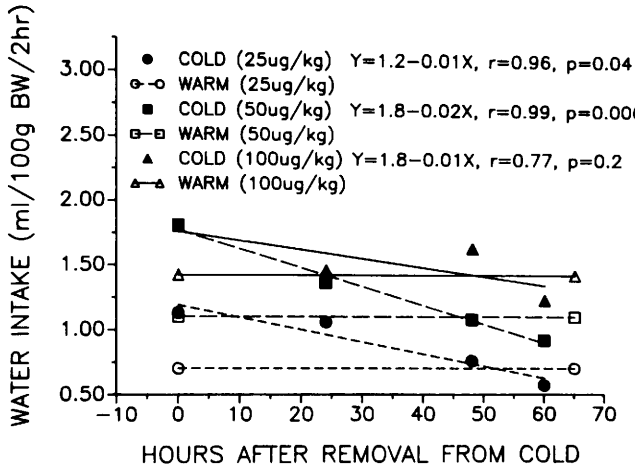

Figure 6. Linear regressions of water intake versus hours after removal from cold are shown for the cold-treated groups administered the three doses of Ang II. Water intakes for similarly treated warm-acclimated control groups are shown as a straight line in the figure. Note the decrease in water intake with time after removal from cold for the groups given the three doses of Ang II. The intersection of the regression line with the straight line of its respective control group represents the time after removal from cold for Ang II-induced water intake of the cold-treated group to return to control level.

An earlier study from this laboratory reported similar observations in rats removed from cold for 2 hr, not only with Ang II-induced drinking, but with isoproterenol-, serotonin-, and hypertonic saline-induced drinking as well (16). The results uniformly failed to show that prior exposure to cold affected water intake induced by the compounds mentioned above. In retrospect, it would appear that an increased dipsogenic responsiveness to Ang II, and possibly the other dipsogenic agents as well, was most likely masked by the postcold (thermogenic) drinking response.

An earlier study also showed that plasma renin activity in rats removed from cold increased significantly above that observed during exposure to cold (10). Hence, the postcold drinking response was attributed to an increased concentration of Ang II in plasma. Strengthening this suggestion was the fact that the postcold (thermogenic) drink could be prevented by administration of the angiotensin I-converting enzyme inhibitor, captopril, to rats prior to removal from cold (1, 2). By 24 hr after removal from cold, an increased dipsogenic responsiveness to administration of Ang II was observed in the cold-treated rats. These results are consistent with the possibility that an up-regulation of Ang II receptors occurred sometime be-

tween 2 and 24 hr after removal from cold. This remains to be verified.

The present data support the results of previous studies in which a dose-response relationship was observed between dose of Ang II administered peripherally and the drinking response induced by it (17). The present data also support an earlier suggestion that the drinking response of cold-exposed rats is more sensitive to acute administration of Ang II than is that of warm-acclimated controls whether the Ang II is administered either centrally or peripherally (18). Earlier studies from this laboratory showed a direct relationship between water intake in 1 hr after subcutaneous administration of Ang II and the number of Ang II receptors in the diencephalon of the brain of deoxycorticosterone acetate-treated rats (19). By analogy, the results of the present study suggest that the increased drinking response of cold-treated rats to Ang II may be related to an upregulation of Ang II receptors in the diencephalon of the brain. A possibility exists that the increased dipsogenic response to Ang II may help to maintain body fluids in the cold-exposed rat. Earlier studies from this laboratory have shown that cold-exposed rats are dehydrated relative to warm-adapted controls in that serum osmolality and serum chloride concentration are significantly increased (3). This could result from reduced prandial drinking (i.e., decreased water intake for a given food intake) during exposure to cold (2, 3). Hence, the importance of both the postcold drinking response and the increased dipsogenic responsiveness to Ang II may be to restore to normal the changes in body fluid that occurred during chronic exposure to cold.

This work was supported by Grant HL 39154-07 from the National Heart, Lung, and Blood Institute, Bethesda, MD. We thank Dr. Paula Papanek for her help in carrying out Study 1.

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