Plasma arginine-vasopressin following experimental stroke: effect of osmotherapy

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CEREBRAL EDEMA REPRESENTS a major cause of morbidity and mortality in patients with ischemic stroke, predominantly from lethal intracranial compartmental shifts that result in herniation syndromes and compromise vital brain stem function (8, 10, 14–16, 29, 32). Over the past several decades, osmotherapy has remained the cornerstone of medical therapy for cerebral edema (10, 28, 31). Acute administration of osmotic agents produces a potent antiedema action, predominantly on undamaged brain regions with an intact blood-brain barrier (BBB), theoretically causing rapid egress of water from the interstitial and extracellular space into the intravascular compartment, resulting in improved intracranial elastance (10, 15, 16, 29, 31, 37, 49). In addition to causing “dehydration” of the brain, osmotic agents exert beneficial nonosmotic cerebral effects, such as augmentation of cerebral blood flow (CBF) (30, 38), modulation of inflammatory molecules (4, 27), scavenging of free radicals, and modulation of cerebrospinal fluid dynamics (formation and reabsorption) (29).

A pathophysiological role for neurohumoral factors, specifically the nonapeptide arginine-vasopressin (AVP) secreted by the hypothalamo-neurohypophyseal system, has been implicated in a variety of brain injury paradigms, including subarachnoid hemorrhage (21, 22) and traumatic brain injury (19). Relatively few studies have reported this relationship in ischemic stroke (5, 6). Small (parvocellular) vasopressin-containing neurons in the anterior hypothalamus are known to give rise to a complex fiber system that extends throughout the brain (11, 27). The existence of extrahypophyseal vasopressinergic pathways within the brain has been demonstrated and may allow for independent release and function of central vs. systemic AVP (7). AVP influences nonneuronal brain cells (glia) by regulating water balance via adjustment of astrocytic water permeability (27). Recent immunohistochemical and autoradiographic studies have revealed that its presence is robust in brain and is affected by changes in plasma osmolarity (5, 6, 7, 11, 17, 27, 42).

We have shown that maintenance of a hyperosmolar state with continuous intravenous (IV) infusion of hypertonic saline (HS) ameliorates cerebral edema associated with experimental ischemic stroke (46, 47). Although the serum osmolality in patients with acute brain injury is recommended to be 300–320 osmol/l (34, 35, 45), the optimal serum osmolality required to exert the most effective osmotic gradient for the anti-edema action with specific osmotic agents remains under investigation. Emerging evidence from experimental studies suggests that a serum osmolality of >350 osmol/l for the treatment of ischemia-evoked cerebral edema may be more beneficial (12, 47).

In the present study, using a well-characterized model of transient focal ischemia, we sought to determine 1) the temporal profile of plasma AVP, and 2) the effect of osmotherapy on plasma AVP levels. Furthermore, we studied the effects of induction and maintenance of a graded hyperosmolar state with HS and mannitol treatment on stroke volume and ischemia-evoked cerebral edema.

MATERIALS AND METHODS

General preparation and animal surgery. The experimental protocol was approved by the Institutional Animal Care and Use Commit-
tet and conforms to the National Institutes of Health guidelines for the care and use of animals in research. All techniques are as previously described (46, 47). In brief, adult male Wistar rats (280–320 g; n = 159) were anesthetized with halothane (1.0–2.0%) in oxygen-enriched air and allowed to ventilate spontaneously. Using aseptic surgical techniques, the right femoral artery was cannulated to monitor arterial blood pressure and arterial blood gases, and the femoral vein was cannulated for vascular access. After cannulation, both catheters were exteriorized in the posterior midthorax and suture fixed on to a swivel at the middorsal region. This tethering system allows the animal to move freely following emergence from anesthesia while receiving continuous infusion of drugs and desired solutions with a programmable infusion pump at a desired rate. Rectal temperatures (37.5–38.5°C) were maintained with a heating lamp throughout the surgical procedures, and the animals were housed in separate cages at ambient room temperature following emergence from anesthesia.

Focal ischemia and assessment of stroke volume. Transient focal ischemia was produced by middle cerebral artery occlusion (MCAO) (2 h) using an intraluminal suture technique, as previously described (24), with modifications (3, 46, 47). Adequacy of vascular occlusion and reperfusion was determined by laser-Doppler flowmetry (LDF) over the ipsilateral parietal cortex, as previously described (3, 46, 47) (coordinates: 2 mm posterior and 6 mm lateral to bregma) at baseline, during MCAO, and at 30 min of reperfusion. On successful reperfusion, rats were allowed to emerge from anesthesia in separate cages and given free access to food and water. Rats that did not demonstrate a significant reduction of the LDF signal (≤40% of baseline) during MCAO were not included in the final analysis. This degree of reduction in LDF signal is necessary to ensure consistent infarction volume in our model of MCAO (3). Similarly, rats that did not demonstrate return of LDF signal on reperfusion were not included in the final analysis. At 72 h of reperfusion, rats were deeply anesthetized with 5% halothane and decapitated. The brain was harvested and the final analysis. At 72 h of reperfusion, rats were deeply anesthetized with 5% halothane and decapitated. The brain was harvested and

Physiological parameters and mean LDF measurements among groups were determined by one-way ANOVA with post hoc Newman-Keuls test. Mortality rates were compared with logistic regression analysis. The criterion for statistical significance was P < 0.05.

RESULTS

In the first series of experiments, physiological parameters, including mean arterial blood pressure (MAP), pH, arterial carbon dioxide partial pressure (Paco2), oxygen partial pressure (PaO2), and rectal temperatures, were within normal ranges in all experimental groups at baseline, during MCAO, and at 30 min of reperfusion (Table 1). LDF signal during MCAO was not different among different treatment groups (Table 1). Mortality rates before completion of the experiment were as follows: 0 of 5 in surgical-sham controls, 3 of 13 in rats for 24-h end point, 5 of 15 in rats for 48-h end point, 6 of 16 in rats for 72-h end point, and 2 of 12 in rats for 96-h end point. Plasma AVP levels were significantly elevated at 24 h (42 ± 10 pg/ml; n = 10), 48 h (50 ± 28 pg/ml; n = 10), and 72 h (110 ± 47 pg/ml; n = 9), but not at 96 h (22 ± 15 pg/ml; n = 10) following MCAO, compared with sham-operated controls (14 ± 7 pg/ml; n = 5) (Table 1). Plasma osmolarity was not different among experimental groups (Table 1). TTC-determined infarct volume (corrected for brain swelling) was not different among experimental groups, and plasma AVP levels did not demonstrate correlation with infarct volume (data not shown). Histopathological examination of the hypothalamus in rats (n = 4) treated with NS for 72 h of reperfusion did not demonstrate any neuronal injury in anterior hypothalamic nuclei associated with AVP production (paraventricular nuclei, supraoptic nuclei, suprachiasmatic nuclei, medial nuclei of the

Assessment of plasma AVP levels. At death, a sample of blood was obtained and analyzed for AVP in the plasma, as previously described (41), with modifications utilizing the commercially available DSL-1800 AVP RIA kit (Diagnostic Systems Laboratories, Webster, TX).

Experimental groups. In the first series of experiments, rats were killed at 24, 48, 72, and 96 h (n = 10 each) following MCAO, and plasma AVP levels were determined. Surgical shams that underwent all surgical procedures, including neck surgery without MCAO, were used as controls (n = 5). All rats received a continuous IV infusion of 0.3 ml/h of 0.9% saline (NS; 308 osmol/l). In the second series of experiments, rats subjected to transient MCAO were treated in a blinded, randomized fashion to receive a continuous IV infusion of 0.3 ml/h of NS (n = 12), 3% HS (1,027 osmol/l; n = 13), 7.5% HS (2,310 osmol/l; n = 10), or 2 g/kg of 20% mannitol (1,098 osmol/l; n = 12) IV bolus every 6 h. Rats treated with mannitol were given a continuous IV infusion of NS (0.3 ml/h). HS was instituted as a mixture of acetate-chloride (50:50; pH = 6.5–7.0) to avoid hyperchloremic acidosis. Treatments were started at 6 h of reperfusion and continued until 72 h of reperfusion. In a third series of experiments, rats were subjected to 2-h MCAO and treated in a blinded, randomized fashion with a continuous IV infusion of 0.3 ml/h of NS (n = 15), 3% HS (n = 12), 7.5% HS (n = 11), or 20% mannitol (n = 13). Treatments were begun at 6 h of reperfusion, and brain edema was determined at 72 h of reperfusion. Surgical shams were used as controls (n = 10). In all three series of experiments, rats were allowed to emerge from anesthesia at 30 min of reperfusion. Rats were housed in separate cages at room temperature (22–24°C) and during emergence from anesthesia and thereafter until they were euthanized.

Statistical analysis. All values are expressed as means ± SD. Physiological parameters and mean LDF measurements among groups were subjected to repeated-measures ANOVA. Differences in plasma AVP levels and infarct volume among treatment groups were determined by one-way ANOVA with post hoc Newman-Keuls test. Mortality rates were compared with logistic regression analysis. The criterion for statistical significance was P < 0.05.
Table 1. Physiological variables at baseline, during ischemia (2-h MCAO), and 30 min of reperfusion in various treatment groups

<table>
<thead>
<tr>
<th></th>
<th>Surgical Shams</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
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<tbody>
<tr>
<td></td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>96 ± 14</td>
<td>95 ± 11</td>
<td>98 ± 12</td>
<td>98 ± 11</td>
<td>96 ± 10</td>
</tr>
<tr>
<td>Ischemia</td>
<td>100 ± 12</td>
<td>98 ± 8</td>
<td>100 ± 10</td>
<td>102 ± 14</td>
<td>98 ± 12</td>
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<tr>
<td>Reperfusion</td>
<td>98 ± 8</td>
<td>96 ± 10</td>
<td>98 ± 14</td>
<td>102 ± 10</td>
<td>100 ± 8</td>
</tr>
<tr>
<td>LDF signal during MCAO (%)</td>
<td>26 ± 10</td>
<td>28 ± 8</td>
<td>28 ± 10</td>
<td>30 ± 11</td>
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<tr>
<td>pH</td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>7.38 ± 0.11</td>
<td>7.36 ± 0.14</td>
<td>7.35 ± 0.08</td>
<td>7.34 ± 0.08</td>
<td>7.34 ± 0.10</td>
</tr>
<tr>
<td>Ischemia</td>
<td>7.37 ± 0.08</td>
<td>7.36 ± 0.12</td>
<td>7.36 ± 0.12</td>
<td>7.36 ± 0.12</td>
<td>7.36 ± 0.15</td>
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<tr>
<td>Reperfusion</td>
<td>7.38 ± 0.12</td>
<td>7.37 ± 0.08</td>
<td>7.35 ± 0.15</td>
<td>7.38 ± 0.11</td>
<td>7.35 ± 0.14</td>
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<td>PaCO₂, Torr</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
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<td>44 ± 12</td>
<td>44 ± 8</td>
<td>42 ± 11</td>
<td>44 ± 8</td>
</tr>
<tr>
<td>Ischemia</td>
<td>45 ± 10</td>
<td>46 ± 8</td>
<td>47 ± 9</td>
<td>45 ± 9</td>
<td>41 ± 12</td>
</tr>
<tr>
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<td>44 ± 7</td>
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<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>164 ± 38</td>
<td>155 ± 30</td>
<td>162 ± 34</td>
<td>160 ± 42</td>
<td>151 ± 31</td>
</tr>
<tr>
<td>Ischemia</td>
<td>162 ± 40</td>
<td>166 ± 41</td>
<td>158 ± 33</td>
<td>161 ± 31</td>
<td>160 ± 41</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>152 ± 32</td>
<td>160 ± 34</td>
<td>147 ± 38</td>
<td>150 ± 32</td>
<td>158 ± 42</td>
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<td>Rectal temperature, °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>37.2 ± 0.9</td>
<td>37.1 ± 1.2</td>
<td>37.5 ± 1.3</td>
<td>37.6 ± 1.2</td>
<td>37.4 ± 0.9</td>
</tr>
<tr>
<td>Ischemia</td>
<td>38.3 ± 0.8</td>
<td>38.4 ± 1.1</td>
<td>38.3 ± 1.2</td>
<td>38.3 ± 1.1</td>
<td>38.3 ± 1.1</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>38.1 ± 1.0</td>
<td>38.0 ± 0.9</td>
<td>37.8 ± 0.8</td>
<td>38.0 ± 1.1</td>
<td>37.9 ± 1.2</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>88 ± 20</td>
<td>87 ± 18</td>
<td>85 ± 22</td>
<td>100 ± 24</td>
<td>99 ± 16</td>
</tr>
<tr>
<td>Ischemia</td>
<td>82 ± 28</td>
<td>88 ± 26</td>
<td>84 ± 32</td>
<td>94 ± 20</td>
<td>82 ± 22</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>94 ± 32</td>
<td>84 ± 28</td>
<td>86 ± 30</td>
<td>95 ± 18</td>
<td>95 ± 30</td>
</tr>
<tr>
<td>Serum osmolality, mosmol/l</td>
<td>290 ± 9</td>
<td>286 ± 10</td>
<td>284 ± 12</td>
<td>288 ± 9</td>
<td>294 ± 14</td>
</tr>
<tr>
<td>Plasma AVP, ng/ml</td>
<td>14 ± 7</td>
<td>42 ± 21*</td>
<td>50 ± 28*</td>
<td>110 ± 47*</td>
<td>22 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of animals. MABP, mean arterial blood pressure; LDF, laser-Doppler flowmetry; MCAO, middle cerebral artery occlusion; PaCO₂, arterial PCO₂; PaO₂, arterial PO₂; AVP, arginine-vasopressin. *P < 0.05 vs. surgical sham controls.

diagonal band of Broca) (Fig. 1), although there were varying degrees of ischemic damage in the lateral hypothalamus.

In the second series of experiments, physiological parameters (MABP, pH, PaCO₂, PaO₂, and rectal temperatures) were within normal ranges in all experimental groups at baseline, during MCAO, and at 30 min of reperfusion (data not shown). Mortality rates before completion of the experiment were as follows: 3 of 12 in rats treated with NS, 3 of 13 in rats treated with 3% HS, 4 of 12 in rats treated with mannitol, and 1 of 10 in rats treated with 7.5% HS. One rat with NS treatment, two with 3% HS, and one with 7.5% HS treatment did not meet the LDF criterion for successful MCAO or reperfusion. Thus eight rats in each of the experimental groups were included in the final analysis. Average LDF signal reduction from baseline during MCAO was not different among experimental groups (NS: 28 ± 9%; 3% HS: 27 ± 10%; mannitol: 30 ± 11%; 7.5% HS: 27 ± 9%). At the completion of treatments (72 h of reperfusion), plasma AVP levels were significantly attenuated by 10.22 ± 0.33, compared with that achieved with NS (308 ± 8 mosmol/l), 20% mannitol (336 ± 14 mosmol/l), and 3% HS (332 ± 12 mosmol/l) (Fig. 2). TTC-determined infarct volume, corrected for brain swelling, was significantly attenuated in CP complex in rats treated with 7.5% HS (cortex: 44 ± 6%; CP: 59 ± 6%) compared with NS (cortex: 44 ± 8%; CP: 78 ± 5%), 3% HS (cortex: 39 ± 8%; CP: 66 ± 6%), and mannitol (cortex: 38 ± 8%; CP: 70 ± 5%), but there were no differences in total hemispheric infarct volume in various treatment groups (NS: 20 ± 5%; 3% HS: 19 ± 4%; 7.5% HS: 17 ± 4%; 20% mannitol: 18 ± 4%).

In the third series of experiments, physiological parameters (MABP, pH, PaCO₂, PaO₂, and rectal temperatures) were within normal ranges in all experimental groups at baseline, during MCAO, and at 30 min of reperfusion (data not shown). Mortality rates before the end of the experiment (between 48 and 72 h; surgical shams 0 of 10; NS: 5 of 15; 20% mannitol: 3 of 13; 3% HS: 2 of 12; 7.5% HS: 1 of 11) were significantly higher in NS-treated rats compared with surgical shams. Thus 10 rats in each treatment group were included in the final analysis in this series of experiment. All rats met LDF criterion for successful MCAO. Water content in the ipsilateral and contralateral hemispheres was comparable in surgical sham controls. Treatment with 7.5% HS attenuated cerebral edema in both hemispheres at 72 h of reperfusion (serum osmolality: 358 ± 20 mosmol/l) compared with that achieved with NS (308 ± 8 mosmol/l), 20% mannitol (336 ± 14 mosmol/l), and 3% HS (332 ± 12 mosmol/l) (Fig. 3). Serum sodium was significantly elevated with 7.5% HS treatment, whereas potassium, urea nitrogen, and creatinine were within normal limits in all treatment groups (Table 2).

DISCUSSION

This study demonstrates two novel and important findings: 1) a distinct profile in plasma AVP levels following experimental focal ischemia that is independent of brain injury volume; and 2) institution and maintenance of a hyperosmolar state with HS to levels well beyond those suggested and reported in the literature attenuate ischemia-evoked brain edema as well as lower plasma AVP levels. Furthermore, our
Histopathological studies did not demonstrate direct injury to the anterior hypothalamus at the time when peak plasma levels of AVP are demonstrated. These data may have implications for the role of AVP in ischemic stroke and highlight the interaction of osmotherapy with plasma AVP levels following large ischemic strokes.

Cerebral edema following ischemic stroke. Our laboratory previously demonstrated (46, 47) that increases in water content in the ipsilateral as well as contralateral hemispheres following large experimental ischemic stroke are responsive to treatment with HS at concentrations used in the present study. The mechanisms of cerebral edema following focal ischemia are complex. In addition to the classic, simplistic pathobiology of ischemia-evoked cerebral edema that includes a cytotoxic component (secondary to postischemic energy failure) and a vasogenic component (secondary to breakdown of the BBB) (10, 15, 16, 29, 32), other mechanisms are under investigation. Some of these include impedance of cerebral venous return from cerebral swelling; intrahemispheric diastasis or hypometabolism (2, 25, 36); role of inflammatory mediators (1, 15); neurohumoral responses, including AVP release (5, 6, 11, 17, 25, 26, 42); induction of proteins, such as the VEGF (48); and upregulation of water channels, predominantly aquaporin-4 (44).

AVP in ischemic stroke. In the present study, we focused our attention on neurohumoral responses and the role of AVP in the pathophysiology of ischemic stroke. AVP is robustly present in the brain and regulates water balance by adjusting water permeability in glial cells (5). Several lines of evidence suggest that AVP may play an important role in events that follow cerebral ischemia. Elevated AVP levels have been reported in experimental ischemia (5), as well as in the serum and cerebrospinal fluid from patients with ischemic stroke (6, 42). Intracerebroventricular injections of AVP exacerbate acute ischemic brain edema, whereas intracerebroventricular injection of AVP antiserum significantly decreases cerebral edema (17). It has been suggested that AVP may play an important role in inhibiting the Na\(^+\)-K\(^+\)-ATPase activity of the cerebral cell membrane via AVP receptors mediated by cAMP and cGMP (17). Indirect evidence with the use of pharmacolo-

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Fig. 1. Cresyl violet staining of hypothalamus demonstrating relationship of the largest studied middle cerebral artery infarct to hypothalamic nuclei. A: coronal section through the forebrain shows the paraventricular nuclei separated by the third ventricle. The control side is on the left (control) side is shown along with the adjacent optic tract. C: neurons in the supraoptic nucleus on the right (ischemic) side show normal neuronal morphology, although there are reactive cells in the adjacent neuropil.

Fig. 2. Plasma arginine-vasopressin (AVP) (means ± SD) levels in rats treated with 0.9% saline (NS; n = 8), 3% hypertonic saline (HS; n = 8), 20% mannitol (n = 8), and 7.5% HS (n = 8). Treatments were initiated at 6 h and continued until 72 h of reperfusion following 2-h middle cerebral artery occlusion (MCAO). *P < 0.05 vs. NS, 3% HS, and mannitol treatment.

Fig. 3. Water content in the ipsilateral (ischemic) and contralateral (nonischemic) hemispheres in surgical sham controls (n = 10) and rats subjected to 2-h MCAO and treated with NS (n = 10), 3% HS (n = 10), 20% mannitol (n = 10), and 7.5% HS (n = 10). Treatments were initiated at 6 h of reperfusion and continued for 72 h. P < 0.01 vs. corresponding hemisphere (*contralateral and †ipsilateral) in experimental groups that received NS, P < 0.05 vs. corresponding hemisphere (#contralateral and ¶ipsilateral) in rats treated with mannitol.
Table 2. Serum electrolytes in various experimental groups at 72 h of reperfusion following 2-h MCAO

<table>
<thead>
<tr>
<th></th>
<th>NS</th>
<th>Mannitol</th>
<th>3% HS</th>
<th>7.5% HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea nitrogen, mg/dl</td>
<td>15.8±8.2</td>
<td>15.5±3.0</td>
<td>15.0±5.4</td>
<td>24.0±7.7</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.22±0.04</td>
<td>0.22±0.04</td>
<td>0.24±0.05</td>
<td>0.30±0.09</td>
</tr>
<tr>
<td>Sodium, meq/l</td>
<td>148±4*</td>
<td>144±4*</td>
<td>147±4*</td>
<td>172±13</td>
</tr>
<tr>
<td>Potassium, meq/l</td>
<td>4.7±0.4</td>
<td>4.1±0.6</td>
<td>4.2±0.5</td>
<td>4.3±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. NS, 0.9% saline; HS, hypertonic saline. *P < 0.001 vs. 7.5% HS. Normal range: blood urea nitrogen: 12–25.8 mg/dl; creatinine: 0.39–2.29 mg/dl; sodium: 129–150 meq/l; potassium: 4.6–6.0 meq/l (33).

logical agents that inhibit AVP release support a therapeutic target for the treatment of cerebral edema. For example, the kappa-opioid receptor agonist RU-51599 and AVP inhibitor with potent aquarectic activity, characterized by pure water diuresis, reduce brain edema following experimental stroke (20), and a selective nonpeptide V1 receptor antagonist (OPC-21268) has been shown to reduce cerebral edema associated with cold lesion (7), which simulates traumatic brain injury. We did not discern the source of AVP in our model of focal ischemia; however, we did not demonstrate any direct neuronal injury to the anterior hypothalamic nuclei, which is the major source of this hormone. Vasopressin-containing neurons in the anterior hypothalamus give rise to a complex, intrinsic fiber system that can modulate aquaporin-mediated water flux and hence play a crucial role in brain water and ion homeostasis (27).

In the present study, AVP levels were significantly elevated and continued to rise to a maximum at 72 h following focal ischemia. This temporal profile corresponds to and coincides with the maximal cerebral edema typically seen 48–72 h following ischemic stroke in our animal model (46, 47). While previous studies have demonstrated that the degree of elevation in AVP is proportional to the severity of ischemic brain injury (6), we did not observe a correlation between infarct volume and plasma AVP levels in our study. Our histopathological studies did not demonstrate direct injury to the anterior hypothalamus, suggesting that there are other sources of ischemic brain injury.

Osmotherapy and AVP. HS solutions are being increasingly utilized clinically as a therapeutic modality for cerebral edema in a variety of brain injury paradigms (10, 16, 30, 31, 37–40, 49). Owing to its better toxicity profile, and because sodium chloride (reflection coefficient = 1.0) (13) is completely excluded from brain with an intact BBB, it has been proposed that HS may be a more favorable osmotic agent than the conventional agent mannitol (reflection coefficient = 0.9). Furthermore, HS may be a more desirable agent for maintaining a “euvolemic hypervosmolar” state in a variety of brain injury paradigms (10, 16, 46, 47). Our laboratory has previously demonstrated that HS therapy, when instituted at the onset of reperfusion following transient focal ischemia (2 h), worsens TTC-determined infarct volume at the 24-h end point (9) but attenuates cerebral edema when treatment is delayed for 24 h following experimental stroke (46, 47). While we have no direct evidence for mechanism(s) responsible for this set of observations, the mechanism of this detrimental effect was not due to changes in regional CBF (9). Little is known about the differential response of neurons and glia to HS solutions during an “evolving” cerebral infarction. We have postulated that a hyperosmolar state impends the recovery of neurons from ischemia during the early phases of its evolution. This is based on in vitro studies that have demonstrated that hypertonic-hyperoncotic saline differentially affects healthy and glutamate-injured hippocampal neurons and astrocytes (18). There may be competing effects of HS solutions in ischemic stroke, and the beneficial osmotic effects on stroke-associated cerebral edema may be dependent on timing of the onset and duration of therapy in relation to “maturation” of the lesion following ischemic stroke. Based on the observed beneficial effects of HS when therapy was delayed from the onset of ischemia (46, 47), we instituted osmotherapy at 6 h of MCAO and continued treatment until the period of peak plasma levels of AVP (72 h).

Continuous 3% HS and 7.5% HS therapy were utilized to maintain a constant osmotic gradient to cause egress of water from the brain. In contrast to our laboratory’s previous study (9), while total hemispheric infarct volume was not different at 72 h of reperfusion, injury was significantly attenuated in the ipsilateral and contralateral cerebral hemispheres (350 osmol/l). Although we did not discern any differences in mortality rates in our experimental groups, ischemia-evoked cerebral edema was significantly attenuated in the ipsilateral and contralateral cerebral hemispheres, more significantly so with 7.5% HS compared with mannitol and 3% HS treatments. In the present study, we observed attenuation of plasma AVP levels only with 7.5% HS therapy (plasma osmolality in the range of 350–360 mosmol/l). We utilized 3% HS to create a graded hyperosmolar state and as a control for 20% mannitol, because the two solutions have comparable osmotic load (1,027 vs. 1,098 mosmol/l). While our study did not discern the mechanisms of attenuated levels of AVP with 7.5% HS treatment, we speculate that institution of osmotherapy with target plasma osmolalities >350 mosmol/l in our well-characterized model of ischemic stroke attenuates brain and plasma AVP levels, which, consequently, leads to decreased cerebral edema.

Our study has some limitations. Although we instituted IV fluids to ensure euvoemia in our experiments, we have limited measures of hydration status in our animal model. We cannot comment on the diuretic effects of HS, because we did not monitor urinary output in our rat model. It is plausible that HS has diuretic effects, resulting from attenuated plasma AVP levels at the higher serum osmolalities. However, urea nitrogen and creatinine levels were within normal limits in various treatment groups, indicating no systemic dehydration in any of the experimental groups. We did not assess behavioral outcomes in our study because of technical considerations as a result of the tethering system for institution of continuous IV infusions in our experimental model. We cannot comment on the possible rebound effects on cerebral edema following
cessation of osmotherapy in our experimental paradigm. During prolonged elevation of plasma osmolarity, with ongoing osmotherapy for brain edema over days, excess brain electrolytes are replaced by organic solutes (“idiogenic osmotes”; myo-inositol, taurine, glycerylphosphorylcholine, and betaine) for volume regulation and cotransported along with sodium from the extracellular to the intracellular compartment (29, 43). This osmotic compensation, as well as penetration and accumulation of osmotic agents into the brain tissue, particularly in regions of disrupted BBB, may explain prolonged cerebral edema and delayed “rebound edema” when osmotherapy is withdrawn (29). While this phenomenon has been well described with mannitol use, it has not been studied with HS therapy, but is theoretically possible. We did not measure regional brain AVP levels, and it is plausible that extra-central nervous system sites of AVP production are reflected in our data.

In conclusion, our data demonstrate that plasma AVP levels are elevated following ischemic stroke, with peak levels corresponding to the time period of maximal ischemia-evoked cerebral edema. Osmotherapy to target levels >350 osmol/l attenuates plasma AVP levels that may further augment anti-edema effects. The precise mechanism(s) of the role of AVP and clinical significance of agents that attenuate its release following ischemic stroke require further study for the treatment of accompanying cerebral edema.

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