Effect of intracerebroventricular angiotensin II on body weight and food intake in adult rats

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Porter, James P., and Kristen R. Potratz. Effect of intracerebroventricular angiotensin II on body weight and food intake in adult rats. Am J Physiol Regul Integr Comp Physiol 287: R422–R428, 2004. First published April 29, 2004; 10.1152/ajpregu.00537.2003.—We recently reported that intracerebroventricular infusions of ANG II decreased food intake and increased energy expenditure in young rats. The aim of the present study was to determine if intracerebroventricular ANG II has similar effects in adult rats. The time course of the effect was also investigated with the idea that at earlier time points, a potential role for increased hypothalamic expression of corticotropin-releasing hormone (CRH) in the anorexia could be established. Finally, the contribution of ANG II-induced water drinking to the decrease in food intake was directly investigated. Rats received intracerebroventricular saline or ANG II using osmotic minipumps. Food intake, water intake, and body weight were measured daily. Experiments were terminated 2, 5, or 11 days after the beginning of the infusions. ANG II (32 ng·kg⁻¹·min⁻¹) produced a transient decrease in food intake that lasted for 4–5 days although body weight continued to be decreased for the entire experiment most likely due to increased energy expenditure as evidenced by increased uncoupling protein-1 mRNA expression in brown adipose tissue. At 11 days, the expression of CRH mRNA was decreased. At 2 days, CRH expression was not suppressed even though body weight was decreased. The decrease in food intake and body weight was identical whether or not rats were allowed to increase water consumption. These data suggest that in adult rats ANG II acts within the brain to affect food intake and energy expenditure in a manner that is not related to water intake.

It is now well known that ANG II has effects on food intake and energy expenditure (4–7). We recently showed in young 3-wk-old rats that chronic intracerebroventricular infusion of ANG II decreased the rate of weight gain, in part by decreasing food intake, and in part by increasing energy expenditure (14). Systemic infusion of identical doses of ANG II had no effect, suggesting that the response was mediated by an action of the peptide within the brain. However, it was not known if the observed effects of ANG II were somehow associated with the fact that the rats were young and in a rapid growing phase. For example, a potential effect of intracerebroventricular ANG II to inhibit growth hormone release could have contributed to the decrease in weight gain (9, 17). A primary aim of the present study, therefore, was to determine if intracerebroventricular infusion of ANG II had similar effects on body weight and energy expenditure in adult rats that were beyond the rapid growing phase.

A possible connection between brain ANG II and food intake involves corticotropin-releasing hormone (CRH) in the paraventricular nucleus of the hypothalamus (PVN). CRH is a well-known anorexic peptide, and CRH-containing neurons in the PVN are known to express the ANG II type 1 (AT₁) receptor (1, 10, 16). Our earlier study looked at CRH mRNA expression in the PVN at the time of termination of the experiment after a 10-day intracerebroventricular infusion of ANG II. The expression of CRH mRNA was, if anything, decreased at that time point. A second aim of the present investigation was to look at earlier time points to see if the decreased food intake imparted by intracerebroventricular ANG II could be correlated with increased expression of CRH mRNA in the PVN.

Brain ANG II is a potent dypsogen (18), and in our earlier study we showed that there was no apparent correlation between water intake and food intake. The final aim of the present investigation was to show definitively that the effect of intracerebroventricular ANG II to decrease food intake did not involve its effect to increase water intake.

The data show that intracerebroventricular infusion of ANG II in adult rats decreased body weight. For the first few days of infusion, the decrease in body weight appeared to be due to decreased food intake that was not accompanied by a relative increase in CRH mRNA in the PVN. The anorexic effect of intracerebroventricular ANG II was transient, only lasting for 4–5 days. After that, the lower body weight was due to increased energy expenditure. Rats that were not allowed to increase their water intake in response to intracerebroventricular ANG II showed food intake effects, body weight effects, and energy expenditure effects that were similar to rats that were allowed to increase their water consumption. Portions of this work were presented in abstract form (15).

MATERIALS AND METHODS

Experiments were performed using male Sprague Dawley rats (beginning wt, 240–280 g) obtained from a commercial vendor (Harlan Sprague Dawley). The rats were individually housed in hanging metal cages and were fed standard rodent diet (Harlan Teklad, 8604). The animal room was maintained at 24°C with a 12:12-h light-dark cycle. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Brigham Young University.

All rats were prepared with a chronic intracerebroventricular infusion cannula as follows. Anesthesia was induced with a mixture of

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Effect of ICV ANG II on Body Weight in Adult Rats

R243

ketamine (125 mg/kg) and acepromazine (1.5 mg/kg) given intraperitoneally. A small hole was drilled through the skull over the right lateral cerebroventricle. Using a stereotaxic instrument (Kopf), an infusion cannula (Alzet Brain Infusion Kit II) connected to a primed minipump (Alzet, 2002) was lowered into the ventricle and cemented to the skull using cyanoacrylate adhesive. The minipump was inserted under the skin between the scapulas, and the incision was closed. The minipumps delivered solution at a rate of 0.5 μl/h and were filled with either sterile saline or ANG II. The ANG II was delivered at a dose of 8 ng/min (32 ng·kg⁻¹·h⁻¹ to start). This dose was arbitrarily chosen to be approximately one tenth the highest dose (on a per kg basis) used in our earlier study with young rats. The lower dose was chosen because of a report that older rats are more responsive to the weight-reducing effects of systemic ANG II (4). In the 2-day study (see below), the catheter between the minipump and infusion cannula was prefiled with sterile saline rather than ANG II to give the rats at least 2 days to recover from the surgery before the ANG II reached the cerebroventricles.

Daily food intake, water intake, and body weight were determined each afternoon (1600–1700) in rats prepared as outlined above. Food intake was measured by subtracting the weight of the remaining pellets and crumbs (collected below the food trough in a plastic weight boat taped to the cage bottom so as to minimize contamination with urine or feces) from the weight control the previous day before (at ~1700). If crumbs were wet, the weight boat was replaced and the crumbs were allowed to air-dry for 1 day before weighing. Water intake was determined using a graduated cylinder. Experiments were run for 2 (after the ANG II reached the cerebroventricles), 5, or 11 days. For each experiment there were at least three groups of rats. One group received intracerebroventricular ANG II and was allowed to eat ad libitum. Another group received intracerebroventricular saline and was allowed to eat ad libitum. The third group was a pair-fed control. These rats received intracerebroventricular saline and were allowed to eat only the amount of food consumed by the ANG II-infusion group. The pair-fed group ran 1 day behind the other groups. Pair feeding was done at the end of each day. Water was given ad libitum. In a 11-day experiment, a fourth group received intracerebroventricular ANG II and ate ad libitum but was not allowed to consume the amount of water drunk by the intracerebroventricular saline, ad libitum-food intake group on the pair-fed group (i.e., water intake was increased on day 2). In the 2-day study, a separate group (n = 5) of rats served as an unoperated control. These rats also ate and drank ad libitum. Feeding was done at ~1700, just before lights out (1730), so that the pair-fed rats (who began eating immediately) and the ad libitum-fed rats would begin their meals at approximately the same time.

At the end of a given experiment, the rats were again anesthetized with the ketamine-acepromazine cocktail (same dose as above). Due to a shortage of ketamine, the 5-day experimental groups were anesthetized with pentobarbital sodium (50 mg/kg). A catheter (PE-50, heat fused to PE-10) was immediately inserted into a femoral artery, and mean arterial pressure was monitored for 2–3 min. The rats were then perfused through the left cardiac ventricle with 30–40 ml of sterile saline. Nose-anus length was determined, and then the brain was removed and frozen in tissue molds fixed in Optimal Cutting Temperature compound (Sakura, Torrance, CA) that were floating in 100% ethanol cooled with dry ice. The epididymal fat pad was removed bilaterally and weighed. The brown adipose tissue (BAT) between the scapulas was also removed, weighed, and immediately frozen in liquid nitrogen for subsequent determination of uncoupling protein-1 (UCP-1) mRNA expression using RT-PCR.

In situ hybridization. Coronal sections (20 μm) through the PVN were cut with a cryostat and thaw-mounted onto slides (Superfrost Plus, Fisher). Placement of the intracerebroventricular cannula at the top of the lateral ventricle was verified by visual inspection during sectioning. The sections were subsequently fixed (4% buffered parafomaldehyde), acetylated, and dehydrated. Hybridization, in situ, was carried out overnight at 57°C using a [32P]UTP-labeled (1 × 10⁶ cpm per section) antisense riboprobe to CRH mRNA (Dr. K. Mayo, Northwestern Univ., Evanston, IL). Unincorporated probe was removed by incubating the slides in RNase (14 μg/ml, Sigma) for 30 min followed by washes in buffer without RNase, 1× standard saline citrate (SSC; room temperature) and 0.5× SSC (60°C). Initial visualization of the hybridized probe utilized an overnight exposure to 60 min of the chemiluminescent film (Hybide MP, Amersham). The slides were subsequently dipped in NTB-2 emulsion and exposed in light-tight boxes for 48 h. The emulsion was then developed and fixed, and the sections were lightly stained with cresyl violet, dehydrated in ethanol, placed in xylene, and coverslipped using Permount.

Expression of UCP-1 mRNA using relative RT-PCR. Total RNA was isolated from BAT after homogenization in Trizol (Invitrogen) reagent. RNA (2 μg) was reverse transcribed to cDNA using 200 U of Superscript II (Invitrogen) in the presence of 1 μl of dNTP mix (10.0 μM each), 0.4 μl of random decamers (250 μM, Ambion), 2 μl of DTT (0.1 M), 40 U of RNase inhibitor (Invitrogen), and 2 μl of 10× buffer (100 mM Tris·HCl, pH 8.3, 500 mM KCl, and 15 mM MgCl₂). Each tube had a final volume of 20 μl and was incubated at 42°C for 2 min. Reaction was then cooled on ice and the RNA was extracted using 4 μl of a 324-bp fragment that was used as an internal control. Each PCR reaction was adjusted to 20 μl of the RT reaction product combined with 1 μl of the UCP-1 primer pairs, 1 μM of 18S primer pairs (Ambion), 0.625 μl of the dNTP mix, 1 μCi [32P]dCTP, 5 μl of a 10× buffer (200 mM Tris·HCl, pH 8.4, 500 mM KCl), 1.4 mM MgCl₂, and 0.4 μl of JumpStart Taq DNA polymerase (Sigma). Each tube had a final volume of 50 μl and was subjected to the following optimized temperature profile for amplification, 94°C (20 s), 60°C (30 s), 72°C (45 s) repeated 22 times followed by a final elongation period (72°C) for 10 min. The UCP-1 primer pairs (197-bp fragment) used were 5'-GTGAAGGTCTGAAATGGAAGC (sense) and 5'-AGGCG-CCTCTTCATGAGGTC (antisense) (8). The 18S RNA pairs amplified a 324-bp fragment that was used as an internal control. Each PCR reaction was subjected to nondenaturing PAGE, and gels were exposed to autoradiographic film for 24 h.

Data analysis. The effect of 2, 5, or 11 days of intracerebroventricular ANG II infusion on daily food intake and body weight was compared with the corresponding saline-infused, pair-fed control and pair-watered control (only 11-day experiment) or nonsurgery control (only 2-day experiment) groups using two-way ANOVA for repeated measures (SigmaStat, Jandel). Because every test yielded a significant interaction, post hoc pairwise analysis of all individual cells was performed using the Student-Newman-Keuls method. Differences in nose-anus length and epididymal fat pad weight, and mean arterial pressure were compared using one-way ANOVA. For the relative RT-PCR data, the optical density (OD) of the UCP-1 band was determined using SigmaGel (Jandel). The OD value was then standardized by dividing it by the OD value of its corresponding 18S band. This OD ratio was then set at 1 for saline-control animals, and the OD ratio of the experimental and pair-fed rats was adjusted accordingly. Differences in these averaged values were determined using one-way ANOVA. For all analyses, P < 0.05 was considered to be significant.

Semi-quantitative analysis of the emulsion-dipped slides was carried out under dark field using MicroSuite (Olympus) image analysis. For each brain, sections through the PVN were imaged. For each image, both sides of the PVN were outlined as regions of interest, and mean optical densities were determined. A region of identical area just ventral to the PVN was used to determine the background. The final optical density values (region of interest minus background) times the area (μm²) for each section of each saline control rat were averaged and set at 1. The values in the ANG II, pair-fed, and unoperated groups where then adjusted accordingly. Differences in these averaged values were determined using one-way ANOVA.
RESULTS

In the 2-day experiment, the ANG II reached the cerebroventricles on the 2nd day after surgery. This was evidenced by the increase in water intake observed on the third afternoon. Body weight in all three operated groups was significantly less than the unoperated control on all postoperative days. Body weight in the ANG II-infused rats and pair-fed rats was decreased compared with the saline group on the 2nd day after ANG II reached the cerebroventricles [treatment, $F(3,19) = 3.19, P = 0.047$; time, $F(4,76) = 21.3, P < 0.001$; interaction, $F(12,76) = 6.19, P < 0.001$] (Fig. 1A). Body weight in the intracerebroventriculair saline group on the last day was not different from its presurgery initial weight. Food consumption was significantly decreased in all operated groups on the first postoperative day. In the ANG II-infused and pair-fed groups, food consumption on days 1 and 2 was less than the intracerebroventricular saline group [treatment, $F(3,19) = 7.57, P < 0.002$; time, $F(3,57) = 31.87, P < 0.001$; interaction, $F(9,57) = 4.83, P < 0.001$] (Fig. 1B). All operated groups had epididymal fat pad weights less than the unoperated control. Among the operated groups there were no differences (Table 1).

In the 5-day experiment, body weight was decreased in the ANG II-infused and pair-fed rats from day 2 on [treatment, $F(2,11) = 7.1, P < 0.011$; time, $F(5,55) = 48.2, P < 0.001$; interaction, $F(11,55) = 29.9, P < 0.001$] (Fig. 2A). On days 4 and 5, body weight in the ANG II-infused rats was also less than in the pair-fed group. Food consumption was decreased in the ANG II-infused and pair-fed groups from day 1 to day 4 [treatment, $F(2,11) = 419.8, P < 0.001$; time, $F(4,44) = 98.7, P < 0.001$; interaction, $F(8,44) = 5.0, P < 0.001$] (Fig. 2B). On day 5 there were no differences in food intake among the groups. At the time of death, there were no differences in epididymal fat pad weight or nose-anus length among the groups (Table 1). Average daily water intake was ~3.5 times greater, and mean arterial pressure was ~20% higher (under pentobarbital sodium anesthesia) in the ANG II-infused rats (Table 2).

In the 11-day experiment, intracerebroventricular ANG II produced a significant decrease in body weight compared with the intracerebroventricular saline group [treatment, $F(3,16) = 6.1, P < 0.006$; time, $F(10,160) = 39.5, P < 0.001$; interaction, $F(30,160) = 8.6, P < 0.001$] (Fig. 3A). The effect

Table 1. Effect of intracerebroventricular infusions on epididymal fat pad weight and nose-anus length at the time of termination of each experiment

<table>
<thead>
<tr>
<th></th>
<th>2 Day</th>
<th>5 Day</th>
<th>11 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal fat pad, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICV saline</td>
<td>1.4±0.05*</td>
<td>1.8±0.1</td>
<td>2.3±0.09</td>
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<tr>
<td>ICV ANG II</td>
<td>1.2±0.09*</td>
<td>1.4±0.10</td>
<td>1.5±0.15†</td>
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<tr>
<td>ICV saline-pair fed</td>
<td>1.3±0.10*</td>
<td>1.6±0.12</td>
<td>1.6±0.16†</td>
</tr>
<tr>
<td>ICV ANG II-pair watered</td>
<td>1.6±0.18†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No surgery</td>
<td>1.6±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose-anus length, cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICV saline</td>
<td>ND</td>
<td>20.9±0.21</td>
<td>21.6±0.12</td>
</tr>
<tr>
<td>ICV ANG II</td>
<td>ND</td>
<td>20.7±0.13</td>
<td>20.1±0.24†</td>
</tr>
<tr>
<td>ICV saline-pair fed</td>
<td>ND</td>
<td>20.9±0.05</td>
<td>20.9±0.17†</td>
</tr>
<tr>
<td>ICV ANG II-pair watered</td>
<td>20.5±0.28†</td>
<td></td>
<td></td>
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</table>

Values are means ± SE. *$P < 0.05$ vs. no surgery; †$P < 0.05$ vs. intracerebroventricular (icv) saline; ND, not done

1). In this experiment, nose-anus length was not determined. Water intake was ~3.5 times greater in the ANG II-infused rats (Table 2). There were no differences in mean arterial pressure among the four groups.

In the 5-day experiment, body weight was decreased in the ANG II-infused and pair-fed rats from day 2 on [treatment, $F(2,11) = 7.1, P < 0.011$; time, $F(5,55) = 48.2, P < 0.001$; interaction, $F(11,55) = 29.9, P < 0.001$] (Fig. 2A). On days 4 and 5, body weight in the ANG II-infused rats was also less than in the pair-fed group. Food consumption was decreased in the ANG II-infused and pair-fed groups from day 1 to day 4 [treatment, $F(2,11) = 419.8, P < 0.001$; time, $F(4,44) = 98.7, P < 0.001$; interaction, $F(8,44) = 5.0, P < 0.001$] (Fig. 2B). On day 5 there were no differences in food intake among the groups. At the time of death, there were no differences in epididymal fat pad weight or nose-anus length among the groups (Table 1). Average daily water intake was ~3.5 times greater, and mean arterial pressure was ~20% higher (under pentobarbital sodium anesthesia) in the ANG II-infused rats (Table 2).

In the 11-day experiment, intracerebroventricular ANG II produced a significant decrease in body weight compared with the intracerebroventricular saline group [treatment, $F(3,16) = 6.1, P < 0.006$; time, $F(10,160) = 39.5, P < 0.001$; interaction, $F(30,160) = 8.6, P < 0.001$] (Fig. 3A). The effect

Table 2. Effect of intracerebroventricular infusions on MAP and average daily water intake

<table>
<thead>
<tr>
<th></th>
<th>2 Day</th>
<th>5 Day</th>
<th>11 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICV saline</td>
<td>99.3±1.7</td>
<td>110.7±3.1</td>
<td>111.9±2.5</td>
</tr>
<tr>
<td>ICV ANG II</td>
<td>104.5±6.2</td>
<td>132.3±2.9*</td>
<td>111.5±6.8</td>
</tr>
<tr>
<td>ICV saline-pair fed</td>
<td>102.8±3.0</td>
<td>109.0±3.5</td>
<td>122.5±6.5</td>
</tr>
<tr>
<td>No surgery</td>
<td>102.2±4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water intake, ml/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICV saline</td>
<td>36.4±0.8</td>
<td>36.6±2.3</td>
<td>38.8±0.6</td>
</tr>
<tr>
<td>ICV ANG II</td>
<td>131.8±21.4*</td>
<td>129.5±8.5*</td>
<td>114.1±15.7*</td>
</tr>
<tr>
<td>ICV ANG II-pair watered</td>
<td>39.4±8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICV saline-pair fed</td>
<td>34.4±2.1</td>
<td>32.1±1.1</td>
<td>31.1±1.2</td>
</tr>
<tr>
<td>No surgery</td>
<td>35.1±1.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$ compared with all other groups. The 5-day rats were anesthetized with pentobarbital sodium, which may explain the increase in mean arterial pressure (MAP) with icv ANG II.
Persisted from day 3 to the end of the experiment. Rats that received intracerebroventricular ANG II but were not allowed to increase their water drinking exhibited a similar pattern of decreased body weight gain. The pair-fed rats also had a decreased body weight during this same period. On day 10, body weight in the ANG II-infused rats and ANG II-pair-watered rats was also less than the pair-fed group. Food consumption in the ANG II-infused rats was significantly decreased from the first day of the experiment (treatment, $F(3,16) = 8.1, P < 0.002$; time, $F(9,144) = 81.3, P < 0.001$; interaction, $F(27,144) = 2.4, P < 0.001$). However, the anorexia was transient. From day 6 on there were no longer any differences in food intake compared with control (Fig. 3B). ANG II-pair-watered rats had decreased food intake beginning on day 2 that persisted through day 5. These rats tended to have lower food consumption compared with control throughout the rest of the experiment, but the difference was only statistically significant on day 8. Consumption of pair-fed, ANG II-infused, and ANG II-pair-watered groups was not different at any time point. At termination of the experiment, epididymal fat pad wet weight and nose-anus length were significantly decreased in the ANG II-infused, pair-fed, and ANG II-pair-watered groups (Table 1). There were no differences in epididymal fat pad weight or nose-anus length among the three experimental groups. Average daily water intake was approximately three times greater than control in the ANG II-infused group (Table 2). There were no other differences in daily water intake among the other groups. Mean arterial pressure in the anesthetized rats just before death was not different in any group.

At the time of termination of the 5-day and 11-day experiments, UCP-1 mRNA expression in BAT was greater in the ANG II-infused rats than in the other two groups (Fig. 4). In the 2-day experiment, there were no statistical differences in expression of UCP-1 mRNA in BAT of operated rats. All operated groups exhibited increased UCP-1 mRNA expression compared with the unoperated group.

CRH mRNA expression in the PVN of ANG II-infused rats was significantly decreased compared with the intracerebroventricular saline group at the time of termination of the 11-day experiment (Fig. 5). It should be noted that usable data were only obtained from one of the pair-fed rats (sections cut from the other brains in this group were all anterior to the PVN) so no statistical comparison could be made. However, in the 5-day experiment, it was clear that CRH mRNA expression in the PVN of the ANG II-infused rats was decreased compared with both the intracerebroventricular saline group and the pair-fed group (Fig. 5). In the 2-day experiment, there were no differences in CRH mRNA expression in any group. (Fig. 5).

**DISCUSSION**

There are several reports that have investigated the effect of systemically infused ANG II on body weight and food intake (4–7). It is clear that systemic doses ranging from 175 to 500...
The results also show that the ability of intracerebroventricular ANG II to decrease body weight is not limited to young rats in a rapid growing phase but is also present in adult rats. In our earlier study, 3-wk-old control rats gained ~100% of their initial body weight over a 12-day period (14). The older control rats used in the present study also gained weight, but the increase was only ~20% of their initial body weight over a similar period of time. The results with intracerebroventricular ANG II in adult rats are consistent with a report that systemically infused ANG II has a greater effect to reduce body weight in older rats (~300 g) vs. younger rats (~200 g), although weanling rats were not investigated in that report (4).

It is not known if decreased IGF-1 as reported by others after systemic infusion of ANG II (4, 5) contributed to the decreased body weight after intracerebroventricular infusion of ANG II. It is surprising that the anorexic effect of intracerebroventricular ANG II at the dose used was only transient, lasting just 4–5 days. We previously showed a longer effect of intracerebroventricular ANG II to decrease food intake in young weanling rats (14). In that study, food intake was decreased for the entire 10 days of the experiment with the high dose (16.7 ng/min) of intracerebroventricular ANG II but exhibited a biphasic effect with the low dose (4.2 ng/min). In that case, food intake was decreased for 5 days, then returned to control levels only to decrease again for days 9–11. The timing of the initial return to control food intake in that study was identical to the duration of the anorexia in the present study. Perhaps the difference was due to the different doses used. On a per-kilogram basis, the dose used in the present study with adult rats was one tenth that of the high dose and one third that of the low dose used with the young rats. A lower dose could result in a shorter duration of effect on food intake. It is unlikely that transient nature of the effect was due to failure of the minipumps to deliver solution throughout the experiment because water intake continued to be increased throughout the entire experiment. This raises the possibility that prior studies that utilized higher systemic or central doses may have overestimated the potential for ANG II to decrease food intake.

Despite the return to control levels of food consumption in the 11-day and 5-day experiments, body weight continued to increase throughout the 11-day infusion (4, 5) contributed to the decreased body weight in older rats (~300 g) vs. younger rats (~200 g), although weanling rats were not investigated in that report (4). It is not known if decreased IGF-1 as reported by others after systemic infusion of ANG II (4, 5) contributed to the decreased body weight after intracerebroventricular infusion of ANG II.

However, the magnitude and timing of the contribution of decreases in food intake to the lower body weight is not as clear. An early report by Cassis et al. (7) suggested that decreases in food intake contributed little to the decrease in body weight. Rather, it was postulated that sympathetically mediated increases in peripheral metabolism were primarily responsible. In a subsequent report, the anorexia produced by systemic ANG II was attributed to 63% of the decrease in body weight during a 14-day infusion (6). The effect of ANG II to decrease food intake persisted for as long as 28 days. Brink et al. (5) reported that 74% of the decrease in body weight was due to an anorexigenic effect of ANG II. A subsequent report suggested that the decrease in body weight not attributable to anorexia may be due to decreased muscle protein accumulation secondary to increased catabolism (4). This underscores the potential for confounding factors to arise from the periphery when studying the effect of systemic ANG II on food intake (presumably a central neural effect). In the present investigation, the ANG II was given directly into the cerebroventricles in an attempt to reduce variables associated with peripheral effects of the peptide. The results suggest that low doses of ANG II acting centrally only produce a transient decrease in food intake. The prolonged effect of central ANG II to decrease body weight is likely due to increased energy expenditure.

The results also show that the ability of intracerebroventricular ANG II to decrease body weight is not limited to young rats in a rapid growing phase but is also present in adult rats. In our earlier study, 3-wk-old control rats gained ~100% of their initial body weight over a 12-day period (14). The older control rats used in the present study also gained weight, but the increase was only ~20% of their initial body weight over a similar period of time. The results with intracerebroventricular ANG II in adult rats are consistent with a report that systemically infused ANG II has a greater effect to reduce body weight in older rats (~300 g) vs. younger rats (~200 g), although weanling rats were not investigated in that report (4).

It is not known if decreased IGF-1 as reported by others after systemic infusion of ANG II (4, 5) contributed to the decreased body weight after intracerebroventricular infusion of ANG II.
be decreased throughout the entire experiment in the rats receiving intracerebroventricular ANG II. This suggests that additional factors contributed to the lower body weight. In both cases, UCP-1 mRNA expression was increased in ANG II-infused rats compared with the other two groups at the time of death. This is consistent with the effect of intracerebroventricular ANG II in young rats and suggests that increased thermogenesis may have been responsible for the persistently lower body weight. Sympathetically induced thermogenesis in BAT is dependent on UCP-1 (11). The effect of intracerebroventricular ANG II likely resulted from increased sympathetic outflow. The importance of decreased food intake vs. increased energy expenditure appears to depend on the timing. In the 2-day experiment, food intake was still lower in the ANG II-infused rats, but UCP-1 mRNA expression in BAT was not increased at the termination of the protocol, suggesting a predominant role of the anorexia for at least 2 days. Sometime between 2 days and 5 days of infusion, increased thermogenesis appeared to take over in importance at a time when the anorexic effect was abating.

A recent report showed that chronic subcutaneous infusion of a pressor dose of ANG II had a time-dependent effect on oxygen consumption in adult rats (6). Depending on the dose, oxygen consumption was significantly decreased for 4–10 days and then gradually returned to control or even significantly greater than control at 14–18 days. The changes in oxygen consumption were not due to decreased food intake because comparisons were made to pair-fed rats. If UCP-1 mRNA expression in BAT is equated with oxygen consumption, a similar time-dependent trend was seen in the present study with intracerebroventricular infusion of ANG II. However, the timing and magnitude of the effect were different. No early decrease in UCP-1 mRNA expression was observed in the 2-day group, but by 5 days of intracerebroventricular infusion and beyond, the ANG II appeared to increase thermogenesis, perhaps via increased sympathetic outflow to BAT. The earlier increase (5 vs. 16 days) in energy expenditure in our study could have been because intracerebroventricular ANG II has greater access to the necessary receptors in the brain that lead to sympathoexitation.

The expression of CRH mRNA in the PVN was decreased at the time of death in the ANG II-infused rats from the 11-day and 5-day experiments. In both cases, the difference in CRH expression was not due to differences in food intake because the anorexia had already abated. Lower body weight in the rats receiving ANG II could be partly responsible, but the pair-fed rats also had a lower body weight (although not as great as with ANG II) and yet did not exhibit a decrease in CRH expression compared with the intracerebroventricular saline control group. It is possible that chronic increases in brain ANG II had a direct effect in the PVN to downregulate CRH mRNA synthesis or increase its breakdown. Plasma corticosterone levels were not determined in the present investigation, but centrally administered ANG II has been reported to increase corticosterone via its ability to increase the secretion of vasopressin (13, 20). An alternate possibility for the decreased CRH mRNA in the PVN could simply be increased negative feedback by circulating corticosterone.

The 2-day experiment was performed such that rats were killed at a time when food intake in the ANG II-infused rats was still significantly decreased. A delay in delivery of the ANG II to the cerebroventricles was employed to eliminate confounding factors associated with surgery-induced anorexia and weight loss. By the fourth postoperative day (2 days after the ANG II began to reach the cerebroventricles), body weight in the intracerebroventricular saline group had returned to presurgery levels, although it was still lower than the unoperated rats. Food intake in the intracerebroventricular saline group was not different from the unoperated group from the second postoperative day on. Hence, the confounding effects of surgery were minimized. We had anticipated that the expression of CRH mRNA in the PVN after 2 days of intracerebroventricular ANG II might be increased to help explain the decrease in food consumption. However, there was no relative increase in CRH mRNA expression in the intracerebroventricular ANG II-infused group. We did not look at CRH mRNA expression any earlier than 2 days postinfusion. Acute intracerebroventricular ANG II is known to increase CRH mRNA expression and CRH release (1, 13). Hence, a role for CRH in the anorexia before day 2 cannot be ruled out.

ANG II is well known to increase drinking behavior (18). It is not clearly known to what degree this increase in water consumption affects feeding behavior in rats receiving either systemic or central infusion of ANG II. In our previous report using young rats, the lower dose of intracerebroventricular ANG II had variable effects on water intake. When daily food intake and daily water intake for each rat for each day were correlated, no relationship was detected (14). However, correlation does not establish a cause-and-effect relationship. In the present study, a group of intracerebroventricular ANG II-infused rats was included in the 11-day experiment that was not allowed to increase water consumption. This group was given the same amount of water each day as that consumed by the intracerebroventricular saline group on the previous day. The pattern of food intake, body weight, and UCP-1 mRNA expression (data not shown) in this pair-watered group was virtually identical to that of the intracerebroventricular ANG II group that was allowed to increase water intake. If anything, the pair-watered group tended to consume less food. These data clearly show that the effect of central ANG II to decrease food intake is independent of its effect on water intake.

In summary, the present experiments show that the effect of chronic intracerebroventricular infusion of ANG II in adult rats on food intake and thermogenesis is time dependent at the dose used. For the first few days the decrease in body weight produced by brain ANG II appeared to be mediated primarily by decreased food intake and did not involve increased thermogenesis. We could find no evidence of an early increase in CRH mRNA in the PVN to explain the anorexia. By the fifth day of infusion the decrease in food consumption was no longer present, but thermogenesis was increased and remained elevated for at least 11 days. During this period, the expression of CRH mRNA in the PVN was decreased. The mechanism for the shift from an early contribution of anorexia to a late contribution of increased energy expenditure is not known.

Perspectives

ANG II is not a peptide that is typically associated with the day-to-day regulation of food intake and energy expenditure (19), although a role for ANG II in this regard has not been ruled out. However, there are abnormal conditions where ANG
II is increased, such as heart failure (12), where body wasting is a concern (2, 3). Infusion of ANG II into the cerebroventricles was used to model such conditions where brain levels of the peptide may also be abnormally high. The results are consistent with those reported for peripheral infusions of ANG II and suggest that the anorexic and thermogenic effects of ANG II involve a key central nervous component.

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