Differential effects of intravenous hyperosmotic solutes on drinking latency and c-Fos expression in the circumventricular organs and hypothalamus of the rat

Jacqueline M. Ho,1 Dannielle K. Zierath,1 Anna V. Savos,1 Dominic J. Femiano,1 John E. Bassett,1 Michael J. McKinley,2 and Douglas A. Fitts1

1Department of Psychology, University of Washington, Seattle, Washington; and 2Howard Florey Institute, University of Melbourne, Melbourne, Australia

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Ho JM, Zierath DK, Savos AV, Femiano DJ, Bassett JE, McKinley MJ, Fitts DA. Differential effects of intravenous hyperosmotic solutes on drinking latency and c-Fos expression in the circumventricular organs and hypothalamus of the rat. Am J Physiol Regul Integr Comp Physiol 292: R1690–R1698, 2007. First published December 28, 2006; doi:10.1152/ajpregu.00547.2006.—Hyperosmotic intravenous infusions of NaCl are more potent for inducing drinking and vasopressin (AVP) secretion than equally osmotic solutions of glucose or urea. The fact that all three solutes increased cerebrospinal fluid osmolality and sodium concentration led the investigators to conclude that critical sodium receptors or osmoreceptors for stimulating drinking and AVP secretion were outside the blood-brain barrier (BBB) in the circumventricular organs (CVOs). We tested an obvious prediction of this hypothesis: that all three solutes should increase c-Fos-like immunoreactivity (Fos-ir) in the supraoptic and paraventricular nuclei of the hypothalamus (SON and PVN) consistently occurred with short latency during hyperosmotic NaCl infusions only. Fos-ir in the forebrain CVOs, the subfornical organ, and organum vasculosum laminae terminalis was consistently elevated only by hyperosmotic NaCl. However, all three hyperosmotic solutes potentely stimulated Fos-ir in the supraoptic and paraventricular nuclei of the hypothalamus inside the BBB. Hyperosmotic NaCl greatly elevated Fos-ir in the area postrema, but even glucose and urea caused moderate elevations that may be related to volume expansion rather than osmolality. The data provide strong support for the conclusion that the osmoreceptors controlling drinking are located in the CVOs.

THE BRAIN AREAS THAT CONTAIN ELECTROPHYSIOLOGICALLY DEFINED OSMORECEPTIVE CELLS THAT FUNCTION IN THE REGULATION OF FLUID AND ELECTROLYTE BALANCE INCLUDE THE CIRCUMVENTRICULAR ORGANS (CVOs), THE MEDIAN PREOPTIC NUCLEUS (MnPO), THE HYPOTHALAMIC SUPRAOPTIC AND PARAVENTRICULAR NUCLEI (SON AND PVN), AND THE NUCLEUS OF THE SOLITARY TRACT (1, 17, 18, 33, 34, 40, 43). THESE AREAS ARE INTRICATELY INTERCONNECTED BY NEURAL PATHWAYS INTO A NETWORK SUBSERVING FLUID BALANCE AS ORIGINALLY DESCRIBED BY MISELIS (29). CANDIDATE MECHANISMS FOR OSMORECEPTORS HAVE BEEN IDENTIFIED AMONG AQUAPORINS, THE VANILLOID FAMILY OF TRANSIENT RECEPTOR POTENTIAL CHANNELS, AND MECHANICAL GATING OF SODIUM CURRENTS (3, 22, 44, 45). HOWEVER, ELECTROPHYSIOLOGICAL STUDIES IN SINGLE NEURONS OR BRAIN SLICES DO NOT DIFFERENTIATE WHICH OSMORECEPTORS ARE MORE IMPORTANT FOR FUNCTIONS SUCH AS AVP SECRETION OR DRINKING.

THE CONCEPT THAT THE CRITICAL OSMORECEPTORS FOR THESE FUNCTIONS ARE LOCATED IN THE CVOs AND IN THE PERIPHERAL NERVOUS SYSTEM DENDRAL FROM THREE INFLUENTIAL STUDIES THAT DEMONSTRATED DRINKING IN SHEEP, DOGS, AND RATS WAS ELICITED BY INTRAVENOUS HYPEROSMOTIC NaCl OR SUCROSE BUT NOT BY UREA OR GLUCOSE (8, 26, 41). THE GENERICALLY ACCEPTED EXPLANATION FOR THIS IS THAT SODIUM AND SUCROSE ARE BOTH EXCLUDED FROM ENTRY INTO CELLS AND THEREFORE DEHYDRATE THEM, WHEREAS UREA AND GLUCOSE ARE PARTICLES THAT EASILY PENEETRATE CELL MEMBRANES AND DO NOT DEHYDRATE CELLS (10, 12). HOWEVER, NONE OF THESE PARTICLES RAPIDLY CROSS THE BLOOD-BRAIN BARRIER (BBB) WITHIN THE FEW MINUTES NECESSARY TO STIMULATE DRINKING AND AVP SECRETION. ALL WERE FOUND TO INCREASE CEREBROSPINAL FLUID (CSF) SODIUM CONCENTRATION OR OSMOLALITY, INCLUDING HYPEROSMOTIC INFUSIONS OF PERMEANT MOLECULES THAT DID NOT CAUSE DRINKING. THIS RULED OUT SPECIFIC CEREBROSPINAL FLUID SODIUM RECEPTORS INSIDE THE BBB, AND, IF IMPORTANT OSMORECEPTORS FOR TRIGGERING AVP SECRETION AND DRINKING RESIDE ON THE BRAIN SIDE OF THE BBB, THEY SHOULD HAVE BEEN STIMULATED EQUALLY BY NaCl AND UREA. THIS LED ALL THREE LABORATORIES TO CONCLUDE THAT THE CRITICAL OSMORECEPTORS FOR THESE FUNCTIONS LAY OUTSIDE THE BBB, AND THIS FOCUSED ATTENTION ON THE CVOs AND PERIPHERAL NERVES.

A STRONG PREDICTION FROM THIS HYPOTHESIS IS THAT OSMORECEPTORS INSIDE THE BBB, SUCH AS IN THE HYPOTHALAMUS, SHOULD BE ACTIVATED BY ANY OF THESE HYPEROSMOTIC SOLUTES DELIVERED INTRAVENOUSLY, WHEREAS OSMORECEPTORS OUTSIDE THE BBB, SUCH AS IN THE CVOs, SHOULD BE ACTIVATED MORE VIGOROUSLY BY EFFECTIVE OSMOTIC PARTICLES, SUCH AS NaCl, AND LESS VIGOROUSLY BY PERMEABLE SOLUTES, SUCH AS GLUCOSE OR UREA. WE USED c-FOS-LIKE IMMUNOREACTIVITY (FOS-IR), WHICH HAS BEEN USED EXTENSIVELY TO MAP RESPONSIVE AREAS OF THE BRAIN AFTER OSMOTIC STIMULI (11, 13, 15, 19, 24, 28, 30, 35, 38, 39), AS A MEASURE OF NEURAL ACTIVITY TO PROVIDE A DIRECT TEST OF THIS PREDICTION.

METHODS

Animals

The subjects included in the study were 47 male Long-Evans rats weighing 380–550 g. They were maintained individually in hanging wire mesh cages with Harlan Teklad rat chow and tap water ad libitum. The temperature was controlled at 23°C, and the rats were

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maintained on a 12:12-h light-dark cycle. Each rat was used only once. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington.

**Catheterization Surgery**

Polyethylene catheters were implanted using an aseptic technique into the left femoral vein and artery 3 days before the beginning of the experiment under halothane anesthesia for arterial blood sampling and for intravenous infusions of 0.15 M NaCl, 1.5 M NaCl, 3 M glucose, or 3 M urea. Thus, the osmotic concentrations of all hyperosmotic solutions were about 3.0 Osm/l. 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.

**Experiment**

Immediately after surgery, rats were relocated to cylindrical plastic infusion cages where they recovered for 3 days until the day of the experiment. At that time, the arterial and venous PE-50 lines were connected for arterial blood sampling and intravenous infusions. Rats were allowed to settle down from the brief handling, and rat chow was taken away. Regular water bottles were replaced with water burettes, and rats were allowed to drink freely. Drinking latencies were recorded at the start of infusion, but water was immediately removed if a rat began to drink so it would not affect Fos expression in the brain. Rats that did not drink by the end of the experiment were assigned a drinking latency of 90 min. Baseline 0.7-ml blood samples were taken away. Regular water bottles were replaced with water burettes, and rats were allowed to drink freely. Drinking latencies were recorded at the start of infusion, but water was immediately removed if a rat began to drink so it would not affect Fos expression in the brain. Rats that did not drink by the end of the experiment were assigned a drinking latency of 90 min. Baseline 0.7-ml blood samples were taken at the start of the experiment, followed by an 11- or 22-min intravenous infusion. The rate of infusion was maintained at 0.14 ml/min with an infusion pump, and the infusate contained isotonic 0.15 M NaCl, 1.5 M NaCl, 3 M glucose, or 3 M urea (n = 3 for all 11-min groups; n = 3, n = 4, n = 4, n = 4, respectively, for 22-min groups). Additional 0.7-ml blood samples were taken at 30 min after the beginning of the infusion and again at 90 min. Rats were perfused for analysis of Fos-ir at 90 min. The blood was centrifuged immediately, and plasma was frozen for later determination of osmolality in duplicate 20-μl samples by freezing-point depression (model 3300; Advanced Instruments).

**Histology and Fos-ir Analysis**

Fos-ir was demonstrated using the avidin–biotin–peroxidase technique. Rats were deeply anesthetized with Nembutal and perfused through the heart with PBS (0.1 M, pH 7.4) for exsanguination followed by 4% paraformaldehyde in PBS. Brains were removed and postfixed in 4% paraformaldehyde overnight and then in a 25% sucrose and PBS solution for an additional 24 h for cryoprotection. The tissue was sectioned coronally on a freezing microtome at 50 μm. Sections were rinsed in PBS, soaked in a methanol–hydrogen peroxide solution for 20 min, soaked in 3% goat serum for 60 min, and incubated chilled with primary Fos antibody (rabbit polyclonal IgG, diluted 1:14,000; Santa Cruz Biotechnology), goat serum, and Triton X-100 for 48 h. Rinsed sections were then incubated in biotinylated goat anti-rabbit antibody for 1 h and processed using the Vectastain ABC kit (Vector Laboratories) for 1 h. They were rinsed again, treated with hydrogen peroxide with diaminobenzidine as the chromogen for 2–3 min, mounted on subbed slides and dried before cover slipping. All treatments were included in each batch.

The nuclei of interest were areas suspected of containing osmoreceptors [the hypothalamic SON and PVN, subfornical organ (SFO), organum vasculosum laminae terminalis (OVLT), and area postrema (AP)] and two integrative areas related to these structures that are not known to contain osmoreceptors (the MnPO and central nucleus of the amygdala). For SFO and OVLT, all Fos grains were counted within the borders of the nucleus in all sections. The data were averaged separately for sections from the rostral and caudal SFO, as well as for the dorsal cap and the main body of OVLT. For PVN, the data were averaged from at least three bilateral sections containing the largest lateral magnocellular subnucleus at about −1.80 mm from bregma (31). To standardize counting of three bilateral subregions of PVN, Fos-positive cells were counted within a circle 167 μm in diameter in the lateral magnocellular PVN and dorsal cap regions and within a square 200 μm on a side in the ventromedial parvocellular PVN. The SON was counted bilaterally and averaged for at least three sections.

All sections of the AP were counted and grouped into the most rostral, medial, and caudal parts of the nucleus. All sections of the MnPO were counted and grouped according to whether the nucleus was dorsal or ventral to the anterior commissure. At least three sections of the central nucleus of the amygdala were counted bilaterally within a circle 251 μm in diameter at −2.12–2.30 mm caudal to bregma (31). All nuclei were photographed by using a light microscope and digital camera and were analyzed in a computer running Scion image-processing software. For SON, SFO, OVLT, AP, and MnPO, the area of the nucleus was measured by the imaging software after tracing its outline with a drawing tool. The density of Fos-ir was then calculated by dividing the total number of Fos-positive cells by the area. The densities are reported as the number of cells in a square 100 μm on a side.

**CSF Extraction**

CSF extraction occurred in a separate set of 20 catheterized rats. Subjects were anesthetized under halothane and suspended from the ear and incisor bars of an elevated stereotaxic apparatus. The cisterna magna was surgically exposed. Rats received a 10-min intravenous infusion of 1.5 M NaCl, 3 M glucose, 3 M urea, or 0.15 M NaCl (n = 5 for all groups). This time point was selected because all rats in the hyperosmotic NaCl group of the main study had begun drinking water by 10 min. The rate of infusion was maintained at 0.14 ml/min with an infusion pump. Following the infusion, the cisterna magna was punctured with a 30-gauge needle and 70 μl of CSF was sampled by gravity feed into a PE-10 tube. Blood samples of 0.7 ml were taken prior to anesthesia and at the time of CSF extraction about 20 min later. The rat was then euthanized while it was still under anesthesia. Plasma and CSF samples were frozen for later analysis of osmolality in duplicate by freezing-point depression.

**Statistics**

Data were analyzed using ANOVA, and follow-up planned comparisons were made using Fisher’s least significant difference test following a significant F ratio. When the assumptions for ANOVA were violated (e.g., latency data), nonparametric statistics were used. Nonparametric tests included Kruskal-Wallis as an omnibus test for significance among many means, followed by Mann-Whitney U-tests when the omnibus test was significant. P < 0.05 was required for significance.

**RESULTS**

**Drinking Latency**

Consistent with the findings of earlier studies (7, 10, 12, 24, 39), we found that NaCl was more effective than glucose or urea in eliciting drinking in this experiment. Figure 1 displays the cumulative percentage of animals drinking at 10-min intervals and the median latencies for the four solute infusion conditions. The data were analyzed with nonparametric statistics because of the large number of animals that did not drink at all and therefore were assigned a value of >90 min instead of a true latency. Because nonparametric tests would never yield a significant effect with sample sizes of three and four,
we combined the data for the two infusion durations to achieve sample sizes of six or seven. The Kruskal-Wallis test was significant, indicating a difference in the mean ranks for drinking latency among the four groups \[H_{9273}(3) = 10.24, P = 0.017\]. Follow-up Mann-Whitney \(U\)-tests determined that the hyperosmotic NaCl group had a significantly shorter drinking latency than all other groups (\(P\) = 0.018 vs. the next fastest drinkers infused with urea; \(P\) = 0.003 vs. the slowest drinkers infused with isotonic saline). The glucose (\(P\) = 0.47) and urea (\(P\) = 0.15) groups did not differ from isotonic.

### Plasma Osmolality

The changes in plasma osmolalities for the four solute infusion conditions and two durations are shown in Fig. 2. Raw data from plasma samples taken at time 0, 30, and 90 min relative to the start of infusion demonstrate a three-way interaction of solute treatment, sample time, and infusion duration [\(F(6, 38) = 3.50, P = 0.007\)]. The initial plasma levels were not significantly different (range of means, 299–303 mOsm/kg), so the effects are displayed as change scores in the figure.

At the 30-min time point, NaCl and urea raised plasma osmolality in the 11-min infusions, and all solutes significantly elevated plasma osmolality relative to the isotonic group in the 22-min infusions. Urea infusion produced significantly higher plasma osmolalities than NaCl and glucose at the 30-min time point after both infusion durations. NaCl produced significantly higher osmolalities than glucose only after the 22-min infusion.

At the 90-min time point, there was a significant tendency for plasma osmolality to decline slightly from baseline after isotonic infusions of both durations. Osmolality after glucose infusions always declined to baseline by 90 min, but the osmolalities after hyperosmotic sodium and urea did not.

### Fos-ir Outside the BBB

The data for Fos expression in the CVOs are presented as representative photomicrographs of groups receiving 22-min infusions in Fig. 3 and as means ± SE of all groups in Fig. 4. Data for the SFO, OVLT, and AP were analyzed with solute and duration of infusion as between-subjects factors and the subregion of the nucleus as a within-subjects factor. All three showed an interaction between solute and nucleic subregion \([F(3, 19) = 3.13, P = 0.050; F(3, 19) = 16.49, P < 0.001; and F(6, 38) = 6.80, P < 0.001\], respectively). Regions that showed a significantly higher response to osmotic stimulation were the rostral stalk of the SFO, the dorsal cap of the OVLT, and the medial and caudal AP. Except for the medial AP, which was nearly identical to the caudal AP, these regions are displayed in Figs. 3 and 4. Data for the remaining regions are displayed in Table 1. Fos-ir density in rostral SFO was significantly enhanced only by hyperosmotic infusions of NaCl. The dorsal cap of the OVLT was strongly stimulated by hyperosmotic NaCl. It was also moderately stimulated by urea, but this effect was significantly weaker than with NaCl. Caudal AP
(Fig. 4) and medial AP (Table 1) both experienced stimulation from NaCl, and glucose and urea produced significant but less substantial effects relative to hyperosmotic NaCl. Thus, hyperosmotic NaCl produced the greatest response by far in the CVOs among all of the solutes tested.

**Fos-ir Inside the BBB**

The data for Fos expression in the hypothalamic nuclei are presented as representative photomicrographs of groups receiving 22-min infusions in Fig. 5 and as means ± SE of all groups in Fig. 6. In the SON, an interaction occurred between solute treatment and infusion duration $[F(3, 19) = 4.09, P = 0.021]$. Twenty-two-minute intravenous infusions into animals that received hyperosmotic NaCl, glucose, or urea elicited significantly more Fos expression in the SON than did isotonic infusions into control animals. NaCl and urea significantly elevated levels of Fos-ir in the 11-min infusions, but glucose did not. Data for Fos expression in the PVN were analyzed with subregion as a within-subjects factor, and the analysis revealed a three-way interaction between treatment, infusion duration, and nucleus subregion $[F(6, 38) = 2.59, P = 0.033]$. The significant response was in the lateral magnocellular PVN (PVN-lm), and this is shown in Fig. 6. The other regions are displayed in Table 1. PVN-lm demonstrated a pattern of Fos expression induced by the different solutes identical to that in the SON. Thus, all hyperosmotic solutes at both durations of infusion activated neurons in these two hypothalamic nuclei except for the 11-min infusion of glucose.

MnPO was analyzed with subregion (dorsal and ventral) as a within-subjects variable. The two parts behaved similarly, so the data are presented in Table 1 for the entire nucleus. The only significant effect was the main effect for solute $[F(3, 19) = 7.77, P = 0.001]$. Further analysis of the means combined for duration and subregion revealed denser Fos expression in the hyperosmotic NaCl (4.82 ± 0.57) and urea (3.42 ± 1.11) groups than in the hyperosmotic glucose (1.02 ± 0.24) and isotonic NaCl groups (0.73 ± 0.14).

The analysis of the central nucleus of the amygdala demonstrated strong effects of solute $[F(3, 19) = 7.61, P = 0.002]$ and duration $[F(1, 19) = 19.86, P < 0.001]$, but the interaction was not significant ($P = 0.10$). The data are displayed in Table 1. Analysis of the means combined for duration revealed that the hyperosmotic NaCl infusion (7.07 ± 1.52) generated significantly more Fos expression...
than hyperosmotic glucose (4.00 ± 1.33), urea (3.00 ± 0.73), or isotonic saline (1.43 ± 0.27). On average, the 22-min infusions produced three times more Fos expression than the 11-min infusions, although most of this effect appeared to be caused by the three hyperosmotic groups (see Table 1).

**CSF Experiment**

Figure 7 shows baseline plasma osmolalities as well as postinfusion plasma and CSF osmolalities. The data were analyzed with solute infusion as a between-subjects factor and fluid sample (preinfusion plasma, postinfusion plasma, postinfusion CSF) as a within-subjects variable. The interaction for solute and fluid sample was significant \( F(6, 32) = 12.57, P < 0.001 \). Baseline plasma osmolalities did not differ among the four groups. Rats receiving isotonic saline had elevated postinfusion plasma and CSF osmolality relative to the presurgical plasma value, indicating that some feature of the anesthetic and procedure elevated osmolality. Compared with these postinfusion values of the isotonic saline group, all groups receiving a hyperosmotic infusion had elevated plasma osmolality. However, only the urea-infused group had significantly elevated CSF osmolality by the end of the 10-min infusion.

**DISCUSSION**

We infused hyperosmotic NaCl, urea, or glucose intravenously and measured the latency to drink and the quantity of Fos-ir in the sensory CVOs, hypothalamus, MnPO, and amygdala. We did not include impermeant molecules other than NaCl, so we refer in this discussion to sodium-receptors and osmoreceptors interchangeably as “osmoreceptors.” We hypothesized that if NaCl was more effective than the other solutes in eliciting drinking, it should also be more effective at eliciting Fos expression in some populations of sodium- or osmoreceptive cells, such as those in the CVOs. Our behavioral data confirmed that infusions of hyperosmotic NaCl caused rats to drink with significantly shorter latencies than the other solutes. We could not allow the rats to continue drinking after the first sip because it would have made the Fos data uninterpretable. However, we assume that the earlier drinking latencies that we observed with NaCl would have corresponded to the larger intakes that others observed with NaCl in several experiments (8, 10, 12, 26, 41). All three hyperosmotic solutes caused substantial Fos expression in the SON and PVN of the hypothalamus, but hyperosmotic NaCl caused far more Fos expression than glucose or urea in the CVOs. The data strongly support the notion that the osmoreceptors that are most important for eliciting drinking under the present conditions are located in the CVOs.

Infusions of hyperosmotic NaCl, regardless of infusion duration, consistently and robustly elevated Fos expression in all nuclei of interest. This was expected, as NaCl is excluded from brain and cellular compartments and thus should cause widespread dehydration. Glucose and urea, while slow to cross the BBB, have relatively more rapid access into peripheral cells. The result of their slow penetration into the brain was predicted to stimulate osmosensitive cells in SON and PVN of the hypothalamus, but hyperosmotic NaCl caused far more Fos expression than glucose or urea in the CVOs. The data strongly support the notion that the osmoreceptors that are most important for eliciting drinking under the present conditions are located in the CVOs.

In the lateral magnocellular part of the PVN, hyperosmotic NaCl for 22 min elicited a significantly greater response than either glucose or urea. This might indicate that NaCl was more effective than glucose or urea in dehydrating osmoreceptors in the hypothalamus, as well as in the CVOs. However, another interpretation is likely. In addition to their own osmoreceptors
Table 1. Density of Fos-like immunoreactivity (cells per 10,000 μm²) in brain regions included in the analysis but omitted from Figs. 4 and 6

<table>
<thead>
<tr>
<th>Effect</th>
<th>Duration</th>
<th>SFOb</th>
<th>OVLTb</th>
<th>APr</th>
<th>APm</th>
<th>PVNdc</th>
<th>PVNvm</th>
<th>MnPO</th>
<th>CeA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic</td>
<td>11</td>
<td>0.5 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.6</td>
<td>0.6 ± 0.4</td>
<td>7.7 ± 5.9</td>
<td>8.9 ± 6.3</td>
<td>0.5 ± 0.1</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>22</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>1.9 ± 0.5</td>
<td>1.4 ± 0.3</td>
<td>6.5 ± 2.6</td>
<td>10.5 ± 3.7</td>
<td>1.0 ± 0.2</td>
<td>1.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>11</td>
<td>0.1 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>0.4 ± 0.1</td>
<td>1.3 ± 0.4</td>
<td>2.7 ± 0.6</td>
<td>0.2 ± 0.1</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>22</td>
<td>0.4 ± 0.3</td>
<td>2.6 ± 1.0</td>
<td>2.8 ± 0.9</td>
<td>5.7 ± 1.5</td>
<td>14.8 ± 5.8</td>
<td>19.6 ± 5.7</td>
<td>1.6 ± 0.3</td>
<td>6.2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>11</td>
<td>0.5 ± 0.3</td>
<td>3.3 ± 1.6</td>
<td>1.0 ± 0.8</td>
<td>1.1 ± 0.5</td>
<td>5.2 ± 2.3</td>
<td>11.8 ± 4.0</td>
<td>4.2 ± 2.9</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>22</td>
<td>0.2 ± 0.1</td>
<td>2.8 ± 0.9</td>
<td>5.5 ± 0.9</td>
<td>6.2 ± 1.0</td>
<td>10.7 ± 1.8</td>
<td>18.2 ± 2.8</td>
<td>2.9 ± 0.5</td>
<td>4.1 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>11</td>
<td>0.5 ± 0.1</td>
<td>2.7 ± 1.3</td>
<td>1.9 ± 1.0</td>
<td>7.1 ± 2.1</td>
<td>5.3 ± 2.0</td>
<td>16.2 ± 5.2</td>
<td>3.5 ± 0.8</td>
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<tr>
<td>22</td>
<td>3.9 ± 1.7</td>
<td>4.8 ± 1.6</td>
<td>5.3 ± 1.5</td>
<td>12.2 ± 1.7</td>
<td>12.2 ± 6.6</td>
<td>17.4 ± 6.2</td>
<td>5.8 ± 0.6</td>
<td>9.6 ± 1.6</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, duration of infusion. Region abbreviations and significant effects: SFOb, central body of subfornical organs [NaCl > others]; OVLTb, body of organum vasculosum laminae terminalis [NaCl > Iso, NaCl > Glu, Urea > Iso]; APr, rostral area postrema [NaCl > Iso, NaCl > Glu, Urea > Iso, Urea > Glu]; APm, medial AP [NaCl > others, Urea > Glu, Urea > Iso]; PVNdc, dorsal cap of paraventricular nucleus; PVNvm, ventromedial PVN [none significant]; MnPO, entire median preoptic nucleus [NaCl > Iso, NaCl > Glu, Urea > Iso, Urea > Glu]; CeA, central nucleus of the amygdala [NaCl > others, Glu > Iso]. Effect abbreviations: S, solute; R, region; I, solute × region interaction; 3-way, solute × subregion × duration.

Table continues...

The CVOs responded strongly only to hyperosmotic infusions of NaCl. When data from 11- and 22-min infusions were combined, Fos expression resulting from glucose and urea in rostral SFO did not differ from the control (Figs. 3 and 4). However, urea did moderately increase Fos expression in the OVLT. Although the osmotic doses of each of the solutes were identical and generated identical increases in plasma osmolality at the end of a 10-min infusion (Fig. 7), the postinfusion handling of each of these solutes differed dramatically. Glucose was apparently cleared from the plasma much more quickly than either NaCl or urea, and NaCl was cleared more quickly than urea (Fig. 2). Thus, urea produced: 1) a greater elevation of plasma osmolality than NaCl or glucose (Fig. 2), 2) a rise in CSF osmolality quicker than either NaCl or glucose (Fig. 7), and 3) the second shortest median drinking latency (Fig. 1). This is consistent with a previous study conducted in...
conscious and unconscious dogs in which urea produced the greatest rise in CSF osmolality and in CSF sodium concentration among equiosmolar solutes (41). Furthermore, high concentrations of intravascular urea have been reported to cause some drinking in rats and sheep (10, 26). Thus, urea provides a weak stimulus for thirst and some Fos expression in OVLT, and this suggests that high plasma concentrations of urea cause a greater dehydration of osmoreceptors than glucose.

The MnPO contains dense projections from both of the forebrain sensory CVOs, and it is perhaps not surprising that the pattern of Fos expression in that structure more closely resembles that of the OVLT and AP than the hypothalamus, i.e., hyperosmotic NaCl and urea stimulated Fos expression, but glucose did not, even at the 22-min duration. It is difficult to draw solid conclusions about the amygdala. Sodium and glucose both elevated Fos expression, similar to the pattern in the hypothalamus, but the mean elevation produced by urea was not significantly different from the mean of the isotonic group despite being double its value.

Why does urea produce such a sustained elevation of plasma osmolality? When urea is taken up by cells, the increase of intracellular urea reduces the concentration gradient favoring the entry of more urea and favors a buildup of urea in the plasma. When glucose is taken up by cells, it is subject to metabolism either anabolically or catabolically inside the cell, and this helps to maintain a high concentration gradient favoring further entry of glucose. This difference could explain the more rapid disappearance of glucose from the plasma, as well as the small difference between these two permeant solutes in producing drinking and Fos expression in the brain.

Entry of urea into cells is dependent on a family of urea transporters (2, 5), and presumably, only those cells that contain urea transporters would be protected from the dehydrating effects of a hyperosmotic infusion of urea. In the brain, urea transporters may be found on astrocytes rather than on neurons (2). The fact that urea caused less of a stimulus for thirst and brain Fos expression than NaCl implies that the osmoreceptive neurons in the CVOs do contain at least some urea transporters. Otherwise, the high plasma osmolality induced by urea should have strongly activated these osmoreceptors. On the other hand, urea did mildly stimulate Fos expression in OVLT, and this might have resulted from a relative paucity of urea transporters. Another possibility is that astrocytes themselves are critically involved in osmotic signaling (7).

We have no ready explanation for how urea produces such a rapid rise in CSF osmolality, but it could result either from a more rapid osmotic dehydration of the brain or from a more rapid entry of urea into the brain. The fact that sodium concentration, as well as osmolality, was increased in the CSF of dogs after urea infusions (41) suggests the former. However, urea has a much lower reflection coefficient ($s_{H11005}/s_{H11005}$) than mannitol ($s_{H11005}/s_{H11005}$) or NaCl ($s_{H11005}/s_{H11005}$), and this may argue for the latter explanation. Urea has been used in osmotherapy after ischemic stroke or traumatic brain injury, but because urea crosses the intact portion of the BBB more readily than NaCl or mannitol and equilibrates more quickly between the brain and intravascular compartment (16), it is more likely to produce a rebound rise in intracranial pressure (20). If urea transporters function in the movement of urea across the BBB in either direction, however, a selective blockade of this move-
ment could drastically change the reflection coefficient of urea and enhance its usefulness as an osmotherapeutic agent because of its slow disappearance from plasma (Fig. 2).

Data from our CSF study demonstrate that plasma osmolality was elevated by the anesthetic and surgical procedures alone because the isotonic-infused group experienced an increase over the ~20-min procedure. For this reason, the post-infusion plasma and CSF levels of isotonic controls served as the baseline for animals in this procedure. Relative to this control group, all hyperosmotic solutes elevated plasma osmolality equally by the end of the infusion. Only urea significantly elevated CSF osmolality by the end of the 10-min infusion. McKinley et al. (26) examined the time course of changes in CSF sodium concentration in sheep and found at the 10-min time point that an infusion of 2 M urea had significantly elevated CSF sodium, whereas an infusion of 1 M NaCl had not yet quite done so.

Even though urea elevated CSF osmolality sooner than NaCl, it was not more effective than NaCl in causing drinking. Hyperosmotic NaCl in the main study always elicited drinking within 10 min (Fig. 1), and a 10-min infusion of hyperosmotic NaCl did not significantly increase CSF osmolality (Fig. 7). These findings imply that the critical osmoreceptors for thirst were situated where they could sense the plasma and not necessarily the CSF, i.e., in the CVOs.

Volume expansion, rather than osmolality, in the hyperosmotic groups might also have contributed to some of the Fos-ir observed in the hypothalamus and AP (14, 32). Studies of c-Fos expression after isotonic blood volume expansion used much larger volumes of infused fluid than we did [e.g., 2 ml/100 g in 60 s (Ref. 14) or 10 ml/100 g in 10 min (Ref. 32) compared with 1.5 or 3 ml/rat in 11 or 22 min, respectively, in the current study]. The infusions in our study were bracketed by 0.7-ml blood samples that would have mitigated their effect on blood volume. All of the infused molecules were small enough to cross the capillary barrier, but we cannot rule out some shift of extracellular fluid from the interstitium into the vasculature after all hyperosmotic infusions. We attempted to determine changes in plasma volume by measuring plasma protein concentrations by refractometry in this study, but unfortunately we found that high concentrations of glucose or urea per se could increase the refractometer reading significantly. We did not measure blood pressure, but it is doubtful that arterial pressure was greatly altered during the procedure. Hochstenbach and Ciriello (17) infused 1.4 M NaCl for 10 min at 5.5 ml/kg in intact rats and observed ~2-mm increase in mean arterial pressure during the infusion that returned to normal at the end of the infusion.

Lesions of CVOs have been reported to alter osmotic sensing related to drinking and AVP secretion (6, 9, 11, 27, 28, 36, 38, 42). In rats, SFO lesions or disconnections consistently blunted, but did not abolish, drinking in response to intragastric loads of hyperosmotic NaCl or food (9, 11, 36, 37, 38). Lesioned rats took longer (>15 min) to begin drinking, but once they began drinking, they usually caught up in total intake by about 90 min after the load. This suggests that one function of the SFO is to provide a quick response to a rapid rise in plasma osmolality. It also suggests that other osmoreceptive areas, including those inside the BBB can eventually be sufficiently stimulated to compensate for the loss of the SFO.

In rats, a lesion of the SFO was superior to a lesion of the OVLT in reducing the drinking response to intragastric hyperosmotic NaCl, and a combined lesion of SFO and OVLT produced the same deficit as a lesion of SFO alone (9). In dogs, a lesion of OVLT did abolish drinking in response to intravenous hyperosmotic NaCl (42). In sheep, a lesion of either CVO blunted the osmotic drinking response, but it was eliminated only by a lesion of the entire lamina terminalis (27). There appears to be redundancy and some species differences in the osmotic functions of the anterior sensory CVOs.

In this study, we assumed that the only feature of the infused solutes to have an effect on Fos expression in neurons was their potential for activating either osmoreceptive neurons or neurons downstream from osmoreceptors. It is possible that cells within these regions of interest became activated and expressed Fos-ir in response to specific molecules, such as glucose or urea. This is unlikely, because these solutes were more notable for their lack of activation of Fos expression in these nuclei rather than the other way around. Furthermore, glucose can become as effective as NaCl for inducing thirst and activating Fos expression in neurons in the CVOs and hypothalamic SON and PVN under conditions of streptozotocin-induced diabetes, which causes cells to become less permeable to glucose because of a lack of insulin (25). Presumably, the same would occur with hyperosmotic urea infusions if urea transport were blocked peripherally (5).

Taken altogether, the data indicate a primary role for CVOs in mediating a thirst response during a rapid rise of plasma osmolality. Although all solutes stimulated Fos expression in the hypothalamic nuclei inside the BBB and raised plasma osmolality, only hyperosmotic NaCl induced drinking with short latency. Not surprisingly, NaCl was the only solute to stimulate Fos expression in the CVOs without fail. Thus, the data support the hypothesis that the osmoreceptors controlling drinking are located in the CVOs.

GRANTS

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