Hypertonic Saline Worsens Infarct Volume After Transient Focal Ischemia in Rats

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Background and Purpose—Hypertonic saline (HS) has been advocated as a hyperosmolar agent for the treatment of cerebral edema, especially after traumatic brain injury. We tested the hypothesis that continuous intravenous HS administered during reperfusion from transient focal cerebral ischemia attenuates infarct volume.

Methods—Halothane-anesthetized male Wistar rats were subjected to 2 hours of middle cerebral artery occlusion (MCAO) by the intraluminal occlusion technique. At the onset of reperfusion, rats received a 10-mL/kg intravenous bolus of 0.9% saline (SAL, n=8) or 7.5% SAL (chloride:acetate 50:50, n=8) followed by a continuous infusion for 22 hours. In a second series of experiments, ischemic damage was determined in cohorts treated with equivolumetric 3% saline (n=8) or 20% mannitol (n=8). In a third series, regional cerebral blood flow was measured ([14C]iodoantipyrine autoradiography) at 6 hours of reperfusion in 7.5%-SAL–treated (n=5) or SAL-treated (n=5) animals.

Results—In SAL rats, serum Na+ was 137±3 and 138±2 mEq/L (mean±SEM) at baseline and 22 hours of reperfusion, respectively. In 7.5% SAL, serum Na+ was 136±2 and 154±2 mEq/L at baseline and reperfusion, respectively. Physiological variables and reduction in laser-Doppler signal during MCAO and early reperfusion were not different between the 2 treatment groups. Cortical infarct volume was larger in 7.5%-SAL–treated rats (121±14 mm³; 30±3% of contralateral cortex; P<0.05) than in SAL (64±15 mm³; 16±4% of contralateral cortex). Striatal infarct volume was unchanged by HS therapy. Ipsilateral cortical tissue volume was increased relative to the contralateral side (by 26±5% with SAL; by 41±5% with 7.5% SAL). In contrast, ischemic damage was unaffected by 3%-SAL or 20%-mannitol treatment compared with SAL. Regional cerebral blood flow during reperfusion was heterogeneous in all animals, but there was no evidence of postischemic hypoperfusion or blood flow maldistribution in 7.5%-SAL–treated animals.

Conclusions—These data demonstrate that hypernatremia resulting from postischemic HS infusion worsens cortical infarct volume in transient focal cerebral ischemia. The deleterious effect is not linked to exacerbation of delayed hypoperfusion during early reperfusion (6 hours); however, blood flow defects at later recovery time points remain to be excluded. These results may have implications for HS therapy in clinical ischemic stroke. (Stroke. 2000;31:1694-1701.)

Key Words: infarction ■ ischemia, focal ■ mannitol ■ osmolar concentration ■ reperfusion ■ saline solution, hypertonic

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smotherapy remains the mainstay of pharmacological intervention in neurological and neurosurgical intensive care for the treatment of cerebral edema and intracranial hypertension of diverse causes. Through the years, a variety of osmotic agents have been used in clinical practice, including mannitol, glycerol, urea, and sorbitol.1–3 Mannitol has been the osmotic agent of choice since the 1960s because it ordinarily does not cross the blood-brain barrier (reflection coefficient=0.9).1 Osmotic agents have potent antiedema action, presumably by drawing water from interstitial and intracellular spaces into the intravascular compartment.1–3 However, these agents have other nonosmotic cerebral effects, as has been demonstrated in a variety of animal models. For example, mannitol

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protects the brain by reducing blood viscosity, with consequent improved microvascular cerebral blood flow (CBF).3–5 by free radical scavenging properties,6 and by decreasing cerebrospinal fluid formation and reabsorption.7

More recently, hypertonic saline (HS) solutions have received renewed attention and use in clinical practice. Sodium chloride is completely excluded from the intact blood-brain barrier (reflection coefficient=1.0) and is theoretically a better osmotic agent than mannitol.4 Naive animals that are hemodiluted with hypertonic lactated Ringer’s solu-
tion (osmolarity of 480 mOsm/L) experience decreased intracranial pressure and total brain water content, along with increased CBF and resulting enhanced oxygen delivery.\textsuperscript{8–10} Furthermore, several case series and randomized clinical trials have demonstrated improved outcome with HS therapy in traumatic brain injury.\textsuperscript{11–14} Therefore, it has been hypothesized that HS therapy could be beneficial in cerebral ischemia and stroke.

The purpose of the present study was to test the hypothesis that inducing systemic hypernatremia during reperfusion decreases infarct volume after middle cerebral artery occlusion (MCAO). We also determined whether continuous HS infusion affects regional CBF during reperfusion and whether HS therapy causes histopathological injury independently of ischemia to neurons and glia in the rat.

### Materials and Methods

#### General Preparation and Animal Surgery

The experimental protocol was approved by the Institutional Animal Care and Use Committee and conforms to the National Institutes of Health guidelines for the care and use of animals in research. All techniques are as previously described.\textsuperscript{15} In brief, adult male Wistar rats (290 to 330 g) were anesthetized with halothane (1.0% to 2.0%) in oxygen-enriched air and allowed to ventilate spontaneously. Under 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 mid thorax. Rectal and temporalis muscle temperatures were maintained with a heating lamp throughout the surgical procedures.

#### Focal Ischemia and Reperfusion

All experiments were performed by a single individual (I.H.). Cortical perfusion was measured by laser Doppler flowmetry (LDF) as previously described\textsuperscript{15} (coordinates: 2 mm posterior and 6 mm lateral to bregma) (Moor Instruments Ltd, model MBF3D). To allow continuous monitoring of LDF, the headpiece of the stereotaxic frame was modified to allow for free rotation around the longitudinal axis of the rat and was equipped with a snout mask for spontaneous ventilation and with a holder for the LDF probe. The probe was positioned over an area devoid of large cortical blood vessels, and its position was not changed throughout the experiment. The LDF signal was allowed to stabilize over a 30-minute period before baseline measurements were obtained. Transient focal ischemia (2 hours) was produced by MCAO with an intraluminal suture technique as previously described.\textsuperscript{15,17} At the end of ischemia, reperfusion was produced by withdrawal of the intraluminal suture; this was associated with rapid restoration of the LDF signal. Rats that did not demonstrate a significant reduction of the LDF signal (<40% of baseline) during MCAO or rapid restoration of the LDF signal during reperfusion were excluded from the study. LDF measurements were averaged over 5-minute periods at 5, 15, 30, 60, 90, and 120 minutes of MCAO and at 15 minutes of reperfusion.

Rats were assigned to 2 treatment groups and received either 0.9% saline (SAL) (308 mOsm/L) or 7.5% SAL (2310 mOsm/L) on reperfusion, rats were deeply anesthetized with halothane (1.0% to 2.0%) and the MCA was occluded for 2 hours and treated with continuous 7.5%-SAL (n = 5) or SAL (n = 5) infusion with the onset of reperfusion as in the previous cohorts. At 6 hours of reperfusion, rats were reanesthetized with halothane; arterial blood pressure and blood gases were measured. \([^{14}C]\)IAP (40 µCi, New England Nuclear) in 0.8 mL of isotonic saline was infused intravenously for 45 seconds. During infusion, fifteen 10- to 20-µL samples of free-flowing arterial blood from the femoral artery catheter were collected in heparin-coated sample tubes. With the filament still in place, the rat was decapitated 45 seconds after the start of infusion. One postdecapitation arterial blood sample was also collected. The brain was quickly removed and frozen at –50°C in 2-methylbutane on dry ice. Each brain was sectioned by cryostat into coronal sections 20 µm thick at –20°C and thaw-mounted onto cover glasses. Sections were apposed for 1 week to film (Kodak, Bio-Max MR) with \(^{14}C\) standards. The concentrations of \([^{14}C]\)IAP in blood samples were determined by liquid scintillation spectrophotometry (Beckman, model 3801) after decolorization with 0.2 mL of tissue solubilizer (Soluene-350, Packard Instruments Co). Autoradiographic images representing 5 different coronal levels (+2.2, +0.2, −1.8, −3.8, and −5.8 mm from the bregma, 6 to 9 images at each coronal level) were digitized, and CBF was determined with image analysis software (Inquiry, Louts Associates). Rates of CBF were calculated by the Kety-Schmidt modification of the Fick principle as previously described.\textsuperscript{15} Standard coefficients for diffusion equilibrium and tissue:blood partition were used,\textsuperscript{15} with the assumption that these variables are unchanged by ischemia and reperfusion. Theoretical assessment of tracer techniques suggest that tissue:blood partition coefficients for diffusible tracers change little with cerebral ischemia.\textsuperscript{20}

The individual (S.J.M.) performing the CBF data analysis was blinded to the treatment groups. Two methods of analysis were used to determine CBF. First, discrete areas were measured by sampling of 0.08-mm\(^2\) squares within those regions most vulnerable to MCA occlusion: frontal and parietal cortex and medial and lateral caudoputamen. Flow rates were then averaged from squares assayed from 6 to 9 consecutive brain slices at each of 3 coronal levels (+2.2, +0.2, and −1.8 mm from the bregma). In the second method, areas for increasing incremental ranges of flow rates were determined by digital image scanning and perimetric measurements for selected slices in the entire ischemic hemisphere. Areas were averaged over 3 images from each brain level (+2.2, +0.2, −1.8, −3.8, and −5.8 mm from the bregma) and then numerically integrated across the 5 coronal levels to obtain an estimate of tissue volume for each incremental range of CBF.
TABLE 1. Summary of Selected Physiological Variables at Baseline (Before Ischemia), During Ischemia, and at 15 Minutes of Reperfusion (After Ischemia) in 4 Treatment Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>0.9% Saline</th>
<th>3% Saline</th>
<th>7.5% Saline</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>84±1</td>
<td>86±3</td>
<td>83±2</td>
<td>83±2</td>
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<tr>
<td>Ischemia</td>
<td>87±1</td>
<td>87±2</td>
<td>87±1</td>
<td>89±2</td>
</tr>
<tr>
<td>Postischemia</td>
<td>89±2</td>
<td>92±3</td>
<td>88±2</td>
<td>94±3</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>7.37±0.01</td>
<td>7.38±0.00</td>
<td>7.39±0.01</td>
<td>7.39±0.01</td>
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<tr>
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<td>7.36±0.01</td>
<td>7.39±0.01</td>
<td>7.37±0.01</td>
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<tr>
<td>Postischemia</td>
<td>7.45±0.01</td>
<td>7.48±0.01</td>
<td>7.51±0.01</td>
<td>7.47±0.01</td>
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<td>PaCO₂, mm Hg</td>
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<tr>
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<td>45.3±1.0</td>
<td>44.2±1.5</td>
<td>44.9±1.0</td>
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<td>Ischemia</td>
<td>50.5±1.3</td>
<td>51.5±1.0</td>
<td>45.3±1.7</td>
<td>50.4±1.9</td>
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<tr>
<td>Postischemia</td>
<td>38.1±0.9</td>
<td>36.9±2.0</td>
<td>35.2±1.2</td>
<td>38.0±1.4</td>
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<tr>
<td>PaO₂, mm Hg</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>131.7±12.9</td>
<td>128.7±9.5</td>
<td>154.3±15.5</td>
<td>133.7±11.2</td>
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<td>Ischemia</td>
<td>120.2±9.0</td>
<td>98.3±5.2</td>
<td>125.1±14.9</td>
<td>97.1±4.1</td>
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<td>Postischemia</td>
<td>83.1±1.9</td>
<td>88.3±3.0</td>
<td>88.7±2.7</td>
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<tr>
<td>Rectal temperature, °C</td>
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<tr>
<td>Preischemia</td>
<td>37.9±0.1</td>
<td>37.9±0.1</td>
<td>37.8±0.1</td>
<td>37.9±0.1</td>
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<tr>
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<td>38.0±0.1</td>
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<tr>
<td>Postischemia</td>
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<td>37.8±0.0</td>
<td>37.7±0.1</td>
<td>37.6±0.1</td>
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<td>Temporalis muscle temperature, °C</td>
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<tr>
<td>Preischemia</td>
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<td>36.9±0.1</td>
<td>36.9±0.1</td>
<td>37.0±0.1</td>
</tr>
<tr>
<td>Ischemia</td>
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<td>36.9±0.1</td>
<td>36.8±0.0</td>
<td>36.9±0.0</td>
</tr>
<tr>
<td>Postischemia</td>
<td>36.8±0.1</td>
<td>37.0±0.1</td>
<td>36.9±0.1</td>
<td>37.0±0.1</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure. Values are mean±SEM. n=8 per group.

Histopathology

To test whether HS infusion in the absence of MCAO causes injury, separate naive nonischemic rats were treated with an intravenous bolus of 10 mL/kg of either 0.9%, 3%, or 7.5% SAL (n=3 per group) followed by a continuous infusion at a rate of 0.5 mL/h for 4 days. Rats were then killed by decapitation under 5% halothane anesthesia and perfused with 10% neutral buffered formalin. The brains were postfixed in formalin and cut coronally at 2-mm intervals. The coronal slices were embedded in paraffin, cut at 10 μm, and stained with hematoxylin-eosin (H-E), H-E with Luxol fast blue counterstain for myelin, and an antibody to glial fibrillary acidic protein (Dako). Histopathological studies were interpreted by an individual (B.J.C.) who was blinded to the 2 treatment groups.

Statistical Analysis

All values are expressed as mean±SEM. Physiological parameters and mean LDF measurements among groups were subjected to repeated-measures ANOVA. Differences in infarct volume, cerebral edema, and autoradiographic regional CBF were determined by 1-way ANOVA. Post hoc comparisons were made with the Newman-Keuls test. The criterion for statistical significance was P<0.05.

Results

Effect of HS on Infarct Volume

Mean arterial blood pressure, arterial carbon dioxide (PaCO₂) and oxygen (PaO₂), pH, and temporalis muscle temperatures were within normal physiological ranges in all groups throughout the ischemic insult (Table 1). No rats died before the end of the experimental protocol. Two rats treated with 7.5% SAL and 1 treated with SAL were excluded because the residual LDF signal was not sustained below 40% during 2 hours of MCAO. Eight rats in each treatment group successfully completed the experimental protocol. Baseline serum Na⁺ and osmolality were similar in all treatment groups. However, serum Na⁺ and osmolality were increased at 2 hours (148±0.2 mEq/L; 305±2 mOsm/L) and 22 hours (154±2 mEq/L; 338±7 mOsm/L) of reperfusion in 7.5%-SAL-treated rats but not in SAL-treated rats (135±1 mEq/L; 293 mOsm/L and 138±1 mEq/L; 301 mOsm/L, respectively) (Table 2). Hemoglobin concentration, hematocrit, and serum glucose levels were not different among groups. The residual LDF signal averaged over the 2-hour MCAO period was similar in the 0.9%-saline–treated controls (27±3% of baseline) and the 7.5%-SAL–treated group (20±3%). On withdrawal of the monofilament, LDF signal returned rapidly to baseline values within 5 minutes in both groups (Figure 1).

Triphenyltetrazolium chloride–determined infarct volume of contralateral cerebral cortex measured at 22 hours of reperfusion was larger in rats treated with 7.5% SAL (121±14 mm³; 30±3% of contralateral cortex; P<0.05) than in rats treated with SAL (64±15 mm³; 16±4% of contralateral cortex) (Figure 2). There were no significant differences
among groups for infarct volume in the caudoputamen complex. Total hemispheric infarct was larger in rats treated with 7.5% SAL (158 ± 17 mm³; 22 ± 2% of contralateral hemisphere; \( P, 0.05 \)) than in rats treated with SAL (93 ± 19 mm³; 12 ± 3% of contralateral hemisphere). Total tissue volume of the ipsilateral cerebral cortex was increased 26 ± 5% in the SAL group and 41 ± 5% in the 7.5%-SAL group relative to the contralateral cortex.

In the 3%-SAL- or 20%-mannitol–treatment groups, there were no differences in physiological parameters (Table 1) or in serum sodium and osmolality (Table 2). The residual LDF signals during MCAO and early reperfusion in the 3%-SAL group (32 ± 1%) and in the 20%-mannitol group (29 ± 3%) were similar to that of the SAL group (27 ± 3%). The infarct volumes of the cortex and caudoputamen in the 3%-SAL group did not differ from those of the 20%-mannitol or SAL groups (Figure 3).

**Effect of SAL Versus 7.5% SAL on Cerebral Edema After MCAO at 22 Hours**

Physiological variables were maintained within normal values, and there were no differences between treatment groups

| TABLE 2. Summary of Serum Na⁺, Osmolality, Hemoglobin, Hematocrit, and Glucose Levels at Baseline and 2 and 22 Hours of Reperfusion in 4 Treatment Groups |
|-----------------------------------------------|---------------|---------------|---------------|---------------|
| **Serum Na⁺, mEq/L**                          | 0.9% Saline   | 3% Saline     | 7.5% Saline   | Mannitol      |
| Baseline                                      | 137 ± 1       | 136 ± 1       | 136 ± 1       | 137 ± 1       |
| 2 h reperfusion                               | 135 ± 1       | 138 ± 0       | 148 ± 0†      | 136 ± 1       |
| 22 h reperfusion                              | 138 ± 1       | 140 ± 1       | 154 ± 2†      | 139 ± 1       |
| **Serum osmolality, mOsm/L**                  |               |               |               |               |
| Baseline                                      | 297 ± 2       | 298 ± 2       | 299 ± 1       | 300 ± 1       |
| 2 h reperfusion                               | 293 ± 2       | 295 ± 2       | 305 ± 2†      | 299 ± 2       |
| 22 h reperfusion                              | 301 ± 2       | 303 ± 2       | 338 ± 7†      | 303 ± 2       |
| **Hemoglobin, g/dL**                          |               |               |               |               |
| Baseline                                      | 12.6 ± 0.4    | 11.9 ± 0.4    | 12.0 ± 0.2    | 12.4 ± 0.3    |
| 2 h reperfusion                               | 11.6 ± 0.5    | 12.2 ± 0.5    | 12.0 ± 0.3    | 12.6 ± 0.5    |
| 22 h reperfusion                              | 12.9 ± 0.3    | 11.9 ± 0.3    | 13.9 ± 0.4    | 12.9 ± 0.3    |
| **Hematocrit, %**                             |               |               |               |               |
| Baseline                                      | 37 ± 1        | 37 ± 1        | 35 ± 1        | 37 ± 1        |
| 2 h reperfusion                               | 34 ± 1        | 36 ± 1        | 35 ± 1        | 37 ± 1        |
| 22 h reperfusion                              | 37 ± 1        | 35 ± 1        | 38 ± 1        | 37 ± 1        |
| **Glucose, mg/dL**                            |               |               |               |               |
| Baseline                                      | 89 ± 7        | 107 ± 8       | 96 ± 5        | 116 ± 7       |
| 2 h reperfusion                               | 80 ± 10       | 75 ± 3        | 78 ± 7        | 90 ± 6        |
| 22 h reperfusion                              | 106 ± 9       | 87 ± 5        | 103 ± 11      | 93 ± 10       |

Values are mean ± SEM. n = 8 per group.

* \( P < 0.05 \) vs baseline; † \( P < 0.05 \) vs 0.9% saline.

**Figure 1.** Residual laser Doppler flowmetry after occlusion (LDF signal), expressed as percent of preischemic baseline signal in rats treated with SAL, 3% SAL, 7.5% SAL, and 20% mannitol (mean ± SEM, n = 8 per group).

**Figure 2.** Infarct volume in the ipsilateral hemisphere, cerebral cortex, and striatum (caudoputamen) in SAL- or 7.5%-SAL–treated rats (n = 8 per group). * \( P < 0.05 \) between the 2 treatment groups.
Physiological variables and baseline serum Na$^+$

Before or during MCAO or in early reperfusion. The ipsilateral LDF signal during MCAO decreased rapidly to ≈30% of baseline values and remained at this level for the duration of occlusion in both groups. One rat in the 7.5%-SAL group was excluded because the residual LDF signal during MCAO was not sustained below 40% of baseline values. Six rats in each treatment group successfully completed the experimental protocol. Serum Na$^+$ and osmolality in animals treated with SAL were similar between groups before MCAO. As anticipated, treatment with 7.5% SAL was associated with an increase in serum Na$^+$ at both 2 hours (SAL: 136±4 mEq/L; 7.5%-SAL: 144±1 mEq/L, P<0.05) and 22 hours (SAL: 144±2; 7.5%-SAL: 164±3 mEq/L, P<0.05) and in plasma osmolality at 2 hours (SAL: 298±4 mOsm/L; 7.5%-SAL: 311±4 mOsm/L, P<0.05) and at 22 hours (SAL: 307±2 mOsm/L; 7.5%-SAL: 355±10 mOsm/L, P<0.05). Water content in the contralateral noninjured hemisphere in the 7.5%-SAL group (78.3±0.2%) was significantly less (P<0.05) than in the SAL group (79.0±0.1%) at 22 hours of reperfusion. However, there was no difference in water content in the injured hemisphere between the 7.5%-SAL group (81.3±0.6%) and the SAL group (81.7±1.0%).

Effects of SAL Versus 7.5% SAL on CBF at 6 Hours of Reperfusion After MCAO

Physiological variables and baseline serum Na$^+$ were similar in the SAL- and 7.5%-SAL–treated rats. As expected, serum Na$^+$ levels at 6 hours of reperfusion were 152±2 and 137±1 mEq/L in 7.5%-SAL– and SAL-treated rats, respectively. To examine potential cortical and subcortical perfusion deficits during reperfusion, both absolute CBF within the MCA territory and blood flow distribution were quantified. Absolute CBF in the frontal and parietal cortex at 6 hours of reperfusion was not different in the SAL and 7.5%-SAL groups (Figure 4). Blood flow to medial and lateral sections of the caudoputamen was also similar in both treatment groups. Furthermore, when brain volume was partitioned into blood flow increments of 50 mL·100 g$^{-1}·$min$^{-1}$ throughout the previously ischemic hemisphere, there appeared to be no difference in blood flow distribution during reperfusion (Figure 5). HS therapy did not alter the distribution of tissue volume into low-flow zones (ie, <50 mL·100 g$^{-1}·$min$^{-1}$) compared with SAL. Scattered hypodensities on the autoradiographs were observed in three 7.5%-SAL–treated rats and 1 SAL-treated rat, but there was no visible hemorrhage in tissue sections.

Histopathology

At 24 hours of continuous infusion, serum Na$^+$ was increased in rats with 7.5% SAL (155±3 mEq/L) compared with SAL (141±1 mEq/L) or 3% SAL (142±1 mEq/L). Hypernatremia was sustained over the 4-day infusion period. Light microscopy revealed no gross histological differences among treatment groups. There was no evidence of hypoxic-ischemic injury to gray or white matter or macrophage infiltration or myelin pallor or sponginess to suggest white matter injury or edema. There was no evidence of reactive gliosis in H-E sections. Immunohistochemistry for glial fibrillary acidic protein did not reveal reactive astrocytes.

Discussion

This study demonstrates 2 important findings. First, administration of intravenous HS initiated at the onset of reperfusion after transient focal ischemia is not efficacious as a cerebral resuscitation treatment in this animal model of focal stroke. HS-induced hypernatremia/hyperosmolality did alter tissue outcome after MCAO, but in an unanticipated manner. We achieved serum Na$^+$ (145 to 155 mEq/L) and serum osmolality (>310 mOsm/L) goals with continuous infusion of 7.5% saline but not with 3% saline. Continuous 7.5% saline, but not 3% saline or mannitol, increased cortical and hemispheric infarct volumes as assessed at 22 hours of reperfusion. Because continuous hypernatremia did not cause histopathological neuronal, glial, or myelin injury in the absence of ischemia, the mechanism by which HS treatment damages brain is most likely linked to an interaction with reperfusion pathophysiology. Second, CBF recovery was robust in both saline- and HS-treated animals at 6 hours of reperfusion. Absolute CBF within the ipsilateral MCA territory or in contralateral nonischemic regions was not altered by ongoing 7.5% saline infusion. Therefore, the mechanism of HS-exacerbated tissue damage is not likely to be gross exacerbation of delayed hypoperfusion during early reperfusion (6 hours); however, blood flow defects at later recovery times remain to be excluded.

The basis for osmotherapy for focal or global cerebral edema of diverse causes can hypothetically be lessened by drawing free water from the interstitial space into the vascular compartment in regions of intact blood-brain barrier and possibly in regions of defective barrier function. HS solutions have been shown to enhance CBF and oxygen delivery in animal models, suggesting beneficial properties by vascular mechanisms. Furthermore, HS and mannitol have been shown to decrease intracranial pressure and improve neurological outcome after neurotrauma. In contrast, hypernatremia accentuates brain damage after traumatic brain injury. Thus, HS therapy is increasingly being used as a therapeutic modality in the neurological critical care setting. In our retrospective case series, continuous HS therapy improved cerebral edema in neurotrauma and postoperative neurosurgical patients. However, patients with intracerebral...
hemorrhage and cerebral infarction did not benefit from this therapy, and HS therapy remains unproven in clinical stroke. The present data in animals clearly demonstrate that HS therapy leading to significant hypernatremia can increase tissue damage after vascular occlusion and focal cerebral ischemia. There are limited data on the histopathological effects on the brain with the use of HS solutions. Based on published clinical reports, several complications of HS therapy are possible. Myelinotoxicity is a well-known complication of rapid correction of preexisting hyponatremia. Rapidly induced and sustained severe hypernatremia has also been shown to cause brain myelinolysis in rats, but a large serum Na\(^+\) gradient (ΔNa) (39±8 mEq/L) was necessary to demonstrate myelin injury. The ΔNa in our study was only \( \approx 17 \) mEq/L. Maintenance of this ΔNa for 4 days without ischemia did not cause myelin injury. Furthermore, we did not observe any loss of neurons, gliosis, or sponginess of white matter indicative of cerebral edema in our histopathological examination.

Although hypernatremia alone had no apparent deleterious effect on the brain in this model, treatment with 7.5% SAL during early stroke recovery exacerbated infarction, and the effect was most prominent in the cortex. Physiological variables and intraocclusion LDF reduction were similar among all treatment groups, so it seems unlikely that ischemic intensity was heightened in these rats. Although HS solutions have been shown to increase CBF in the nonischemic cerebral circulation, we wished to exclude grossly enhanced cortical perfusion defects that might be present during the immediate HS treatment period after MCAO. One potential cause of abnormal perfusion in HS animals could be blood-brain barrier opening due to intense endothelial injury, allowing leakage of osmotically active sodium and water into perivascular tissue elements. The present data indicate that overall, CBF recovery was not depressed in HS-treated animals at 6 hours of reperfusion. Furthermore, the volume of hypoperfused tissue (<50 mL/100 g min\(^{-1}\)) was not

Figure 4. Absolute CBF within MCA territory of the ischemic ipsilateral hemisphere and contralateral side. Regions displayed are frontal (F CTX) and parietal cortex (P CTX) and medial (Med) and lateral (Lat) caudoputamen complex (CP) in SAL- (n=5) and 7.5%-SAL– (n=5) treated rats. Regional CBF was measured at 6 hours of reperfusion after 2 hours of MCAO by [\(^{14}\)C]IAP autoradiography.

Figure 5. Distribution of brain tissue volume over CBF ranges in SAL- (n=5) and 7.5%-SAL– (n=5) treated rats. Blood flow rates were measured at 6 hours of reperfusion after 2 hours of MCAO by [\(^{14}\)C]IAP autoradiography.

significantly different between the HS and SAL groups. Nevertheless, some animals in the HS group did have a considerable area of hypoperfusion at 6 hours, as indicated by the larger SEM relative to the mean value at CBF <50 mL/100 g min\(^{-1}\) in Figure 5. CBF recovery, as measured by [\(^{14}\)C]IAP, was characterized by large intraregional variability in all animals, regardless of treatment assignment, at 6 hours of reperfusion. Intrasubject and intragroup blood flow heterogeneity and patchy CBF recovery patterns have been well characterized by other investigators with reversible cerebral ischemia in rat and cat. Although it may have been difficult to show small differences in absolute CBF during reperfusion, the present data clearly do not suggest a gross increase in postischemic hyperperfusion with HS therapy, at least within the window of our observations. Differences in CBF distribution may become more prominent in the later phases of reperfusion in our experimental paradigm. Nevertheless, non–blood flow–associated mechanisms should be considered in the surprisingly deleterious interaction between hypernatremia and reperfusion.

Using wet-to-dry weight comparisons, we examined the possibility that HS therapy paradoxically worsened parenchymal edema rather than dehydrating the injured hemisphere. Cerebral edema was present in all animals at 22 hours of reperfusion, regardless of treatment, because ipsilateral cortical volume increased by 41% and 26% relative to contralateral cortex in the 7.5%-SAL and SAL groups, respectively. Part of this increase in volume can be accounted for by increased brain water. The difference in percent water content between ipsilateral and contralateral hemispheres was 3% in HS-treated animals and 2.7% in the normal saline control group. Consistent with the goal of osmotherapy, we detected lower water content in the noninjured contralateral hemisphere of HS-treated rats and presume that this measurement represents brain regions with intact blood-brain barrier and sodium exclusion. However, there was no evidence that HS therapy worsened (or improved) water accumulation in the injured hemisphere. A greater amount of tissue swelling without a greater increase in water content may imply that the additional tissue volume observed with 7.5% SAL (41% versus 26% in SAL) is due to the greater volume of damaged
and infarcted tissue elements rather than more intense edema formation. Alternatively, augmented edema formation by 7.5% SAL in the ischemic core where blood-brain barrier disruption is anticipated may not have been detected by our measurements of water content within the total ipsilateral hemisphere. It is possible that increases in brain water were not large because of dilution of areas of low water content (noninfarcted regions) with those of high water content (infarcted regions). Edema after cerebral ischemia has both vasogenic and cytotoxic components, and HS may have a differential effect over the 22-hour reperfusion period on these 2 components, ie, improving vasogenic but exacerbating cytotoxic edema. A potential for rebound brain uptake of water with HS treatment secondary to increases in idiogenic tissue osmole also exists. Increased brain osmolality unrelated to tissue electrolyte change or protein extravasation across the blood-brain barrier has also been reported at 3 and 6 hours after MCAO.28

In the present study, the desired end point for serum Na+ was 145 to 155 mmol/L. The rationale for this end point was to achieve a serum osmolality of 310 to 320 mOsm/L, ie, the target for patients with intracranial hypertension and cerebral edema.20 We were able to achieve these goals in our rat model with a continuous infusion of 7.5% SAL. In a further set of experiments, we infused comparable concentrations and equal volumes (10 mL/kg) of 20% mannitol and 3% saline. The goal was to compare the effect of an alternative osmolar agent (mannitol) and a lower level of hypernatremia (3% SAL) with that of 7.5% SAL in our model. Neither agent extended ischemic damage as 7.5% saline therapy did, presumably because increases in osmolality were moderate and mimicked those observed with the control saline treatment. Although evaluation of the potential of mannitol as a neuroprotective agent (mannitol) and a lower level of hypernatremia (3% SAL in the ischemic core where blood-brain barrier dysfunction is anticipated may not have been detected by our measurements of water content within the total ipsilateral hemisphere. It is possible that increases in brain water were not large because of dilution of areas of low water content (noninfarcted regions) with those of high water content (infarcted regions). Edema after cerebral ischemia has both vasogenic and cytotoxic components, and HS may have a differential effect over the 22-hour reperfusion period on these 2 components, ie, improving vasogenic but exacerbating cytotoxic edema. A potential for rebound brain uptake of water with HS treatment secondary to increases in idiogenic tissue osmole also exists. Increased brain osmolality unrelated to tissue electrolyte change or protein extravasation across the blood-brain barrier has also been reported at 3 and 6 hours after MCAO.28

In conclusion, our data demonstrate that continuous HS therapy worsens infarct volume after transient focal ischemia. We cannot exclude the possibility that HS may have therapeutic efficacy if used in a setting of permanent focal ischemia without reflow, if initiated at a later time than immediately after occlusion, or if given as a single large bolus during early reperfusion rather than as a continuous infusion. However, caution is advised in the use of HS for patients who experience an ischemic insult.

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Editorial Comment

The article by Bhardwaj et al is a thoughtfully executed animal study with some surprising observations concerning hypertonic saline administration during reperfusion after transient focal ischemia. The authors investigated the hypothesis that hypertonic saline administration would improve histological and/or functional outcome after middle cerebral artery occlusion and reperfusion in rats. Their data are negative and in fact demonstrate an exacerbation of the damage after focal ischemia in the hypertonic saline–treated animals.

Negative data are often the subject of even greater scrutiny than those that are positive. However, the methods used by this group are above reproach. The rat filament MCAO model was appropriately chosen, as it is, at the moment, the small-animal model that most closely mimics the clinical scenario of thrombolytic-treated human stroke. Readers not familiar with this model should note that the authors’ use of laser Doppler flowmetry of the ipsilateral hemisphere is an effective method for confirming occlusion of the MCA and for knowing when and if the filament has caused perforation of an intracerebral artery. Studies in which this technique has not been used are more prone to methodological error.

The authors also expressed some surprise over the lack of histological effects of mannitol administration. Indeed, one would expect some benefit of mannitol during ischemia by virtue of its effects on blood viscosity, and such benefits have been shown in animals in terms of outcome and brain water content.1–3 An important difference in the current study is the use of mannitol as a continuous infusion, whereas bolus infusion has been the method used in most experimental studies and for patients. Mannitol should not be considered a potent neuroprotective agent during ischemia, and the negative data suggest to me more a reflection of honest data reporting than a problem with the model.

Given that hypertonic saline may exacerbate damage in ischemia/reperfusion, clinicians should avoid such treatment in stroke patients outside the context of a controlled and carefully scrutinized clinical trial.

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Hypertonic Saline Worsens Infarct Volume After Transient Focal Ischemia in Rats
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