Cerebral Na concentration, Na appetite and thirst of sheep: influence of somatostatin and losartan

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Weisinger, R. S., J. R. Blair-West, P. Burns, D. A. Denton, and B. Purcell. Cerebral Na concentration, Na appetite and thirst of sheep: influence of somatostatin and losartan. Am J Physiol Regulatory Integrative Comp Physiol 280: R686–R694, 2001.—Na and water intakes of Na-depleted sheep are influenced by changes in cerebral Na concentration. Somatostatin and losartan blocked both Na and water intakes, whereas somatostatin was most effective. Water intake was increased during intracerebroventricular infusion of hypertonic mannitol. The increased Na appetite caused by intracerebroventricular infusion of hypertonic mannitol was decreased by concurrent intracerebroventricular infusion of either somatostatin or losartan, with somatostatin being most effective. Water intake was increased during intracerebroventricular infusion of hypertonic mannitol and somatostatin. Na intake was decreased and water intake was increased during systemic or intracerebroventricular infusion of hypertonic NaCl. Intracerebroventricular infusion of losartan blocked both Na and water intake, whereas somatostatin did not influence either of these changes in intake. The results further consolidate a role for somatostatin and ANG II in the central mechanisms controlling Na appetite and thirst of sheep.

somatostatin II; sodium intake; water intake

Somatostatin is a peptide originally isolated from ovine hypothalamus (7). Somatostatinergic neurons (10, 14) and somatostatin binding sites (15, 21) have been identified in the circumventricular organs as well as other brain areas implicated in Na appetite and body fluid balance. Previous research (29) demonstrated that the Na intake of Na-depleted sheep was decreased by intracerebroventricular infusion of somatostatin. The enhanced Na intake that occurs during intracerebroventricular infusion of ANG II or subsequent to dehydration was also decreased by somatostatin. These results suggested that somatostatin may be involved in the final common pathway of a central mechanism involved in Na appetite. In addition, the enhanced water intake that occurs during infusion of ANG II or subsequent to dehydration was unaltered by somatostatin, whereas the relatively low water intake that occurs before Na access in Na-depleted sheep was enhanced (29). Assuming that water intake in Na-depleted sheep is inhibited, possibly as a result of osmotic dilution (25), the enhancement of water intake in the Na-depleted sheep suggests that somatostatin may inhibit an inhibitory system involved in thirst. Thus somatostatin appears to act centrally to influence both the Na appetite and thirst mechanisms in sheep.

Changes in brain extracellular fluid (ECF) Na concentration, acting on Na sensors located in brain areas within the blood-brain barrier, have been postulated to be involved in the control of Na depletion-induced Na appetite (19, 30–32, 34, 35). Na depletion-induced Na appetite is enhanced by lowering the Na concentration of both cerebrospinal fluid (CSF) and brain ECF (e.g., intracerebroventricular infusion of hypertonic mannitol (35)). Na depletion-induced Na appetite is decreased by raising the Na concentration of both CSF and brain ECF [e.g., intracerebroventricular or intracarotid infusion of hypertonic NaCl (19, 35)]. Presumably, these changes in cerebral Na concentration impinge on the neural mechanisms involved in the initiation and/or satiation of Na appetite.

Changes in CSF or brain ECF Na concentration acting on Na sensors located in brain areas within and osmoreceptors located in brain areas without the blood-brain barrier have been postulated to be involved in the control of thirst (1, 18). Water intake caused by intracarotid infusion of hypertonic NaCl is attenuated by lowering the Na concentration of both CSF and nearby brain ECF (e.g., intracerebroventricular or intracarotid infusion of hypertonic NaCl (19, 35)). Water intake is stimulated by raising the Na concentration of both CSF and brain ECF (e.g., intracerebroventricular or systemic infusion of hypertonic NaCl (1, 18)). Presumably, these changes in cerebral Na concentration impinge on the neural mechanisms involved in osmotic thirst.

In addition, changes in Na and water intake caused by systemic or intracerebroventricular infusion of hypertonic NaCl have been shown to be dependent on...
brain ANG II. Both the increased water intake (5, 31) and the decreased Na intake (31) are attenuated or prevented by intracerebroventricular infusion of the ANG II type 1 receptor antagonist, losartan (36). In regards to the role of brain ANG II in the enhanced Na appetite caused by intracerebroventricular infusion of hypertonic mannitol, thus far it has been shown that Na intake caused by decreased cerebral Na concentration is not altered by intravenous (33) or intracerebroventricular (30) infusion of the angiotensin-converting enzyme inhibitor, captopril. These results suggest that if ANG II is involved, its formation does not depend on an angiotensin-converting enzyme.

The present experiments aim to determine the influence of somatostatin on the changes in Na appetite of Na-depleted sheep during infusions that alter cerebral Na concentration. The sheep were only moderately Na depleted (Table 1). During the 22-h Na-deprivation period, the sheep lost 300–500 mmol of Na via their parotid fistula, and thus they were only moderately Na depleted (Table 1).

Materials and Methods

Animals

Twenty-three crossbred Merino ewes, 35–45 kg body wt, were used. All sheep were Na depleted as a result of the continuous loss of NaHCO3-rich saliva from a unilateral parotid fistula. In addition, all animals were surgically prepared with a guide tube (17-gauge stainless steel needle, 34 mm long) implanted 3–10 mm above each lateral brain ventricle (29–35). The sheep were oophorectomized and had both carotid arteries exteriorized in skin loops. All surgery was performed while the sheep were under general anesthe-

### Table 1. Saliva and urine loss, total Na loss (urine plus saliva), and daily water intake before the various experiments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Saliva Loss, ml</th>
<th>Urine Loss, ml</th>
<th>Total Na Loss, mmol</th>
<th>Daily Water Intake, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8</td>
<td>2,384 ± 163</td>
<td>472 ± 70</td>
<td>363 ± 19</td>
<td>3,513 ± 169</td>
</tr>
<tr>
<td>0.7 M icv mannitol</td>
<td>8</td>
<td>2,362 ± 209</td>
<td>632 ± 189</td>
<td>358 ± 29</td>
<td>3,400 ± 194</td>
</tr>
<tr>
<td>0.7 M icv mannitol + Som</td>
<td>8</td>
<td>2,138 ± 163</td>
<td>476 ± 86</td>
<td>341 ± 17</td>
<td>3,060 ± 220</td>
</tr>
<tr>
<td>0.7 M icv mannitol + Los</td>
<td>8</td>
<td>2,628 ± 192</td>
<td>458 ± 103</td>
<td>371 ± 28</td>
<td>3,650 ± 276</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12</td>
<td>1,940 ± 145</td>
<td>448 ± 61</td>
<td>373 ± 23</td>
<td>3,170 ± 132</td>
</tr>
<tr>
<td>0.5 M icv NaCl</td>
<td>12</td>
<td>2,150 ± 177</td>
<td>415 ± 52</td>
<td>381 ± 28</td>
<td>3,200 ± 194</td>
</tr>
<tr>
<td>0.5 M icv NaCl + Som</td>
<td>12</td>
<td>1,871 ± 119</td>
<td>502 ± 95</td>
<td>377 ± 28</td>
<td>2,950 ± 160</td>
</tr>
<tr>
<td>Baseline</td>
<td>9</td>
<td>2,208 ± 127</td>
<td>462 ± 47</td>
<td>433 ± 28</td>
<td>3,120 ± 104</td>
</tr>
<tr>
<td>4 M ic NaCl</td>
<td>9</td>
<td>2,122 ± 154</td>
<td>456 ± 61</td>
<td>425 ± 29</td>
<td>3,066 ± 114</td>
</tr>
<tr>
<td>4 M ic NaCl + Som</td>
<td>9</td>
<td>2,164 ± 163</td>
<td>494 ± 49</td>
<td>434 ± 23</td>
<td>3,155 ± 132</td>
</tr>
<tr>
<td>Baseline</td>
<td>5</td>
<td>2,605 ± 262</td>
<td>609 ± 74</td>
<td>430 ± 14</td>
<td>4,480 ± 405</td>
</tr>
<tr>
<td>4 M ic NaCl</td>
<td>5</td>
<td>2,340 ± 196</td>
<td>596 ± 63</td>
<td>427 ± 11</td>
<td>4,470 ± 398</td>
</tr>
<tr>
<td>4 M ic NaCl + Los</td>
<td>5</td>
<td>2,460 ± 238</td>
<td>600 ± 77</td>
<td>454 ± 19</td>
<td>4,570 ± 360</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals had continuous access to water and 2-h access to 600 mM NaHCO3. icv, Intracerebroventricular; ic, intracarotid; Som, somatostatin-28; Los, losartan.

*Infusion and Sampling Procedures*

For intracerebroventricular infusion on the day of experiment, an obturator was removed from one of the guide tubes, and a probe (20-gauge needle attached to a metal Luer-Lok cap) of the appropriate length was inserted through the guide tube and a probe (20-gauge needle attached to a metal Luer-Lok cap) of the appropriate length was inserted through the guide tube into a lateral brain ventricle. The probe was connected via a polyethylene cannula to a 10-ml syringe held in an infusion pump (Perfusor, Braun). The infusions were for 3 h at 1 ml/h. The infusates used [0.7 M mannitol, somatostatin 1–28, molecular wt = 3,149 (SOM-28, Auspep), and losartan (Dupont Merck)] were dissolved in artificial CSF (aCSF): [Na] = 151 mM, [Cl] = 157.5 mM, [K] = 2.8 mM, [Ca] = 1.1 mM, [Mg] = 0.9 mM, and [HPO42−] = 0.5 mM. The hypertonic...
NaCl-aCSF used in experiment 2 was dissolved in aCSF, except that [Na] = 500 mM and [Cl] = 506.5 mM.

A sample of CSF (1–2 ml) was obtained from the lateral ventricle before and after treatment by connecting a cannula, filled with isotonic saline except for a small air bubble at the tip, to the ventricular probe, and then CSF was allowed to siphon into the cannula by gravitational force.

**Intracarotid Infusion and Blood Sampling**

Intracarotid infusion of 4 M NaCl (19, 31, 34) was made through a polyethylene cannula, and an 18-gauge needle was inserted into one carotid artery, while the contralateral carotid artery was occluded by a pneumatic cuff inflated to 300 mmHg pressure. This procedure provides essentially bilateral distribution of the infusate in the brain. The 4 M NaCl-aCSF solution was infused at a rate of 96 ml/h over 30 min with the use of a motor-driven syringe pump (Palmer). A 10-ml blood sample was obtained from a carotid artery before and after infusion of 4 M NaCl.

**Experimental Design**

**Experiment 1. Influence of Somatostatin or Losartan on Changes in Water and Na Intake Caused by Hypertonic Mannitol Solution in Na-depleted Sheep.** Na-depleted sheep (n = 8) were infused intracerebroventriculatly (1 ml/h over 3 h) with 0.7 M mannitol-aCSF alone or together with either somatostatin (50 μg/ml) or losartan (500 μg/ml). The individual or combined infusion began 1 h before and continued until the end of the 2-h Na access period. Infusion of hypertonic mannitol-aCSF causes a marked decrease in Na concentration and a marked increase in osmolality of CSF (30, 32–35). This dose of somatostatin was used because it decreased Na, but not water, intake caused by Na depletion, water deprivation, and intracerebroventricular infusion of a high dose (3.8 μg/h) of ANG II (29). This dose of losartan was used because it decreased water intake caused by intracerebroventricular or intracarotid infusion of hypertonic NaCl (5, 31) and intracerebroventricular infusion of a high dose (3.8 μg/h) of ANG II (31).

Intake of water was measured during the 1-h period before the Na access period, and intake of water and Na was measured during the 2-h Na access period during the various infusions and during comparable 3-h periods when no infusion was given (baseline).

**Experiment 2. Influence of Somatostatin or Losartan on Changes in Water and Na Intake Caused by Hypertonic NaCl Solutions in Na-depleted Sheep.** Na-depleted sheep (n = 12) were infused intracerebroventricularly (1 ml/h over 3 h) with 0.5 M NaCl-aCSF or 0.5 M NaCl-aCSF plus somatostatin (50 μg/ml). The individual or combined infusion began 1 h before and continued until the end of the 2-h Na access period. The infusion of hypertonic 0.5 M NaCl-aCSF causes a marked increase in both Na concentration and osmolality of CSF (31, 32, 35).

Intake of water was measured during the 1-h period before the Na access period, and intake of water and Na was measured during the 2-h Na access period during the various infusions and during comparable 3-h periods when no infusion was given (baseline).

Na-depleted sheep (n = 9) were given an intracarotid infusion of 4 M NaCl alone or together with an intracerebroventricular infusion of somatostatin. The 30-min intracarotid infusion of 4 M NaCl began 10 min before the 2-h Na access period. The 3-h intracerebroventricular infusion of somatostatin 28 (50 μg/ml at 1 ml/h) began 1 h before and continued until the end of the 2-h Na access period. The intracarotid infusion of 4 M NaCl causes a marked increase in both Na concentration and osmolality of plasma and CSF (19).

Na-depleted sheep (n = 7) were given an intracarotid infusion of 4 M NaCl alone or together with an intracerebroventricular infusion of losartan. The 30-min intracarotid infusion of 4 M NaCl began 10 min before the 2-h Na access period. The 3-h intracerebroventricular infusion of losartan (500 μg/ml at 1 ml/h) began 1 h before and continued until the end of the 2-h Na access period.

Intake of water was measured during the 1-h period before the Na access period, and intake of water and Na was measured during the 2-h Na access period during the various infusions and during comparable 3-h periods when no infusion was given (baseline).

**Statistical Analysis**

Unless specified otherwise, a one-way analysis of variance (repeated-measures design, Figs. 1, 2, 3 and Table 1; independent-measures design, Tables 2 and 3) and subsequent least-significant differences tests (Statistica, Statsoft) were used to compare the baseline value to the value(s) obtained during or after each of the treatments or to compare various treatment values. The mean of the values obtained the day before each infusion for each animal was used in determining the baseline value. Data are presented as means ± SE.

**Analytic Procedures**

[Na] and [K] of saliva, urine, and CSF were measured with a Beckman Synchron CX5 Clinical System. Osmolality was measured by a Digimatic Osmometer (Advanced Instruments).

**RESULTS**

**Experiment 1. Influence of Somatostatin or Losartan on Changes in Water and Na Intake Caused by Hypertonic Mannitol Solution in Na-depleted Sheep.** The intracerebroventricular infusion of hypertonic mannitol caused a two- to threefold increase in Na intake, from 334.8 ± 22.0 to 822.3 ± 76.8 mmol (P < 0.001). The intracerebroventricular infusion of somatostatin completely blocked the increase in Na intake caused by the intracerebroventricular infusion of hypertonic mannitol. Intake during the intracerebroventricular infusion of mannitol and somatostatin was decreased (P < 0.001) relative to that during the infusion of mannitol alone, but it was not different from that during the baseline period. The intracerebroventricular infusion of losartan reduced, but did not eliminate, the increase in Na intake caused by the intracerebroventricular infusion of hypertonic mannitol. Intake during the intracerebroventricular infusion of mannitol and losartan was decreased (P < 0.001) relative to that during the infusion of mannitol alone, but it was increased (P < 0.05) relative to intake during the baseline period or during the infusion of mannitol and somatostatin (Fig. 1, top).

Water intake during the 1-h period before and the 2-h period of Na access was not increased, relative to baseline, by the intracerebroventricular infusion of 0.7 M mannitol in aCSF. Relative to baseline, during the combined mannitol and somatostatin infusion, water intake was increased (P < 0.05) during the 1-h period before Na access and was decreased (P < 0.05) during...
vs. mannitol, 111 P described in text. ***P hypotonic mannitol-aCSF and Som, or hypotonic mannitol-aCSF in WATER AND NA INTAKE CAUSED BY HYPERTONIC NaCl INFUSION OF 0.5 M NaCl caused a 50–60% reduction (P < 0.001) in Na intake from 353 ± 6 mmol to 174 ± 27 mmol (Fig. 2, top). The intracerebroventricular infusion of hypertonic NaCl increased (P < 0.01) water intake during the 1-h period before Na access from 71 ± 17 to 733 ± 159 ml (Fig. 2, middle). Intake of water during the 3-h infusion of hypertonic NaCl was 25–30% of total daily water intake. The intracerebroventricular infusion of somatostatin did not influence either the increased water intake or the decreased Na intake caused by intracerebroventricular infusion of 0.5 M NaCl.

The intracarotid infusion of 4 M NaCl caused a 40–50% reduction (P < 0.001) in Na intake. Na intake decreased from 408 ± 35 to 189 ± 29 mmol (Fig. 3, top left). Water intake during the 1-h period before and the 2-h period of Na access was greatly increased (P < 0.001), relative to baseline, by the intracarotid infusion of 4 M NaCl (Fig. 3, middle and bottom left). The main intake of water occurred during the 30-min infusion of hypertonic NaCl and was ~50–60% of daily water intake. The intracerebroventricular infusion of somatostatin did not influence either the increased water intake or the decreased Na intake caused by intracarotid infusion of 4 M NaCl (Fig. 3, left).

The intracarotid infusion of 4 M NaCl caused a 55–60% reduction (P < 0.01) in Na intake. Na intake decreased from 470 ± 53 to 194 ± 49 mmol (Fig. 3, top right). Water intake during the 1-h period before and the 2-h period of Na access was greatly increased (P < 0.01), relative to baseline, by the intracarotid infusion of 4 M NaCl (Fig. 3, middle and bottom right). The main intake of water occurred during the 30-min infusion of hypertonic NaCl and was ~50–60% of daily water intake. The intracerebroventricular infusion of losartan did not influence the increased water intake before Na access, but it blocked the decreased Na intake and increased water intake during the 2-h Na

The intracerebroventricular infusion of somatostatin-28 (Som; 50 µg/h) or losartan (Los; 500 µg/h) on changes in Na and water intake induced by intracerebroventricular infusion of hypertonic (0.7 M) mannitol-artificial cerebrospinal fluid (aCSF) in Na-depleted sheep (n = 8). Infusion of hypertonic mannitol-aCSF, hypertonic mannitol-aCSF and Som, or hypertonic mannitol-aCSF and Los began 1 h before 2-h Na access period. Statistical analysis described in text. ***P < 0.001; *P < 0.05 (vs. baseline (no infusion)); ++ + P < 0.001 (vs. mannitol alone); and #P < 0.05 (mannitol + Som vs. mannitol + Los).

Fig. 1. Effect of 3-h intracerebroventricular infusion of somatostatin-28 (Som; 50 µg/h) or losartan (Los; 500 µg/h) on changes in Na and water intake induced by intracerebroventricular infusion of hypertonic (0.7 M) mannitol-artificial cerebrospinal fluid (aCSF) in Na-depleted sheep (n = 8). Infusion of hypertonic mannitol-aCSF, hypertonic mannitol-aCSF and Som, or hypertonic mannitol-aCSF and Los began 1 h before 2-h Na access period. Statistical analysis described in text. ***P < 0.001; *P < 0.05 (vs. baseline (no infusion)); ++ + P < 0.001 (vs. mannitol alone); and #P < 0.05 (mannitol + Som vs. mannitol + Los).

Daily water intake and Na loss (or Na deficit) before Na access period were similar for baseline and infusion conditions (Table 1). CSF [Na] was decreased (P < 0.05) and osmolality was increased (P < 0.05) during intracerebroventricular infusion of mannitol. The intracerebroventricular infusion of somatostatin or losartan did not influence the changes in CSF composition caused by the intracerebroventricular infusion of 0.7 M mannitol (Table 2).

**Experiment 2.** INFLUENCE OF SOMATOSTATIN ON CHANGES IN WATER AND NA INTAKE CAUSED BY HYPERTONIC NaCl SOLUTIONS IN NA-DEPLETED SHEEP. The intracerebroventricular infusion of 0.5 M NaCl caused a 50–60% reduction (P < 0.001) in Na intake from 353 ± 17 to 142 ± 34 mmol (Fig. 2, top). The intracerebroventricular infusion of hypertonic NaCl increased (P < 0.01) water intake during the 1-h period before Na access from 71 ± 17 to 733 ± 159 ml (Fig. 2, middle). Intake of water during the 3-h infusion of hypertonic NaCl was 25–30% of total daily water intake. The intracerebroventricular infusion of somatostatin did not influence either the increased water intake or the decreased Na intake caused by intracerebroventricular infusion of 0.5 M NaCl.

The intracarotid infusion of 4 M NaCl caused a 40–50% reduction (P < 0.001) in Na intake. Na intake decreased from 408 ± 35 to 189 ± 29 mmol (Fig. 3, top left). Water intake during the 1-h period before and the 2-h period of Na access was greatly increased (P < 0.001), relative to baseline, by the intracarotid infusion of 4 M NaCl (Fig. 3, middle and bottom left). The main intake of water occurred during the 30-min infusion of hypertonic NaCl and was ~50–60% of daily water intake. The intracerebroventricular infusion of somatostatin did not influence either the increased water intake or the decreased Na intake caused by intracarotid infusion of 4 M NaCl (Fig. 3, left).

The intracarotid infusion of 4 M NaCl caused a 55–60% reduction (P < 0.01) in Na intake. Na intake decreased from 470 ± 53 to 194 ± 49 mmol (Fig. 3, top right). Water intake during the 1-h period before and the 2-h period of Na access was greatly increased (P < 0.01), relative to baseline, by the intracarotid infusion of 4 M NaCl (Fig. 3, middle and bottom right). The main intake of water occurred during the 30-min infusion of hypertonic NaCl and was ~50–60% of daily water intake. The intracerebroventricular infusion of losartan did not influence the increased water intake before Na access, but it blocked the decreased Na intake and increased water intake during the 2-h Na

**Table 2. Effect of the various treatments on CSF composition**

<table>
<thead>
<tr>
<th>Infusion</th>
<th>n</th>
<th>Na, mM</th>
<th>K, mM</th>
<th>Osmolality, mosmol/kgH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (baseline)</td>
<td>17</td>
<td>150.2 ± 0.7</td>
<td>3.0 ± 0.1</td>
<td>303 ± 2</td>
</tr>
<tr>
<td>icv hypertonic mannitol</td>
<td>7</td>
<td>128.7 ± 2.3</td>
<td>2.6 ± 0.1*</td>
<td>381 ± 17‡</td>
</tr>
<tr>
<td>icv hypertonic mannitol + Som</td>
<td>5</td>
<td>130.0 ± 2.4†</td>
<td>2.4 ± 0.1‡</td>
<td>369 ± 24‡</td>
</tr>
<tr>
<td>icv hypertonic mannitol + Los</td>
<td>5</td>
<td>125.4 ± 5.7‡</td>
<td>2.5 ± 0.1†</td>
<td>360 ± 21†</td>
</tr>
<tr>
<td>icv hypertonic NaCl + Som</td>
<td>11</td>
<td>174.2 ± 6.2‡</td>
<td>2.7 ± 0.1*</td>
<td>351 ± 12‡</td>
</tr>
<tr>
<td>icv hypertonic NaCl + Los</td>
<td>4</td>
<td>158.9 ± 1.1*</td>
<td>2.7 ± 0.1</td>
<td>320 ± 2.2*</td>
</tr>
<tr>
<td>NaCl + Som</td>
<td>8</td>
<td>165.2 ± 5.8*</td>
<td>2.7 ± 0.1*</td>
<td>335 ± 11*</td>
</tr>
<tr>
<td>4 M ic NaCl + icv aCSF</td>
<td>10</td>
<td>165.3 ± 3.0†</td>
<td>3.1 ± 0.1</td>
<td>330 ± 6*</td>
</tr>
<tr>
<td>4 M NaCl + icv Som</td>
<td>7</td>
<td>165.0 ± 1.7†</td>
<td>2.9 ± 0.1</td>
<td>339 ± 3†</td>
</tr>
<tr>
<td>4 M NaCl + icv Los</td>
<td>4</td>
<td>158.9 ± 1.1*</td>
<td>2.7 ± 0.1</td>
<td>320 ± 2.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals had continuous access to water and 2-h access to 600 mM NaHCO3 (t = 0 to 120 min). †Cerebrospinal fluid (CSF) samples were not obtained in all experiments. Samples (CSF, 1 ml) were obtained before (t = −60 min) and after (t = 20 min, ic, t = 120 min, icv) infusion. Statistical analysis by 1-way analysis of variance, independent measures design, and subsequent least-significant differences (LSD) test. *P < 0.001; †P < 0.01; *P < 0.05 (vs. baseline).
mechanism controlling the initiation of Na intake in Na-depleted sheep. Intracerebroventricular infusion of somatostatin completely blocked the enhanced Na intake caused by lowered cerebral Na concentration in Na-depleted sheep. Lowered cerebral Na concentration acting via Na sensors, located some distance from the brain ventricular system, is one of the mechanisms that has been postulated to be involved in the control of Na appetite of Na-depleted sheep. Previously, it was demonstrated that intracerebroventricular infusion of somatostatin decreased the Na intake of Na-depleted or water-deprived sheep. Furthermore, somatostatin blocked the Na appetite of sheep that increased their Na intake during a 3-h intracerebroventricular infusion of ANG II.

**DISCUSSION**

The results of this study suggest that somatostatin can act as an inhibitory factor impinging on the central mechanism controlling the initiation of Na intake in Na-depleted sheep. Intracerebroventricular infusion of somatostatin completely blocked the enhanced Na intake caused by lowered cerebral Na concentration in Na-depleted sheep. Lowered cerebral Na concentration acting via Na sensors, located some distance from the brain ventricular system, is one of the mechanisms that has been postulated to be involved in the control of Na appetite of Na-depleted sheep. Previously, it was demonstrated that intracerebroventricular infusion of somatostatin decreased the Na intake of Na-depleted or water-deprived sheep. Furthermore, somatostatin blocked the Na appetite of sheep that increased their Na intake during a 3-h intracerebroventricular infusion of ANG II.

**Somatostatin did not influence the decreased Na intake of the Na-depleted sheep infused with hypertonically-infused saline.**

**Fig. 2. Effect of 3-h intracerebroventricular infusion of Som (50 μg/h) on changes in Na and water intake induced by intracerebroventricular infusion of hypertonic NaCl-aCSF (0.5 M NaCl-aCSF, n = 12) in Na-depleted sheep. Infusion of hypertonic NaCl-aCSF or hypertonic NaCl-aCSF and Som began 1 h before 2-h Na access period. Statistical analysis described in text. ***P < 0.001; and **P < 0.01 [vs. baseline (no infusion)].**

**Fig. 3. Effect of 3-h intracerebroventricular infusion of Som (50 μg/h) or Los (500 μg/h) on changes in Na and water intake induced by intracarotid infusion of 4 M NaCl (96 ml/h over 30 min, n = 9) in Na-depleted sheep. Infusion of 4 M NaCl began 10 min before 2-h Na access period. Infusion of Som or Los began 1 h before 2-h Na access period. Statistical analysis described in text. *P < 0.05, **P < 0.01, and ***P < 0.001 [vs. baseline (no infusion)].**
tonic NaCl, either systemically or intracerebroventricularly. The failure of somatostatin to act additively with the elevated cerebral Na concentration to further reduce the Na intake of sheep would be consistent with the proposal that a somatostatinergic system was maximally activated by hypertonic NaCl. In hypovolemic rats, oxytocin and/or atrial natriuretic peptide systems have been shown to be involved in the inhibition of Na appetite by hypertonic stimuli (4). It is conceivable that in Na-depleted sheep, somatostatin is involved in the inhibition of Na appetite caused by intracarotid or intracerebroventricular infusion of hypertonic NaCl. That is, the somatostatinergic mechanism proposed to work in sheep is analogous to the oxytocinergic system proposed in rats.

Somatostatin did not enhance the water intake caused by the infusion, intracerebroventricular or intracarotid, of hypertonic NaCl. Increased water intake caused by increased osmolality, e.g., during intracerebroventricular or intracarotid infusion of hypertonic NaCl, is thought to be mediated by Na sensors and/or osmoreceptors (1, 18) in the thirst system. These sensors are located in tissue on the wall of the brain ventricular system (17, 20) and are different from the Na sensors of the Na appetite system (32, 34, 35). The present results are consistent with previous results.
Table 3. Effect of the various treatments on plasma composition and hematocrit

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Sample Time, min</th>
<th>Na, mM</th>
<th>K, mM</th>
<th>Osmolality, mosmol/kgH2O</th>
<th>Hct, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (baseline)</td>
<td>14</td>
<td>140.9 ± 0.7</td>
<td>4.3 ± 0.1</td>
<td>291 ± 1</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>4 M ic NaCl</td>
<td>14 ± 20</td>
<td>154.8 ± 0.9*</td>
<td>3.6 ± 0.1*</td>
<td>316 ± 1*</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>4 M ic NaCl + icv Som</td>
<td>9 ± 20</td>
<td>153.8 ± 1.2*</td>
<td>3.5 ± 0.1*</td>
<td>313 ± 1*</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>4 M ic NaCl + icv Los</td>
<td>5 ± 20</td>
<td>158.8 ± 3.7*</td>
<td>3.4 ± 0.1*</td>
<td>324 ± 6*</td>
<td>25 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals had continuous access to water and 2-h access to 600 mM NaHCO₃ (t = 0 to 120 min). Samples (blood, 10 ml) were obtained before (t = −60 min) and after (t = 20 min, ic; t = 120 min, icv) infusion. Statistical analysis by 1-way analysis of variance, independent measures design, and subsequent LSD test. *P < 0.001 (vs. baseline). Hct, hematocrit.

showing that somatostatin did not stimulate the water intake of water-deprived sheep or of sheep infused intracerebroventricularly with ANG II (29). On the other hand, somatostatin did stimulate the water intake of Na-depleted sheep (29) and of Na-depleted sheep infused intracerebroventricularly with hypertonic mannitol (present experiment). These results are consistent with the idea that somatostatin stimulates water intake in situations where water intake seems inappropriate, i.e., suppressed or inhibited. During intracerebroventricular infusion of hypertonic mannitol, it seems reasonable to assume that the expected water intake, due to the increased osmolality of CSF, is inhibited by the concomitant decrease in Na concentration of CSF (20). It is conceivable that somatostatin inhibits this inhibitory stimulus of decreased CSF Na concentration, and thus increased water intake is observed.

At present, the mechanism by which somatostatin stimulates water intake, or inhibits Na intake, is unknown. Interestingly, intracerebroventricular infusion of carbachol has been shown to both stimulate water intake and inhibit Na appetite in the Na-replete rat (12). Although, unlike somatostatin in some respects [e.g., carbachol stimulates water intake in Na-replete animals (23)], the possible interaction between cholinergic and somatostatinergic systems in the regulation of Na and water intake requires further investigation.

The results of the present experiments demonstrated that intracerebroventricular infusion of losartan blocks the decrease in Na intake caused by the systemic administration of hypertonic NaCl in the Na-depleted sheep. Previously, intracerebroventricular administration of losartan was shown to block the decrease in Na intake caused by intracerebroventricular infusion of hypertonic NaCl in the Na-depleted sheep (31). As proposed in the model shown in Fig. 4A, the inhibition of Na appetite results from the osmotic stimulation of angiotensinergic mechanisms in the thirst system and subsequent stimulation of somatostatinergic mechanisms that inhibit Na appetite. At present, there is no direct evidence that ANG II stimulates the release of somatostatin. However, ANG II has been shown to stimulate dopaminergic mechanisms, and dopaminergic mechanisms have been shown to regulate somatostatinergic neurons (2, 8, 13, 22). According to the model proposed, the osmotic stimulation of angiotensinergic mechanisms in the neurons subserving thirst not only stimulates water intake but also stimulates the somatostatinergic system that inhibits Na appetite (5, 31, present experiment). Intracerebroventricular administration of losartan, by interfering with the actions of ANG II, therefore causes decreased water intake and increased Na intake. These results were observed in this study.

However, the following ingestive behavior experiments on sheep are an important consideration in this context. Sheep with a parotid fistula (consistently losing Na-rich saliva) were Na deprived for 48 h, water deprived for 24 h, or both (3). It was observed that after concurrent depletion, with slightly raised plasma Na concentration [i.e., from 147.0 ± 11.0 (normal) to 149.8 ± 12.2 (concurrent Na and water depletion)], when the animals were offered both water and NaHCO₃ solution, on 10 of 13 occasions (n = 3 sheep) they drank rapidly from one container and turned and drank from the other. If offered only one solution, when satiated they would take no more, but if offered the other they would drink rapidly to satiate that appetite. Ruminal distension from the first intake, e.g., water, did not negate the satiation of the second deficiency, i.e., Na. The two drive states involved clearly separable satiation processes. It would appear that the stimulation of Na osmoreceptors in the thirst system by 24-h water deprivation was, in general, not sufficient to disclose the presence of a mechanism that consistently inhibited Na intake in severely Na-depleted sheep. In the present experiments, the sheep were not as Na depleted, and the stimulation of the thirst system, i.e., the increase in brain ECF Na concentration and osmolality, was greater than that of the earlier experiments (3). It is conceivable that the inhibition of Na intake by the proposed thirst-stimulated mechanism can be modified by the severity of the Na deficit and/or by the severity of the thirst. On the other hand, it is possible that a large and rapid increase in brain ECF Na concentration and osmolality, as produced by systemic or intracerebroventricular infusion of hypertonic NaCl, is required to reveal the presence of the thirst-induced inhibitory mechanism.

Interestingly, intracerebroventricular infusion of losartan not only reversed the decrease in Na intake caused by the infusion of hypertonic NaCl but also reversed the increase in Na intake caused by the intracerebroventricular infusion of hypertonic mannitol. During the infusion of hypertonic mannitol, the decrease in brain ECF Na concentration [i.e., presumably acting via a decrease in the intracellular Na concen-
activation of the Na sensors subserving Na appetite (19, 30–32, 34, 35), working via angiotensinergic transmission in the neurons subserving Na appetite, increases Na intake (Fig. 4B). Intracerebroventricular infusion of losartan blocks this action of ANG II and thus blocks the increase in Na intake. In the thirst system, decreased Na in the Na sensors acts to minimize ANG II release caused by increased activity of the osmosensors. Thus relatively little water intake occurs, and there is relatively little inhibition of Na appetite due to release of somatostatin. Because neither systemic nor intracerebroventricular administration of captopril affects the increase in Na intake caused by intracerebroventricular infusion of hypertonic mannitol (31, 33), it would appear that the formation of ANG II involved in Na appetite is not dependent on an angiotensin-converting enzyme. Although far from conclusive, the results are compatible with the possibility that ANG II is formed from proANG II and that ANG II is released as a neurotransmitter.

Paradoxically, although intracerebroventricular infusion of losartan blocks the increase in Na intake caused by intracerebroventricular infusion of mannitol (present experiment), it does not interfere with the Na appetite of the Na-depleted sheep. It seems clear that one element in the genesis of the Na intake of the Na-depleted animal is mediated by peripherally generated ANG II acting in brain areas without a blood-brain barrier (28, 30, 31, 33). For example, the decrease in Na appetite of the Na-depleted sheep caused by systemic infusion of captopril is prevented by concurrent intravenous, but not intracerebroventricular, infusion of ANG II (33). The influence of intracerebroventricular losartan on Na intake of sheep caused by reduction of brain ECF Na concentration (during intracerebroventricular infusion of hypertonic mannitol), on the other hand, is consistent with a role for central ANG II. A role of brain ANG II in Na appetite has been shown in studies on rats (24), baboons (6), and mice (11).

One possible explanation for this paradox is that the brain angiotensinergic neurons activated by reduced brain ECF Na concentration are located in brain areas more readily accessible to antagonists infused into CSF than is the brain angiotensinergic system, normally activated by Na depletion. Conceivably, mannitol infusion is activating a system that is activated primarily in cases of severe Na deficiency. Under the moderate (22 h) Na-depletion conditions, given that losartan readily penetrates the CSF-brain barrier (26), the angiotensinergic system involved in Na appetite may be too distant from the brain ventricular system to be adequately blocked by losartan. Thus the role of brain ANG II in Na-depleted sheep is not demonstrated. Another possible factor is that losartan and other ANG II type 1 antagonists, 1-158,809 and EXP 3174, interfere with binding to neurokinin 3 (NK3) receptors (9), and substances that bind to these receptors, i.e., tachykinins, have an inhibitory influence on Na appetite (16, 27). By interfering with the ability of the tachykinins to activate NK3 receptors, it is possible that losartan causes the enhancement of Na intake. That is, losartan has actions on two competing systems that influence Na intake. Losartan causes decreased Na intake by interfering with angiotensinergic mechanisms and stimulates Na intake by interfering with tachykininergic mechanisms. The observed influence of losartan on Na intake is the result of these two actions. Finally, it is possible that different ANG II receptor systems are activated by different stimuli, e.g., Na depletion or reduction of brain ECF Na concentration, and that more specific antagonists will be required to demonstrate the role of ANG II. For example, intracerebroventricular infusion of ANG II type 1 receptor antagonist ZD 7155, but not losartan, decreased the Na appetite of Na-depleted baboons (6), suggesting that the structure, potency, or some other aspect of the antagonist used may be important.

In conclusion, the results of the present experiments further consolidate the view that somatostatinergic and angiotensinergic mechanisms contribute to the regulation of Na appetite and thirst in the Na-depleted sheep (Fig. 4, A and B). We have proposed that osmotic stimulation, via the stimulation of angiotensinergic neurons, causes water intake and, via the angiotensinergic stimulation of somatostatin, inhibits Na appetite. The finding that losartan, but not captopril (30), inhibits the enhanced Na appetite of the Na-depleted sheep infused intracerebroventricularly with hypertonic mannitol suggests that brain ANG II (apparently not formed by conversion from ANG I), possibly as a neurotransmitter, contributes to the initiation of Na appetite. Somatostatin also appears to have an inhibitory action on inhibitory mechanisms involved in thirst.

Perspectives

The results of the present experiments are vital with regard to their contribution to our knowledge of the role of inhibitory factors in the control mechanisms of thirst and Na appetite. Both of these behaviors are involved with the maintenance of body fluid and electrolyte homeostasis and are therefore fundamental to an individual’s health and survival. A breakdown in these systems, as observed in some schizophrenic or elderly individuals, may have grave consequences. It is conceivable that inappropriate functioning of an inhibitory system could explain the over- or underingestion of water that characterizes such individuals. Treatment with drugs that appropriately alter brain somatostatin levels could be of therapeutic value. Clearly, further investigations into the role of brain somatostatin are required before we fully understand its importance. In particular, investigation into the production and/or release of somatostatin in response to various challenges to body fluid homeostasis would add to the understanding of the physiological significance of the findings reported above.

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