Effects of central oxytocin receptor blockade on water and saline intake, mean arterial pressure, and c-Fos expression in rats

Douglas A. Fitts, Simon N. Thornton, Alexandra A. Ruhf, Dannielle K. Zierath, Alan Kim Johnson, and Robert L. Thunhorst. Effects of central oxytocin receptor blockade on water and saline intake, mean arterial pressure, and c-Fos expression in rats. Am J Physiol Regul Integr Comp Physiol 285: R1331–R1339, 2003. First published August 7, 2003; 10.1152/ajpregu.00254.2003.—Central injection of ANG II has been proposed to have dual effects on salt appetite including a direct stimulatory effect and an indirect inhibitory effect through an activation of central oxytocinergic neurons. The inhibition was demonstrated by pretreating rats with central ornithine vasotocin (OVT; oxytocin antagonist) 30 min before a central ANG II injection. The OVT pretreatment produced a large increase in ANG II-induced saline intake. The present paper reports a failure to replicate that influential experiment. However, we also report for the first time that OVT by itself: 1) provokes drinking of both water and saline solution with a latency almost as short as that produced by ANG II; 2) produces a mild pressor response; and 3) increases c-Fos expression in the organum vasculosum laminae terminalis (OVLT) and the median preoptic nucleus (MnPO). Oxytocin activity may provide an inhibitory control of drinking responses as has been suggested, but the inhibition is tonic and includes both water and saline drinking. Inhibition of this tonic activity may stimulate drinking by increasing neural activity in the OVLT and MnPO.

Salt appetite; blood pressure

CIRCULATING ANG II CAN INITIATE salt appetite (10, 32, 35), but several physiological circumstances exist in which an elevation of ANG II does not immediately provoke this behavior. For example, a rapid experimental depletion of intravascular volume using a diuretic or subcutaneous hyperoncotic colloid quickly elevates plasma ANG II levels, but an increase in the intake of hypertonic saline solution is not observed for many hours (12, 14). One proposed explanation for this delay between the rise in ANG II levels and the onset of salt appetite is that the experimental depletion also activates neural oxytocinergic systems that are inhibitory to salt appetite (27, 28, 29, 34). Thus salt appetite emerges only after this oxytocinergic activity is somehow silenced, for instance, by elevated ANG II-induced water intake (27–29).

This physiological sequence is mimicked after an intracerebroventricular (icv) infusion of ANG II: an ANG II infusion immediately generates copious water intake but only gradually elicits salt appetite (12, 14). Blackburn et al. (2) proposed that this delay, before the appearance of salt appetite, resulted from a simultaneous activation of cerebral oxytocinergic activity. They demonstrated this phenomenon by pretreating rats with an icv injection of one of several oxytocin receptor antagonists, including 10 μg ornithine vasotocin (OVT) 30 min before the injection of a 5-μg dose of ANG II. This pretreatment with OVT released a phenomenal increase of hypertonic saline intake after the ANG II injection when compared with rats that received vehicle injections instead of OVT.

Interestingly, no replication of this influential paper has been published. The purpose of this study is to document evidence of a failure to replicate the effect, and also to demonstrate a previously unobserved effect of an icv injection of OVT by itself (i.e., without subsequent ANG II) to generate water intake, salt appetite, and increased arterial pressure. Additional experiments assessed the effects of OVT injection on c-Fos expression in the brain. The nuclei of interest were the hypothalamic vasopressinergic- and oxytocinergic-containing areas, the supraoptic (SON) and paraventricular nuclei (PVN), and the lamina terminalis, consisting of the subfornical organ (SFO), median preoptic nucleus (MnPO), and organum vasculosum laminae terminalis (OVLT). These regions have been well documented to increase Fos-like immunoreactivity (Fos-IR) after water deprivation, sodium depletion, hypertonic saline administration, or ANG II administration (11).

MATERIALS AND METHODS

Animals. All experimental subjects were adult male rats that had continuous access to tap water, 0.3 M NaCl, and a standard chow. Experiment 1 was conducted at the University of Iowa by using Sprague-Dawley rats of either Harlan (n = 14) or Zivic-Miller (n = 44) origin maintained on Purina

http://www.ajpregu.org 0363-6119/03 $5.00 Copyright © 2003 the American Physiological Society R1331
Rat Chow 5012. Experiment 2 was conducted at the University of Washington by using 50 Long-Evans rats maintained on Harlan Teklad rodent diet. The lights were on in the vivaria 12 h per day, and all experiments were conducted during the light phase. All procedures were approved by the Institutional Animal Care and Use Committee at the University in which the work was done.

Drugs. ANG II was obtained from Bachem (Washington experiments) or Sigma (St. Louis, MO) (Iowa experiments). OVT (d(CH2)3-Tyr(Me)2,Orn8-), one of two oxytocin receptor antagonists used successfully by Blackburn et al. (2), was obtained from Bachem (Washington experiments) or Peninsula Laboratories (Iowa experiments). The drugs were mixed freshly before each use with isotonic saline as the vehicle.

Surgery. All rats were fitted with a stainless steel guide cannula in a lateral ventricle by using stereotaxic surgery under general anesthesia using 0.33–0.35 ml/100 g Equithesin. Coordinates at the University of Iowa were anterior-posterior (AP) −1.2, dorsal-ventral (DV) −4.0, and mediolateral (ML) +1.5–2.0 relative to bregma on a leveled skull. The coordinates at the University of Washington were AP −0.5, DV −3.3, and ML +1.4–2.0 relative to bregma on a leveled skull. After recovery from the operation, all rats were allowed at least 1 wk to recover before the experiments. A stainless steel obturator filled the guide cannula when it was not in use for injections.

Cannula patency. The cannulas were tested for patency by using behavioral responses to dipsogenic neurochemicals injected icv through stainless steel injectors that extended 1.0–1.5 mm beyond the end of the guide cannula. At the University of Iowa, carbachol was injected (50 ng in 2 μl), and at the University of Washington, ANG II was injected (40 ng in 2 μl) into hand-held rats. To be included in the study, all rats were required to drink 4 ml in 15 min in their home cages.

Catheterization and blood pressure. A polyethylene catheter was implanted into the left femoral artery 2–3 days before the experiment under halothane anesthesia for measurement of mean arterial pressure (MAP) and heart rate. Catheters were constructed of polyethylene (PE)-10 tubing heat welded to a longer piece of PE-50 tubing; the latter tubing was tunneled to an exit wound between the scapulae. The catheters were filled with 100 U/ml heparin in sterile isotonic saline and obturated until the time of the experiment. Measurements of MAP and heart rate were recorded continuously by computer at 100 Hz (7).

Experiment 1 (University of Iowa). This study was intended to provide a close replication (Experiment 1A) and a variation (Experiment 1B) of the experiment done by Blackburn et al. (2). All food and fluids were removed from the home cages 1 h before testing. Each rat was hand held for a 5-μl icv pretreatment injection of either 10 μg OVT or isotonic saline vehicle. Thirty minutes later, the rat was handled a second time for a 5-μl icv treatment injection of ANG II and then was placed back into the home cage and given access to water and 0.3 M NaCl solution for drinking.

In Experiment 1A, the dose of ANG II was 5 ng, and this was the only treatment these rats received. The subjects were 14 Harlan and 12 Zivic-Miller Sprague-Dawley rats with half of each group receiving a pretreatment of 10 μg of OVT and the other half, vehicle. Intakes of each fluid were recorded at 15, 30, 45, 60, and 120 min.

Blackburn et al. (2) used Zivic-Miller rats, and this accounts for the strain variation in Experiment 1A after Harlan rats were found not to exhibit the expected response. The design of this experiment differed from that of Blackburn et al. in that all rats received ANG II. The original experiment by Blackburn et al. included additional groups treated with OVT or vehicle followed by another vehicle injection; it also included repeated treatments in many animals. The present experiment used each animal only once.

In Experiment 1B, the experiment was repeated twice in the following sequence: 500 ng of ANG II on day 0 and 5 ng on day 6. The subjects were 32 new Zivic-Miller rats with half receiving an icv pretreatment of 10 μg of OVT, and the other half receiving vehicle 30 min before each icv ANG II injection.

Experiment 2 (University of Washington). This study was originally intended to be a variation of the experiment done by Blackburn et al. (2), but we identified effects of OVT missed by the experimental designs used by Blackburn et al. or by the replications at the University of Iowa in Experiment 1. This study examined the effects of OVT by itself on water and saline drinking (Experiment 2A), MAP and heart rate (Experiment 2B), and Fos-IR in the brain (Experiment 2C).

All rats used in Experiment 2 were of the Long-Evans strain.

In Experiment 2A, food was removed from the home cage, and burettes containing water and 0.3 M saline were provided on each cage for 30 min before testing. All rats were given an icv injection as a pretreatment followed by a second injection 30 min later. Burettes of water and saline were available beginning 3 days to rest. They were then moved into cylindrical plastic cages in the experiment room. The rats had previously been familiarized with these cages for 90 min on two occasions. The arterial catheters were connected by polyethylene lines to blood pressure transducers, and the lateral ventricle injectors were connected to 10-μl syringes mounted on Harvard syringe pumps. Food and fluids were not available for consumption at this time. After 30 min of adaptation in the cages, stable baseline blood pressure was recorded for 5 min. The rats then received a 14-s infusion of 10 μg of OVT in 5 μl volume (n = 6) or 5 μl of vehicle alone (n = 3). MAP and heart rate were recorded continuously for 24 min. At the end of the blood pressure recording session, i.e., ~28–30 min after the injection, the rats were allowed access to water for drinking and the latency and amount were recorded for 15 min.

Experiment 2C was designed to determine the effects of a single icv injection of OVT or ANG II on Fos-IR in the rat brain. Nine rats received 10 μg of OVT, three rats received 40 ng of ANG II, three rats received vehicle alone, and two rats received no treatment at all. All injections were 5 μl in volume and were delivered into hand-held rats. The rats were immediately placed back into their home cages for 90 min. Burettes of water and saline were available beginning at least 30 min before the icv injection. Once a rat began to drink either fluid, a latency was recorded, and the fluids were immediately removed from the cage for the remainder of the 90 min for all three of the ANG II-injected rats and for six of the nine OVT-injected rats. The remaining three OVT-in-
Table 1. A summary of differences in experiments

<table>
<thead>
<tr>
<th>Experiment 2A</th>
<th>Strain</th>
<th>Model</th>
<th>Procedure</th>
<th>ANG II Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackburn et al. (2)</td>
<td>ZM</td>
<td>w &amp; b mixed</td>
<td>No fluids until OVT</td>
<td>5 ng</td>
</tr>
<tr>
<td>Experiment 1A</td>
<td>Har, ZM</td>
<td>b</td>
<td>No fluids until OVT</td>
<td>5 ng</td>
</tr>
<tr>
<td>Experiment 1B</td>
<td>ZM</td>
<td>w &amp; b</td>
<td>No fluids until OVT</td>
<td>500, then 5 ng</td>
</tr>
<tr>
<td>Experiment 2A</td>
<td>LE</td>
<td>b</td>
<td>Continuous fluids</td>
<td>40 ng</td>
</tr>
</tbody>
</table>

ZM, Zivic-Miller; Har, Harlan; LE, Long-Evans; w, within-subjects; b, between-subjects; OVT, ornithine vasotocin.

RESULTS

A summary of the differences among the procedures for Blackburn et al. (2) and Experiments 1, A and B, and 2A are given in Table 1.

**Experiment 1.** Results for Experiments 1, A and B are summarized in Fig. 1 and Table 2, respectively, and both failed to confirm any significant OVT-induced enhancement of ANG II-induced water or saline intake. Water and saline intakes induced by ANG II were nearly identical in groups of rats that had been pretreated with OVT or with vehicle.

**Experiment 2A.** The results of the drinking experiment are displayed in Fig. 2. The homogeneity of variance assumption of ANOVA was violated due to the lack of response from the control groups, so the data

---

**Statistics.** Data were analyzed by using ANOVA. A $P < 0.05$ was required for significance. Planned comparisons were made using Fisher’s least significant difference test after a significant $F$-ratio or the Bonferroni test if the $F$ was not significant. When the data did not meet the assumptions for ANOVA because of heterogeneous variances, nonparametric statistics were used. Data are presented as means ± SE unless noted otherwise.
were analyzed using nonparametric tests. The intakes for the first 30 min after the OVT or vehicle injections are shown at the top of Fig. 2 (12 rats per group). Compared with vehicle-injected rats, the OVT-injected rats drank significantly more 0.3 M saline (Mann-Whitney, \( P < 0.05 \)) and more water (\( P < 0.01 \)). The data for the four groups after the second icv injections of ANG II or vehicle are displayed at the bottom of Fig. 2. The cumulative 60-min data were analyzed with a Kruskal-Wallis \( H \)-test, followed by pairwise comparisons by using the Mann-Whitney \( U \)-test when the global test was significant. The overall effect for saline intake was significant, \( H(3) = 12.78, P < 0.01 \), but the OVT-ANG group did not drink more saline than the Veh-ANG group or even than the OVT-Veh group. The groups also differed significantly in the amount of water they drank during the ANG II test, \( H(3) = 18.56, P < 0.01 \). The Veh-ANG group drank more water than any of the other groups, all \( P < 0.05 \), and both of the groups that received OVT on the first injection drank more than the Veh-Veh group, both \( P < 0.01 \). The two OVT groups did not differ significantly from each other.

Experiment 2B. The baseline MAP before the OVT or vehicle injections was \( 132 \pm 2 \) mmHg in the OVT-injected group and \( 126 \pm 1 \) mmHg in the vehicle-injected group. The changes in MAP after the icv injections are displayed in Fig. 3. The average changes in MAP during the entire 24 min after the icv injections were \( +7.5 \pm 3.0, \) range \( +1.5 \) to \( +22.0, \) in the OVT-injected group and \( -1.5 \pm 2.2, \) range \( -5.8 \) to \( +0.7, \) in the vehicle-injected group. Thus all six rats given OVT injections maintained more positive average changes in MAP than the three vehicle-injected controls during the 24 min. Conservative Bonferroni planned comparisons between the baseline MAP and each of the 24 subsequent MAP measurements in each group revealed that MAP was significantly elevated during minutes 10 and 11 after the injection in the OVT group (\( \alpha = 0.00208 \) per test). MAP was never significantly different from baseline in the vehicle group. The average heart rates of the two groups were \( 374 \pm 9 \) beats/min in the OVT group and \( 387 \pm 6 \) in the vehicle group before the injection, and \( 364 \pm 17 \) and \( 380 \pm 8, \) respectively, afterward. Bonferroni planned comparisons revealed no significant changes during any minute in either group.

### Table 2. Intakes of water and 0.3 M NaCl at 60 min after an icv injection of OVT or vehicle, a 30-min delay, and a subsequent icv injection of 500 ng or 5 ng of ANG II in ZM Sprague-Dawley rats

<table>
<thead>
<tr>
<th></th>
<th>500 ng ANG II</th>
<th>5 ng ANG II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>15.7 ± 1.0</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td>Saline</td>
<td>8.8 ± 1.3</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>17.7 ± 1.4</td>
<td>7.3 ± 1.1</td>
</tr>
<tr>
<td>OVT</td>
<td>9.8 ± 1.5</td>
<td>4.4 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. Fluids were not available for drinking during the 30-min delay. The 5-ng treatment was given 6 days after the 500-ng treatment to the same rats. icv, Intracerebroventricular.
After the end of the measurement of MAP, a burette filled with water was placed on each cage for 15 min. Of the six rats in the OVT group, two rats drank within that time and consumed 0.5 and 1.9 ml. Clearly, OVT did not stimulate drinking in these rats when water was withheld for 30 min after the OVT injection.

**Experiment 2C.** Fos-IR in the brain was measured at 90 min after icv injections of OVT, ANG II, or vehicle, or no treatment. Many of the rats receiving icv injections, including the three rats receiving vehicle injections, had apparent Fos-IR in the ependymal lining of the brain ventricles ipsilateral to the injection site, and this was absent in the two rats that received no injection. However, there were no differences in Fos-IR in the regions of interest between the vehicle-injected rats and the untreated rats, so the Fos-IR data were combined into a single unstimulated control group (n = 5).

Latency and drinking data for the experiment are given in Table 3. Only one of the three vehicle-injected rats drank a small amount of water or saline with a long latency. All three OVT injected rats that were allowed to continue drinking ad libitum after the latency measurement drank either water or saline with short latency and consumed some of both fluids for an average of ~9 ml of total fluid in 90 min. Five of the six OVT-injected rats in the nondrinking group began drinking with short latency before the experimenter removed the drinking tubes. The remaining rat in that group did not drink at all, and it was omitted from the analysis of Fos-IR because it presumably received a bad injection. This rat had no Fos-IR in any of the brain regions of interest. Drinking did not significantly affect Fos-IR in the three OVT rats that were allowed to drink compared with the five rats that had their fluids removed as they began drinking. Therefore, the Fos-IR data have been combined for all OVT-injected animals that began drinking (n = 8).

The Fos-IR data are displayed in Fig. 4 for the five control rats that drank little or nothing, eight OVT-injected rats that drank with short latency, and three ANG II-injected rats that drank with short latency.

**Table 3. Latencies to drink either water or saline and fluid intakes by rats receiving a single icv injection of OVT, vehicle, or 40 ng of ANG II before measurement of Fos-IR at 90 min**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>n</th>
<th>Latency (min)</th>
<th>Water Intake (ml/90 min)</th>
<th>Saline Intake (ml/90 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3</td>
<td>1</td>
<td>1.5 ± 0.3</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>OVT</td>
<td>6</td>
<td>5</td>
<td>2.1 ± 0.6</td>
<td>5.1 ± 1.4</td>
<td>4.4 ± 1.5</td>
</tr>
<tr>
<td>OVT</td>
<td>3</td>
<td>3</td>
<td>0.9 ± 0.2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ANG II</td>
<td>3</td>
<td>3</td>
<td>1.5 ± 0.3</td>
<td>2.1 ± 0.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, number of rats tested; n, number drinking; NA, not allowed. Latency or intake data are summarized only for those animals that began drinking (i.e., n drinking). For analysis of Fos-like immunoreactivity (Fos-IR), the data were combined for the 3 OVT rats allowed to drink and the 5 OVT rats that drank water with short latency before the water was removed.

ANG II-injected rats that drank with short latency. Representative photomicrographs are presented in Fig. 5. The regions analyzed were OVLT, SFO, MnPO, PVN, and SON. Two subregions of the MnPO were counted, including the parts ventral and dorsal to the anterior commissure, and these have been analyzed separately. Variances of the groups were heterogeneous, so the data were analyzed by nonparametric statistics including an overall Kruskal-Wallis test for differences among the three groups for each nucleus. If this test was significant, we proceeded to the pairwise comparisons by using the Mann-Whitney U-test. Significant overall tests were found in the OVLT and in both divisions of the MnPO. No significant effects were observed among the group means or even in individual animals for either of the hypothalamic nuclei, the SON or PVN. For display purposes in Fig. 4, the density for the entire nucleus was calculated by summing the total counts of Fos-IR for the three regions and dividing by the total area of the three regions. Two of three animals treated with ANG II had positive Fos-IR responses in the SFO but the effect for the groups was not significant.

Elevation of Fos-IR in the lamina terminalis by ANG II has been well documented, and we observed similar increases here, although the hypothalamic nuclei were not observed to be activated. One-tailed Mann-Whitney U-tests comparing the control rats with the OVT-injected rats revealed significant elevations of Fos-IR by OVT in the OVLT (P = 0.033) and in both the ventral (P = 0.002) and dorsal (P = 0.009) portions of the MnPO. One-tailed tests were used, because we knew a priori that it would be impossible to observe a decrease in Fos-IR from a control value that was essentially zero. Two-tailed Mann-Whitney U-tests revealed that ANG II injections produced significantly more Fos-IR in the OVLT and in the ventral and dorsal parts of the MnPO than did OVT injections (all P = 0.012).
DISCUSSION

Two research teams independently attempted to confirm the results of Blackburn et al. (2) that a blockade of central oxytocin receptors with OVT greatly enhanced saline intake after a subsequent injection of ANG II. Neither team successfully replicated those results; all groups of rats given an icv pretreatment with OVT drank no more saline than vehicle-pretreated groups after they were given ANG II. However, the altered design of Experiment 2A allowed the discovery of a previously unrecognized direct effect of OVT on water and saline intakes. Follow-up experiments

Fig. 5. Photomicrographs of Fos-IR in the OVLT (A, D, and G), SFO (B, E, and H), or dMn (G, F, and I) of rats receiving an icv injection of saline vehicle (A–C), OVT (D–F), or 40 ng of ANG II (G–I). Bar, 0.1 mm.
confirmed the dipsogenic effects of OVT and extended the findings to include a mild pressor response and an activation of Fos-IR in the OVLT and MnPO.

Blackburn et al. (2) reported that a pretreatment with OVT into a lateral ventricle greatly facilitated saline intake when an icv injection of 5 ng of ANG II was given 30 min later. Water and saline fluids were withheld from the rats during the 30 min after the OVT injection and were provided immediately after the ANG II injection. Rats pretreated with OVT drank ~9–12 ml of 0.3 M saline in 1 h after the ANG II injection, whereas the rats pretreated with normal saline vehicle instead of OVT drank only 3 ml of saline. The cumulative intakes of water differed between the groups only at the 45-min time point, and water intakes at 1 h were ~14–18 ml in two OVT groups and ~15 ml in the vehicle-pretreated group. The authors concluded that the OVT injections had a specific influence only on ANG II-induced saline intake and that the small increase in water drinking at 45 min after ANG II in the OVT group probably represented osmoregulatory drinking after the animals had ingested so much concentrated saline earlier in the test.

In experiments reported here, the Iowa team attempted to replicate the essence of that experiment down to the same doses of hormone or antagonist and the same line of rats used (Zivic-Miller) but failed repeatedly to observe any effect of a pretreatment with OVT on ANG II-induced saline or water intakes. The water and saline intakes (Experiment 1A, Fig. 1) were modest compared with the data of Blackburn et al. (2) and were virtually identical whether the rats were pretreated with OVT or vehicle. In a second attempt to replicate (Experiment 1B, Table 2), the Iowa team gave their rats a larger dose of ANG II to determine whether an effect of OVT emerged when rats were stimulated to drink larger amounts like those observed in Blackburn et al. (2). They also gave the rats a second dose of OVT and ANG 6 days later to determine whether the OVT effect at 5 ng of ANG II emerged only after experience with the treatments. This was done because Blackburn’s experiment used some rats for different treatments on successive days. The rats in Experiment 1B drank more at the 500-ng dose of ANG II than those in Experiment 1A drank at 5 ng, and the experience with the 500-ng dose later on generated greater intakes at 5 ng than the intakes observed in Experiment 1A at 5 ng, but in no case did the OVT pretreatment significantly affect the drinking responses after ANG II.

We have no convincing explanation for this failure to replicate a previously reported large effect (2). There are a few remaining differences in the designs of the two experiments (Experiment 1, Ref. 2), including the fact that Blackburn et al. (2) used some of the rats for more than one treatment. They did not specify how many rats received which treatments, thus that part of their experiment could not be repeated exactly.

The initial experiment done at the University of Washington (Experiment 2A) was an attempt to adapt the method of Blackburn et al. (2) to procedures routinely used in our laboratory. These adaptations included the use of Long-Evans rats and a larger dose of ANG II (40 instead of 5 ng), because we knew from past experience that our rats would not drink as much water or saline under control conditions as the intakes reported by Blackburn et al. (2) at 5 ng. We also decided to allow the animals to have free access to water and saline solutions throughout the procedure instead of withholding fluids for 1.5 h before the ANG II injection. The rationale for this was twofold. First, we frequently observe some drinking by normal rats soon after they are provided with fresh water in the home cage. To eliminate this “fresh water effect” from an experiment, we typically allow the rats to drink from test burettes for at least a half hour before we administer a dipsogenic stimulus. Second, we reasoned that allowing the rats access to the fluids after the OVT injection would have no effect on the experiment if the OVT itself was not a stimulus for drinking. On the other hand, if OVT was a stimulus for drinking, this design would allow us to observe that effect. We did indeed observe drinking of both water and saline after icv OVT beginning with a latency similar to that of an icv ANG II injection.

Design of the Blackburn et al. (2) experiment did include a control for the direct effects of OVT on drinking. Specifically, that group was deprived of water for 1 h before receiving an OVT injection. Thirty minutes later, the group was given a vehicle injection instead of ANG II, and was provided with water and saline for drinking. The animals in that group drank little or nothing. Thus it is apparent that the stimulus for drinking is mostly gone by 30 min after an injection of 10 μg of OVT. We verified this in Experiment 2B by allowing the rats to have access to water ~28–30 min after the OVT injection, i.e., after we had finished the blood pressure recordings. There was no difference in drinking between OVT- and vehicle-injected rats. Thus the probable reason that Blackburn et al. failed to notice the direct dipsogenic effects of OVT was the fact that the 30-min delay allowed the effect to dissipate.

The effect of OVT on drinking in our experiment was not limited to saline intake. In hindsight, it would have been easy to document this fact by recording latencies to drink each fluid and taking more frequent measurements of intake. However, we were not expecting such a robust and short-latency response and were surprised to observe it. We have other evidence that OVT directly induced water drinking in addition to saline intake. Multiple observers agreed at the time of OVT injection in Experiment 2A that different rats drank either water or saline first, not always saline followed by water. Four of the OVT-injected rats drank large volumes of water (5–12 ml) without drinking much saline at all during the test (0–0.5 ml). The net concentration of the ingested fluid tended to be hypotonic in the OVT-injected group as a whole (0.06 ± 0.02 M).

Despite the fact that our OVT-injected rats drank both water and saline copiously during the 30 min before the ANG II injection in Experiment 2A, we nevertheless completed the experiment by following up the pretreatment OVT or vehicle injections with either
ANG II or vehicle injections. We did not observe any facilitation of drinking by the OVT pretreatment in the group that later received an injection of ANG II. However, in this case, it is difficult to say that this is a failure to replicate Blackburn et al. (2), because the animals might have experienced a reduced response to ANG II because of the very large volumes of water and saline they had just consumed after the OVT injection.

Our failure to replicate the effect of an oxytocin antagonist on ANG II-induced saline intake is partly supported by a study (22) of the effect of oxytocin antagonists on saline intake in male and female rats subjected to sodium depletion or adrenalectomy. These icv injections of antagonists never caused an increase in saline intake in either model of salt appetite. The data should be interpreted with caution, because both were models of mature salt appetite, i.e., fully developed salt appetite in which all oxytocin activity may have already been suppressed by the normal processes. In that case, an oxytocin antagonist might not be expected to have any effect.

It was surprising in our study that an icv injection of OVT would significantly induce Fos-IR in all of the lamina terminalis except for the SFO. Lesion studies have suggested that the SFO and OVLT sometimes do and sometimes do not support the same functions (5, 6, 11, 19, 23, 24, 26, 31, 33, 35, 36). Direct infusions of ANG II into OVLT always generate both water and saline consumption, but infusions into the SFO generate only water intake (8, 9). Thus the present data demonstrating an activation of Fos-IR in the OVLT and the production of both water and saline intakes are consistent with those infusion data. However, the usual admonitions about interpreting studies of c-Fos expression apply, and we cannot conclude that the SFO did not contribute to the response simply because it did not contain Fos-IR. For example, in a study of osmotic thirst, moderate intragastric loads of hypertonic saline produced Fos-IR in the SON and PNV without producing Fos-IR in the SFO, but nevertheless a knife cut of the connectivity between the SFO and the hypothalamus greatly reduced the Fos-IR in SON and PNV (25). The SFO may participate in these responses in ways that are not reflected by Fos-IR.

One obvious hypothesis to explain these dipsogenic effects of OVT is either a direct or indirect tonic inhibition of angiotensinergic neurons in the lamina terminalis by oxytocinergic neurons. An injection of OVT would then quiet the tonic activity of the oxytocinergic neurons and this would activate the brain ANG system. ANG II in the brain is well known to cause both water and saline drinking, and the effect is particularly robust in the region of the OVLT and ventral MnPO (8, 9) in which both immunoreactive ANG and ANG II receptors are abundant (18). The observation of a small pressor response after OVT injection in Experiment 2B is consistent with the small acute pressor response observed soon after an infusion of ANG II into the OVLT (9).

Dose-response experiments are needed to clarify the relationship between the magnitude of the drinking responses and the amount of Fos-IR produced after ANG II and OVT. The OVT injections generated rather large intakes, but compared with an arbitrary dose of ANG II, the OVT produced considerably less expression of Fos-IR. Possibly, larger doses of OVT could affect ANG II-induced saline intake in the way that Blackburn et al. (2) reported. However, dose-response effects of this antagonist may not be as straightforward as the dose-response effects of an agonist, especially with respect to the brain ANG system. Antagonists of ANG II receptors or of ANG II synthesis can produce unexpected effects depending on the dose of antagonist, the route of administration, and the prior history of the animal (4, 15–17, 30).

The sites of the critical oxytocin receptors mediating this effect are unknown. Oxytocin receptors are heterogeneous and distributed throughout the brain including the preoptic area (13, 20), so the sites of action possibly could be the same areas that contained Fos-IR in Experiment 2C. Another study (1) using icv administration of 10 μg of OVT demonstrated increased feeding behavior with short latency after the injection. Verbalis et al. (34) suggested that central oxytocin activity may inhibit all solute intake. The present experiments broaden this possibility to include an inhibition of all ingestive behaviors including water intake. If so, the phenomenon is probably limited to adult rats, because OVT injections into infant rats did not stimulate 4% salt intake or milk intake in the absence of other stimuli (3).

One problem with the hypothesis, inferred from OVT studies, that oxytocin tonically inhibits all ingestive behaviors is that icv administration of oxytocin agonists do not appear to affect water intake the way that they reduce saline and food intakes [34; however, see Polidori et al. (22)]. This asymmetry of agonist and antagonist effects raises the possibility that the drinking effects of OVT may be acting through an unknown, nonoxytocin pathway. Of course, if this is true for the drinking response, the same could be true for any of the other responses ascribed to oxytocin antagonism using OVT as the antagonist, such as reduced feeding and salt appetite.

We gratefully acknowledge the technical assistance of Julia Freece, Julie Van Bebber, and Terry Beltz.

DISCLOSURES

This study was supported by National Institute of Neurological Disorders and Stroke Grant NS-22274, and National Institute of Mental Health Grants MH-43787, and MH-59239.

REFERENCES

4. El Ghissassi M, Thornton SN, Nicolaides S. Angiotensin II-induced thirst, but not sodium appetite, via AT1 receptors in

AJP-Regul Integr Comp Physiol • VOL 285 • DECEMBER 2003 • www.ajpregu.org