Effect of hyperosmotic solutions on salt excretion and thirst in rats

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Schoorlemmer, G. H. M., A. K. Johnson, and R. L. Thunhorst. Effect of hyperosmotic solutions on salt excretion and thirst in rats. Am J Physiol Regulatory Integrative Comp Physiol 278: R917–R923, 2000.—We investigated urinary changes and thirst induced by infusion of hyperosmotic solutions in freely moving rats. Intracarotid infusions of 0.3 M NaCl (4 ml/20 min, split between both internal carotid arteries) caused a larger increase in excretion of Na+ and K+ than intravenous infusions, indicating that cephalic sensors were involved in the response to intracarotid infusions. Intravenous and intracarotid infusions of hyperosmotic glycerol or urea (300 mM in 150 mM NaCl) had little or no effect, suggesting the sensors were outside the blood-brain barrier (BBB). Intracarotid infusion of hypertonic mannitol (300 mM in 150 mM NaCl) was more effective than intravenous infusion, suggesting that cell volume rather than Na+ concentration of the blood was critical. Similarly, intracarotid infusion (2 ml/20 min, split between both sides), but not intravenous infusion of hypertonic NaCl or mannitol caused thirst. Hyperosmotic glycerol, infused intravenously or into the carotid arteries, did not cause thirst. We conclude that both thirst and electrolyte excretion depend on a cell volume sensor that is located in the head, but outside the BBB.

natriuresis; blood-brain barrier; urea; mannitol

REGULATION OF BODY TONICITY is not only a matter of changes in water intake and excretion, but changes in solute excretion play an important role as well. For example, many mammals increase the excretion of Na+ and K+ during water deprivation (2, 10, 16), which helps maintain constant body tonic.

Blaine et al. (3) found that in sheep, intracarotid infusion of hypertonic NaCl stimulated Na+ excretion, but intravenous infusion was less effective. This suggests that a cephalic sensor accounts for the increase in salt excretion seen during water deprivation. These sensors are thought to be in the hypothalamus close to the anteroventral wall of the third ventricle. Lesions in this area cause a chronic hypernatremia (4) and prevent the natriuresis normally seen during water deprivation (11).

To determine the type of sensor involved, Blaine et al. (3) infused hyperosmotic fructose and sucrose. These solutes, like Na+, do not easily cross the cell membrane. Thus infusion of hyperosmotic sucrose dehydrates the cells but does not increase plasma Na+ concentration ([Na+]p). In fact, such infusions reduce plasma [Na+]p (3) because the blood is diluted with water withdrawn from the cells. Because intracarotid infusion of sucrose and fructose was more effective in stimulating Na+ excretion than intravenous infusion, the authors concluded that cell volume, not plasma [Na+]p, was critical.

It became clear later that the results of Blaine and colleagues (3) may, in fact, be compatible with a mechanism that senses [Na+]p rather than cell volume. Because the blood-brain barrier (BBB) prevents easy entry of fructose and sucrose into the brain tissue, infusion of concentrated fructose or sucrose causes an osmotic gradient that favors movement of water from the brain tissue, across the BBB, into the blood. Indeed, intravascular infusions of hypertonic solutions of sugars that do not cross the BBB cause an increase in [Na+]p of the cerebrospinal fluid (CSF) (5, 12, 19).

Therefore, an Na+ sensor, located on the brain side of the BBB, is compatible with the results of Blaine and colleagues (3).

To investigate the location of the sensor with respect to the BBB, we infused hyperosmotic glycerol and urea, dissolved in 150 mM NaCl. Glycerol and urea do not easily cross the BBB (9). Therefore, these infusions dehydrate tissue on the brain side of the BBB and increase the [Na+]p of the CSF (5, 12, 19). On the other hand, these solutes cross the cell membrane (17), and, therefore, the infusions have little effect on the volume of cells on the blood side of the BBB. If these infusions stimulate electrolyte excretion as effectively as hypertonic NaCl, we can conclude that the sensor is on the brain side of the BBB. If they fail to stimulate electrolyte excretion, we can conclude that the sensor is on the blood side of the BBB. Other researchers (12, 19–21) have used a similar strategy to investigate the location with respect to the BBB of sensors involved in thirst and the secretion of the antidiuretic hormone.

We used carotid infusions to alter tonicity of the brain while minimizing the effect on sensors located in other parts of the body (e.g., in the gastrointestinal tract and liver). We compared intracarotid infusions with intravenous infusions. Intracarotid infusions have a larger effect on tonicity of the head than do intravenous infusions, but the effect on tonicity of the rest of the body, and on blood volume and plasma protein concentration, is the same for intracarotid and intrave-
nous infusions. Thus any difference between intracarotid and intravenous infusion necessarily is due to toxicity or [Na\(^+\)] or [Cl\(^-\)] concentration ([Cl\(^-\)]) of the head. Because such intracarotid infusions have not been used before in rats, we also tested the effect of the infusions on thirst.

**METHODS**

**Animals.** We used male Sprague-Dawley rats from Harlan (Indianapolis, IN) that weighed 350–400 g. Rats were housed individually in a temperature- and humidity-controlled room. Lights were on between 0600 and 1800. Rats had free access to tap water and rat chow (Purina 5012), except during drinking tests and while urine was collected.

**Surgical techniques.** We used an Equithesin-like compound (3.3 ml/kg body wt) (7) for surgical anesthesia. Instruments were sterilized by soaking them in chlorhexidine (Nolvasan, Ft. Dodge Laboratories, Ft. Dodge, IA), and cannulas were soaked for 30 min in 70% ethanol. Drapes were used around incision sites. All cannulas were implanted in a single session (−2 h per rat). Rats were allowed to recover from surgery for at least 10 days. During this period, they regained much or all of the body weight lost after surgery.

**Cannulation of the carotid artery.** We cannulated both carotid arteries because Wood et al. (20) found that hypertonic NaCl is much more dipsogenic when infused into both carotid arteries rather than unilaterally. Carotid cannulas were made of silicone rubber tubing (0.3 mm ID, 0.6 mm OD, Dow Corning, Midland, MI). Because this tubing is very soft, we reinforced it by slipping it over polypropylene tubing (0.3 mm ID, 0.6 mm OD, MRE 025, Braintree Scientific, Braintree, MA). (Silicone rubber expands in xylene or ether and shrinks again when the fluid evaporates.) One millimeter at the cannula tip was not reinforced this way. We made a small ring of silicone glue (Dow Corning silicone sealant) ~3 mm from the cannula tip to help anchor the cannula. To reduce formation of blood clots, the cannula was coated with tridodecylmethylammonium heparin complex (2%, Polysciences, Warrington, PA).

To implant the cannula, we made a 1-cm midline incision through the skin of the throat and dissected a few millimeters around the junction of the common and external carotid artery free from surrounding tissue. We placed two 4–0 silk ligatures around the external carotid artery (Fig. 1) using a small bent needle as a ligation aid. The common and internal carotid arteries were clamped, and a small cut was made in the external carotid artery. The cannula was inserted through the cut and advanced until the tip was at the junction of the common and external carotid arteries (Fig. 1). The cannula was tied in place, and the occipital artery was tied off. The clamp was removed, and the loose end of the cannula was led under the skin to the area between the shoulder blades.

The end of the cannula was connected to a stainless steel elbow (30 g tubing soldered to 23 g) that was fixed with dental cement to a disk of polypropylene mesh (2 cm diameter, 250 µm mesh, CMP-250, Small Parts, Miami Lakes, FL). The mesh was placed under the skin with one end of the elbow protruding through the skin. This end was closed with a plastic cap. The cannula was flushed daily with a mixture of 0.9% NaCl, heparin (100 IU/ml), and penicillin G (2,000 U/ml). Rats recovered well from surgery and regained preoperative body weight. They also had a smooth coat and no obvious motor deficits. On the other hand, in pilot experiments with polyurethane tubing, rats often had a stroke when cannulas were flushed. Most rats were anesthetized and dissected on completion of the experiments to verify that infusions entered the carotid arteries and blood flowed through the internal carotid arteries.

**Venous cannulas.** Venous cannulas were made from silicone rubber tubing (0.6 mm ID, 1.2 mm OD, Dow Corning) connected to polyurethane tubing (0.6 mm ID, 1.0 mm OD, MRE 040 Braintree Scientific). The silicone rubber end was inserted in the femoral vein and advanced 8 cm, which brings the cannula tip into the thoracic vena cava. The polyurethane end was led under the skin to the area between the shoulder blades and connected to an elbow as described for the carotid cannulas.

**Bladder cannulas.** Bladder cannulas were silicone rubber tubing (0.6 mm ID, 1.2 mm OD, Dow Corning) connected to a metal nut. The silicone rubber end was inserted through a hole in the bladder apex. The nut protruded through the skin on the belly. Between experiments, the cannula was closed with a screw inserted in the nut, and the rat could urinate normally. To collect urine, the screw was removed, and the nut was connected to a steel tube. The tube could slide through a slot in the cage bottom allowing the rat to move freely. The residual volume of these cannulas is small (~20 µl), allowing for collection of very small samples.

**Intravenous and intracarotid infusions.** In urine collection experiments, 4 ml fluid was infused in 20 min either in the femoral vein or split between both carotid arteries. The vehicle solution used in these experiments contained 150 mM Na\(^+\), 4 mM K\(^+\), 129 mM Cl\(^-\), 25 mM HCO\(_3\)^\(^-\), 1.25 mM H\(_2\)PO\(_4\)^\(^-\), heparin (1 IU/ml), and HCl (2 µmol/ml). Test solutions contained 150 mM NaCl, 300 mM mannitol, 300 mM urea, or 300 mM glycerol, dissolved in vehicle. These solutions were made fresh just before use. Although the pH was 7.3 when these solutions were made, the pH at the time the fluid entered the body was 7.6–7.7 (apparently some CO\(_2\) escaped). Note that rats did not have access to water during this experiment.

We did two drinking experiments. In the first, we measured water intake during different rates of infusion of 300 mM glycrol, dissolved in vehicle. These solutions were made fresh just before use. Although the pH was 7.3 when these solutions were made, the pH at the time the fluid entered the body was 7.6–7.7 (apparently some CO\(_2\) escaped). Note that rats did not have access to water during this experiment.
NaCl into the carotid arteries. The rates of infusion were 0, 1, 2, or 4 ml/10 min split between both carotid arteries.

In a second experiment, we compared water intake during intravenous and intracarotid infusions of various solutions. The total volume infused was 2 ml/20 min either in the femoral vein or split between both carotid arteries. Infusions contained 150 mM NaCl (vehicle), 300 mM NaCl, 150 mM NaCl + 300 mM glycerol, or 150 mM NaCl + 300 mM mannitol. Solutions used in drinking experiments also contained heparin (1 IU/ml), pH 7.3–7.4. These solutions were kept frozen until needed.

To sterilize the infusion fluids, they were passed through a 0.2-µm filter just before the start of the experiment. Solutions were infused with Hamilton “gastight” syringes mounted on a syringe pump (Harvard 22, Sage 343, or Sage 11). The syringes were connected to the cannulas with polyethylene tubing (Intramedic PE-50, Beckton-Dickinson, Sparks, MD). Rats received one infusion per day administered in a random order. They gained weight between infusions.

Measured variables. Urine volume was measured as weight (1 ml was assumed to weigh 1 g). Osmolality was measured as freezing point depression (µOsmette 5004, Precision Systems, Natick, MA). [Na⁺] and K⁺ concentration ([K⁺]) were measured with a flame photometer (IL 343, Instrumentation Laboratory, Lexington, MA). Water intake was read from inverted glass tubes with 0.1-ml graduations and equipped with metal spouts.

Statistical analysis. Data shown are means ± SE. To analyze effects on excretion, we compared changes caused by intravenous and intracarotid infusions with a paired t-test. To test if this difference between intravenous and intracarotid infusions was different for different solutions, we used a one-way ANOVA with repeated measures, followed by Student-Newman-Keuls test. To compare differences from preinfusion levels, we used Dunnett’s test. To analyze effects on water intake, we compared the difference between water intake during intravenous and intracarotid infusion with a paired t-test. Differences between means were considered significant if the probability that they occurred by chance was < 0.05.

RESULTS

Effects of hypertonic infusions on excretion. Figure 2 shows Na⁺ excretion before, during, and after infusion of hypertonic solutions. Between t = 20 and t = 40 min, 4 ml solution was infused either in vena cava or split between both carotid arteries. *Difference between changes caused by intracarotid and intravenous infusions (paired t-test, n = 8 or 9). Differences from preinfusion values are indicated with + (carotid infusion experiments) and x (intravenous infusion experiments, Dunnett’s test, n = 8 or 9).
pattern of changes in $\text{Na}^+$ and $\text{K}^+$ excretion was similar, but the changes in $\text{K}^+$ excretion were much smaller (Fig. 3).

Changes in urine flow and urine osmolality are shown in Figs. 4 and 5. Hypertonic mannitol and 300 mM NaCl caused a large increase in urine flow, but urine osmolality remained well above that of blood. Intracarotid infusion of 0.3 M NaCl caused a larger increase in urine flow than intravenous infusion.

Effects of hypertonic infusions on thirst. Different rates of infusion of 0.3 M NaCl into the carotid arteries caused dose-dependent water intake (Fig. 6). Intracarotid infusions of hypertonic NaCl or mannitol were equally dipsogenic, but intracarotid infusion of hypertonic glycerol did not cause drinking (Fig. 7). These hypertonic infusions did not cause drinking when infused intravenously. Drinking latencies are shown in Table 1.

**DISCUSSION**

The main findings of these studies are as follows. First, hypertonic NaCl and hypertonic mannitol stimulated excretion of $\text{Na}^+$ and $\text{K}^+$ when infused into the carotid arteries, but intravenous infusions were less effective. Second, although hypertonic NaCl and mannitol stimulated solute excretion, equally hyperosmotic urea and glycerol failed to do so. Third, the same solutes that were most effective in stimulating solute excretion (NaCl and mannitol) were also most effective in stimulating thirst (i.e., water intake).

With respect to thirst, our data confirm work done in other species. Intracarotid infusion of hypertonic NaCl stimulates thirst in goats, dogs, and sheep, but urea is much less effective (12, 14, 20). Glycerol is not an effective dipsogenic stimulus either (14). In rats, intracarotid infusions have not previously been done, but...
intravenous infusion of NaCl caused more thirst than infusion of equally hyperosmotic urea (6).

With respect to Na\(^+\) excretion, there is little conclusive information. In sheep, Blaine et al. (3) found that Na\(^+\) excretion is higher during intracarotid infusions than intravenous delivery of hypertonic sucrose. Neither sucrose nor mannitol easily cross the cell membrane, therefore, the effectiveness of mannitol in the current experiments supports results of Blaine and colleagues. Data from other groups are less conclusive. For example, Na\(^+\) excretion in anesthetized dogs increased during intracarotid (unilateral) infusion, but not during intravenous infusion of hypertonic NaCl (18, 22), which supports our results. These results are not conclusive because it is not clear whether the difference between intravenous and intracarotid infusion is significant. Intracarotid infusion of hypertonic glucose (22) or hypertonic sucrose (18) did not significantly alter excretion of Na\(^+\), but these results are difficult to interpret given the questionable difference between intravenous and intracarotid infusion of hypertonic NaCl.

Our data suggest that the sensors responding to intracarotid infusions are 1) cephalic, 2) outside the BBB, and 3) osmosensitive rather than sensitive to [Na\(^+\)] of the extracellular fluid. Because the characteristics of the sensors for Na\(^+\) excretion and thirst are the same, it is possible that one sensor mediates changes in both responses.

Cephalic sensors. The intracarotid infusions used in these experiments would undoubtedly cause larger changes in tonicity of the head than the intravenous control infusions, but the infusions would not differ in their effect on tonicity of the rest of the body. Therefore, the difference between intravenous and intracarotid infusions of hypertonic NaCl is caused by a sensor in the head. Whether the same sensor also accounts for the increase in Na\(^+\) and K\(^+\) excretion seen during intravenous infusion of hypertonic NaCl is not clear. The intravenous infusions were too small to cause large changes in tonicity of the body fluids; infusion of 4 ml 0.3 M NaCl would increase tonicity of the body fluids of a 350 g rat by <2% (assuming complete mixing).

Sensors outside the BBB. The solutes used in this study differ in their capacity to cross the cell membrane. Na\(^+\) and mannitol do not easily cross the cell membrane, but urea and glycerol do (17). Infusion of hypertonic mannitol causes a fall in plasma [Na\(^+\)] because the blood is diluted with water withdrawn from the cells (12, 19, 21). In contrast, infusion of hypertonic urea does not cause plasma hyponatremia because no water is withdrawn from the cells (12, 19, 21).

Concentrated solutions of Na\(^+\), mannitol, and urea do, however, dehydrate the brain (5, 12, 19) because these solutes do not easily cross the BBB. For example, [Na\(^+\)] of rat CSF, collected 8–20 min after intravenous injection of 2.5 ml 1 M NaCl, 2 M sucrose, or 2 M urea, increased by 6.3, 6.2, and 5.8 mM, respectively (5). Like urea, glycerol crosses the cell membrane, but permeability of the BBB to glycerol is even lower than urea. Clinicians have used systemic infusions of hypertonic glycerol to reduce intracranial pressure in patients suffering from brain edema (9). In conclusion, solutions that dehydrate cells all over the body stimulated thirst and Na\(^+\) excretion, but solutions that dehydrate only tissue inside the BBB failed to do so.

With time, urea and glycerol do cross the BBB. In our experiments, we tried to minimize diffusion of urea and glycerol into the brain tissue by using a relatively short infusion period (20 min). We found that the intracarotid infusions had rapid effects; in the rats that drank

### Table 1. Drinking latency during intracarotid and intravenous infusion of hypertonic solutions

<table>
<thead>
<tr>
<th></th>
<th>150 mM NaCl</th>
<th>300 mM NaCl</th>
<th>300 mM Glycerol + 150 mM NaCl</th>
<th>300 mM Mannitol + 150 mM NaCl</th>
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<tbody>
<tr>
<td>Intravenous</td>
<td>15.3 ± 2.4 (3/8)</td>
<td>12.0 ± 2.6 (5/8)</td>
<td>16.6 ± 1.8 (4/8)</td>
<td>12.9 ± 2.1 (5/8)</td>
</tr>
<tr>
<td>Intracarotid</td>
<td>15.9 ± 2.0 (4/8)</td>
<td>6.9 ± 2.4* (7/8)</td>
<td>13.1 ± 2.6 (4/8)</td>
<td>7.8 ± 2.2 (7/8)</td>
</tr>
</tbody>
</table>

Values are means ± SE in minutes. Latency of 20 min was assigned to rats that did not drink during an infusion. Values in parentheses show number of rats drinking during infusion and number of tested rats. Infusions were 2 ml/20 min, either split between both carotid arteries or into vena cava. *Difference between intracarotid and intravenous infusion (paired t-test).
during the infusion of hypertonic NaCl, the average latency to drink was only 5 min. In the urine collection experiments, already during the first 5 min of infusion of hypertonic NaCl, the intracarotid route of administration tended to be more effective (P = 0.12 for Na⁺ and P = 0.07 for K⁺). The lack of a similarly rapid response to intracarotid infusion of glycerol or urea further supports the idea that there is a sensor located outside the BBB.

Osmoreceptors rather than Na⁺ receptors. Hypertonic mannitol caused more Na⁺ excretion and thirst when infused into the carotid arteries than intravenously. This is compatible with the presence of osmoreceptors outside the BBB as well as both osmoreceptors and Na⁺ receptors inside the BBB. Together, the effects of infusion of hypertonic mannitol, glycerol, and urea suggest that an osmoreceptor (cell volume receptor) located outside the BBB mediates the changes caused by the intracarotid infusions.

In contrast, many (but not all) authors that investigated the sensors responding to infusion of hypertonic and hypotonic solutions into the brain ventricles have concluded that an Na⁺ sensor mediates the response (1) or that both [Na⁺] and tonicity are important (12). Presumably, these infusions act on areas inside the BBB. The ineffectiveness of systemic infusions of hypertonic urea or glycerol to change thirst and Na⁺ excretion suggests that under normal physiological conditions, the osmoreceptors outside the BBB are the more important of the two.

The effect of the infusions on tonicity of the blood perfusing the brain is likely to be small. In rats the size used in these experiments, blood flow through each internal carotid artery is ~1.5 ml/min (8), which is 30 times the infusion rate used in our drinking experiments. Tonicity of the infusion fluids was twice that of normal blood, so the infusions used in the drinking experiment would increase tonicity of carotid blood by ~3.3% (6.6% in urine collection experiments). However, flow through the internal carotid arteries of cannulated rats is higher than normal because the internal carotid arteries will supply blood to areas previously fed by the external carotid arteries. If blood flow through the cannulated carotid artery is 5 ml/min [normal flow through the intact common carotid artery (13)], the infusions used in the drinking experiments would increase tonicity of the blood perfusing the forebrain by 1% (2% in the urine collection experiments). Therefore, it is likely that the infusions caused changes in brain tonicity that are well within the physiological range. Therefore, it is likely that the sensors that mediate the responses to the intracarotid infusions in our experiments are the same as those that mediate the responses seen after stimuli such as water deprivation. However, mixing of infusion fluid and blood may not be complete, especially with slow rates of infusion (15). In that case, local changes in brain tonicity could be larger than anticipated.

Perspectives

Our results indicate that the sensors that mediate osmoregulatory changes in thirst and Na⁺ excretion in rats are similar. Both are located outside the BBB (possibly in one of the circumventricular organs of the brain) and are sensitive to changes in cell volume rather than [Na⁺] of the blood. Neither the mechanism that translates changes in cell volume into an electrical signal, nor the neural circuits that are activated to alter thirst and salt excretion, nor the pathways from brain to kidney that mediate the changes in electrolyte excretion are well understood. It seems likely that the carotid infusion method used in our experiments could be valuable in investigation of these questions.

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