Regulation of brain water during acute glucose-induced hyperosmolality in ovine fetuses, lambs, and adults

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Stonestreet, Barbara S., Katherine H. Petersson, Grazyna B. Sadowska, and Clifford S. Patlak. Regulation of brain water during acute glucose-induced hyperosmolality in ovine fetuses, lambs, and adults. J Appl Physiol 96: 553–560, 2004. First published October 24, 2003; 10.1152/japplphysiol.00617.2003.—We tested the hypothesis that, during acute glucose-induced hyperosmolality, the brain shrinks less than predicted on the basis of an ideal osmometer and that brain volume regulation is present in fetuses, premature and newborn lambs. Brain water responses to glucose-induced hyperosmolality were measured in the cerebral cortex, cerebellum, and medulla of fetuses at 60% of gestation, premature ventilated lambs at 90% of gestation, newborn lambs, and adult sheep. After exposure of the sheep to increases in osmolality with glucose plus NaCl, brain water and electrolytes were measured. The ideal osmometer is a system in which impermeable solutes do not enter or leave in response to an osmotic stress. In the absence of volume regulation, brain solute remains constant as osmolality changes. The osmotically active solute demonstrated direct linear correlations with plasma osmolality in the cerebral cortex of the fetuses at 60% of gestation (r = 0.72, n = 24, P = 0.0001), premature lambs (r = 0.58, n = 22, P = 0.005), newborn lambs (r = 0.57, n = 24, P = 0.004), and adult sheep (r = 0.70, n = 18, P = 0.001). Similar findings were observed in the cerebellum and medulla. Increases in the quantity of osmotically active solute over the range of plasma osmolalities indicate that volume regulation was present in the brain regions of the fetuses, premature lambs, newborn lambs, and adult sheep during glucose-induced hyperosmolality. We conclude that, during glucose-induced hyperosmolality, the brain shrinks less than predicted on the basis of an ideal osmometer and exhibits volume regulation in fetuses at 60% of gestation, premature lambs, newborn lambs, and adult sheep.

brain volume regulation; dehydration; development; electrolytes; maturation; sheep

MAINTENANCE OF BRAIN CELL volume is essential for normal central nervous system functioning (15). The permeability properties of the cerebral capillary endothelium are closer to those of cell membranes than systemic capillaries. This endothelium is permeable to water but highly impermeable to most other plasma constituents. Osmotic forces dominate water flux across the blood-brain barrier, and the volume of the brain is a function of the osmolyte content of the tissue (3). In adult rats, when plasma osmolality increases, water flows across the blood-brain barrier down its concentration gradient from brain tissue to plasma, and brain volume deceases (5). The brain may respond to this stress by gaining osmotically active solutes that limit water loss (5). This phenomenon has been termed brain volume (water) regulation (5).

An ideal osmometer is a system in which impermeable solutes do not enter or leave in response to an osmotic stress (5). In the adult rat, the brain does not behave as an ideal osmometer in response to acute hyperosmolality (5). Water loss and solute gain occur simultaneously such that brain volume stabilizes within 30 min (5). Consequently, the adult brain shrinks less than predicted on the basis of an ideal osmometer, and the brain exhibits volume regulation (5).

Although brain volume regulation has been extensively studied in the adult (7, 10), the ability of the brain to exhibit volume regulation had not been examined during development until our laboratory’s recent work (25). Our laboratory has shown that, during acute mannitol-induced hyperosmolality, brain water loss is maximal and brain volume regulation impaired in most brain regions of fetuses at 60 and 90% of gestation and premature ventilated lambs at 90% of gestation, whereas water loss is limited and volume regulation is present in the brain regions of young lambs and adult sheep (25).

Abnormalities in systemic glucose homeostasis are common during the perinatal period in fetuses of diabetic women, infants with congenital diabetes, infants who are small for gestational age, infants with sepsis, premature infants, and premature infants exposed to corticosteroids (9, 11, 17, 22, 31). These perturbations in glucose homeostasis potentially expose neonates to hyperglycemic hyperosmotic stress, which might affect brain volume (water) regulation. The effect of glucose-induced hyperosmolality on brain volume regulation has not been studied in immature subjects.

Glucose is the major fuel for brain metabolism where it is the primary substrate for energy production (30). Cerebral glucose consumption is particularly high in the newborn, because of the large size of the neonatal brain in relation to body weight (20). Glucose is transported into the brain (8), where it is metabolized. The effects of hyperglycemic hyperosmolality on brain volume regulation may differ considerably from that of mannitol-induced hyperosmolality because mannitol sugar is not transported or metabolized. Transport and metabolism of glucose within the brain might increase the quantity of solute in brain tissue, limiting brain shrinkage in response to a systemic glucose-induced osmotic load.

We tested the hypothesis that, during acute glucose-induced hyperosmolality, the brain shrinks less than predicted on the basis of an ideal osmometer and that brain volume regulation is present in fetuses, premature, and newborn lambs.

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BRAIN WATER REGULATION AND HYPEROSMOLALITY

Table 1. Study groups

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Treatment Group</th>
<th>Recovered From Surgery, days</th>
<th>Study Age, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetuses at 60% of gestation</td>
<td>Glucose</td>
<td>17 (3) (5)</td>
<td>87 (86–88)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>7 (3) (3)</td>
<td>87 (87–88)</td>
</tr>
<tr>
<td>Preterm lambs at 90% of gestation</td>
<td>Glucose</td>
<td>14 (3) (7)</td>
<td>137 (132–142)</td>
</tr>
<tr>
<td>Newborn lambs</td>
<td>Placebo</td>
<td>8 (2) (3)</td>
<td>137 (135–139)</td>
</tr>
<tr>
<td>Adult sheep</td>
<td>Glucose</td>
<td>19 (1) (2)</td>
<td>4 (3–5)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>6 (1) (2)</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>9 (2) (3)</td>
<td>4 (yr)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>9 (3) (5)</td>
<td>4 (yr)</td>
</tr>
</tbody>
</table>

Nos. in parenthesis are range.

MATERIALS AND METHODS

This study was conducted after approval by the Institutional Animal Care and Use Committee of Brown University and Women and Infants’ Hospital of Rhode Island and is in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animal preparation. Surgery was performed under 0.75–2.0% halothane anesthesia as previously described (23). In the fetuses at 60% of gestation, the catheters were placed in the subclavian vein for glucose plus NaCl or placebo (0.154 M NaCl) administration and subclavian artery advanced to the thoracic aorta for blood sample withdrawal, heart rate, and blood pressure monitoring. An amniotic fluid catheter was placed as a referent for fetal arterial blood pressure. A femoral artery catheter was placed in the ewes.

In the premature lambs, study and surgery preparation were performed as previously described (24, 25). For the purpose of this report, the premature lambs at 90% of gestation are designated as premature lambs. Briefly, in the fetuses at 90% of gestation, catheters were placed into a brachial vein for the infusions, and they were placed in a brachial artery and advanced to the thoracic aorta for blood sample withdrawal and blood pressure monitoring. After insertion, the catheters were closed and attached to the skin of the fetus. Then, the head and neck were exposed and an endotracheal tube placed into the trachea to facilitate suctioning and ventilation at delivery. The endotracheal tube remained open in the amniotic cavity to allow for lung liquid egress. The uterus and abdomen of the ewe were then closed. In the fetal and premature lambs, singleton and twin pregnancies were included. When a twin pregnancy was present, only one fetus was studied.

The lambs and adult sheep were intubated under ketamine (10 mg/kg) and maintained with 0.75–2.0% halothane anesthesia. Catheters were placed into a brachial vein for glucose plus NaCl or placebo administration and brachial artery for sampling.

The regional brain water measurements in the placebo-treated animals were obtained from animals in previous studies (25).

Study groups. After recovery from surgery, the fetuses at 60% of gestation (full-term gestation in sheep is 150 days), premature lambs, newborn lambs at 1–5 days of age, and adult sheep were randomly assigned to receive glucose (50% glucose and 0.5 M NaCl) or placebo (0.154 M NaCl) infusions. The NaCl was added to the glucose solution to prevent the plasma and brain sodium and chloride concentrations from decreasing secondary to glucose administration (5, 25). The groups, number of animals in each group, duration of recovery from surgery, and age at study are summarized in Table 1.

In this study, we were not able to study fetal sheep in utero at 90% of gestation, because, when we attempted three studies, the fetuses developed severe metabolic acidosis and died within 20 min after the onset of the glucose infusions. In contrast, the fetuses at 60% of gestation tolerated the infusions. Therefore, we studied the surfactant-treated, ventilated, premature lambs at 90% of gestation, which tolerated the infusions for the duration of the study. We also studied ventilated premature lambs, because ventilated premature infants are at high risk for hyperglycemia and dehydration, which can result in hyperosmolality.

Experimental protocol and methodology. The fetuses were studied while the ewes were standing quietly in a cart. The premature lambs were studied, after delivery by hysterotomy with the ewe under intravenous ketamine anesthesia (15–40 mg/kg). The ewes then were killed with an overdose of pentobarbital sodium (100–200 mg/kg). After delivery, the premature lambs were suctioned via the endotracheal tube, treated with surfactant (100 mg/kg, Survanta, Beractant, Ross Products, Columbus, OH), hand ventilated, and placed on a positive-pressure ventilator (Bio-Med Devices, Flow-Disc MVP-10, Pediatric Respirator, Stamford, CT) (25). Ventilation was begun on room air or oxygen as needed with a respiratory rate of 18–71 breaths/min, a peak inspiratory pressure of 14–43 mmHg, and a positive end-expiratory pressure of 2–10 mmHg. The premature lambs were studied 2 h after they were stabilized on the ventilator. The newborn lambs were studied while blindfolded and resting in a sling, and the adult sheep standing quietly in a cart.

After baseline determinations, 50% glucose and 0.5 M NaCl or placebo (0.154 M NaCl) was administered as an initial rapid intravenous injection, followed by a continuous infusion, to achieve increases in systemic plasma osmolality within each group. The osmolar loads (Table 2) were selected to produce both a wide range of osmolalities for the subjects within each group and a steady-state increase in plasma osmolality within each subject for the duration of the study. In the fetuses at 60% of gestation, glucose plus NaCl or placebo infusions were administered to the fetuses and ewes, because in the initial experiments we found that a given osmolality could not be maintained in the fetuses unless the ewes also received glucose, presumably because of fluid shifts among the fetuses, amniotic fluid, fetal membranes, and ewes (2). The rapid intravenous injections followed by continuous infusions of glucose or placebo were administered directly to the premature, newborn lambs and adult sheep. Plasma osmolalities were obtained at baseline and at 5, 10, 20, 30, 40, 50, and 60 min of study. Arterial blood plasma samples were taken after 60 min of the study for plasma sodium, potassium, and chloride concentrations. The 60-min study interval and exposure to hyperosmolality were considered sufficient for the brain volume to stabilize, on the basis of work in adult rats (5). After exposure to the glucose or

Table 2. Weight, duration of osmolar exposure, and total osmolar load in glucose-infused sheep by group

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Fetuses 60% of Gestation</th>
<th>Preterm Lambs 90% of Gestation</th>
<th>Lambs 3–5 Days of Age</th>
<th>Adult Sheep 4 yr of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>17</td>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.5±0.02</td>
<td>3.5±0.2</td>
<td>4.8±0.3</td>
<td>58±1</td>
</tr>
<tr>
<td>Duration of osmolar exposure, min</td>
<td>98±4*</td>
<td>72±1*</td>
<td>74±0.5*</td>
<td>90±2*†</td>
</tr>
<tr>
<td>Total osmolar load, mosmol</td>
<td>3,217±266†</td>
<td>243±47*</td>
<td>426±243*</td>
<td>2,383±362†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of sheep. Total osmolar load is the load received by the ewe plus fetus or lambs and adult sheep. *P < 0.05 vs. fetuses at 60% of gestation. †P < 0.05 vs. preterm lambs at 90% of gestation.
placebo infusions (Table 2), in the fetal groups, the ewe was given ketamine (15–40 mg/kg) intravenously to achieve a surgical plane of anesthesia. A hysterotomy was performed, and the fetus was withdrawn from the uterus intact and decapitated immediately (24, 26, 27). The ewe was then killed with pentobarbital sodium (100–200 mg/kg). A similar procedure was used in the lambs and adult sheep.

Brain tissue water content and plasma electrolyte concentrations.

The brain was removed within 8–10 min for brain tissue samples of the cerebrum, cerebellum, and medulla. Triplicate 1-g sections of cerebrum, cerebellum, and medulla were obtained for tissue water content and placed in weighed dry glass vials as previously described (5). Tissue samples were dried to a constant weight at 90°C for 72 h to determine sample water content. The dried samples were extracted with 0.75 N HNO3 for 72 h at room temperature. The acid extracts and arterial plasma samples were analyzed for sodium and potassium by atomic absorption spectroscopy (model 2380, Perkin-Elmer, Überlingen, Germany), and plasma was analyzed for chloride by coulometric titration (model CMT10 chloride titrator, Radiometer, Copenhagen, Denmark). Chloride concentrations were not available on the brain tissue extract because of technical difficulties at the onset of the studies.

Plasma osmolality was measured in duplicate on a vapor pressure osmometer (Vapro model 5520, Wescor, Logan, UT).

Calculations and statistical analysis. The osmolar load to the ewe represented the total amount of solute (mosmol) administered to the ewe including the initial rapid injection and the continuous infusion. Similarly, the osmolar load to the fetus was the total amount of solute (mosmol) administered to the fetus as the initial injection and continuous infusion. The total osmolar load was the sum of the osmolar load to the ewe and fetus. In the lambs and adult sheep, the total osmolar load included the initial injection and the continuous infusions given to the lambs or adult sheep (Table 2).

The ideal osmometer is a system in which impermeable solutes do not enter or leave in response to an osmotic stress (5, 25). In the absence of brain volume (water) regulation, the brain behaves on the basis of an ideal osmotic behavior and brain solute (VC) content remains constant as C changes, where V is volume or regional water content (ml/g dry wt brain) that was measured and C is tissue osmolality. To calculate the total regional brain solute (VC, mosmol/g dry wt brain) at the end of the study, it was assumed that equilibrium had been reached, on the basis of previous work (5), such that plasma C = tissue C. For a plot of VC at the end of the study against plasma osmolality, see Figs. 3–5. The plasma osmolality used to calculate VC and on the y-axis is the value at the end of the study.

The 1-h study interval should have been more than sufficient for water loss from the brain to stabilize and to reach a new equilibrium in the four groups of sheep such that plasma C equals tissue C, because in the adult rat water loss was constant and equilibrium reached after only 30 min of hyperosmolality (5).

The equilibrium between plasma and tissue osmolality outlined above (plasma C equals tissue C) is achieved by water movement across the blood-brain barrier, because the barrier is permeable to water but not solutes (3). However, in the case of glucose-induced hyperosmolality, some of the equilibrium is achieved by movement of glucose into the brain, because glucose also is transported across the blood-brain barrier (8).

When osmotic behavior is ideal, VC remains constant as osmolality increases; i.e., the correlation between VC and C is not different from zero (see the dashed lines in Figs. 3–5). When osmotic behavior is nonideal, the volume remains greater than expected for the ideal osmometer. Therefore, brain volume (water) regulation was present when VC was not constant; i.e., there was a significant positive correlation between osmotically active solute and plasma osmolality (see the solid lines in Figs. 3–5).

When volume regulation is present (see Figs. 3–5), it may be shown that the actual water loss, as a percentage of water loss expected under ideal osmotic behavior, may be calculated as

\[(V_1 - V_2)/(V_1 - b) = 100/(1 + m \cdot C/b)\]

where \(V_1\) is the regional brain volume (water content) in each group under normal isotonic conditions, \(V_2\) is the regional brain volume at the end of the acute osmotic stress under conditions of volume regulation, \(b\) is the regional brain volume at the end of the osmotic stress under conditions of ideal osmotic behavior, \(m\) is the slope of the line (see Figs. 3–5), \(C_1\) is the osmolality in each group under normal isotonic conditions, and \(b\) is the intercept of the line (see Figs. 3–5).

All results are expressed as means ± SE. Serial measurements were compared among the glucose-infused groups by two-factor ANOVA for repeated measures with group and time as the factors (Figs. 1 and 2). The least squares linear regression analysis was used to compare the osmotically active regional brain solute with the plasma osmolality values at the end of the study (Figs. 3–5). To determine deviations from an ideal osmometer, the slopes of the regression lines were statistically tested against zero by using Student’s t-tests. The least
squares linear regression analysis also was used to compare the brain electrolyte content to the plasma osmolality at the end of the study. The plasma electrolyte values were compared between the glucose- and placebo-infused fetuses by the Bonferroni-corrected two-group t-test (see Table 4).

RESULTS

Total osmolar load, calculated as the osmolar concentration corrected for the quantity of solution administered to the fetuses plus ewes, lambs, and adult sheep, is summarized in Table 2. Plasma osmolality increased and reached a plateau by 20 min of study (Fig. 1). Although for simplicity the graph illustrates the means ± SE of the plasma osmolality values for each age group, the individual animals within each group increased in a similar manner. However, within each age group, there was a wide range of osmolar values as shown by the individual plasma osmolality values presented in Figs. 3–5. Plasma glucose concentration increased within each group as expected (Fig. 2).

Fig. 2. Plasma glucose concentration plotted against study time for the glucose-infused fetuses at 60% of gestation, preterm lambs at 90% of gestation, newborn lambs at 3–5 days of age, and adult sheep at 4 yr of age. Values are means ± SE. *ANOVA: main effects for age vs. fetuses at 60% of gestation P < 0.05. ANOVA: main effects for age, P < 0.05 vs. newborn lambs at 3–5 days of age.

Fig. 3. Osmotically active solute plotted against plasma osmolality in the cerebrum for fetuses at 60% of gestation (A), preterm lambs at 1–5 days of age (C), and adult sheep (D). ○, Placebo-infused sheep; ●, glucose-infused sheep. The y-axes represent the total regional brain solute and the x-axes the plasma osmolality after 60 min of exposure for each animal. Osmotically active solute represents the regional volume or water content times the plasma osmolality at the end of the study. Fetuses at 60% of gestation: r = 0.72, n = 24, P = 0.0001. Preterm lambs at 90% of gestation: r = 0.58, n = 22, P = 0.005. Newborn lambs at 1–5 days of age: r = 0.57, n = 24, P = 0.004. Adult sheep: r = 0.70, n = 18, P = 0.001. Non-significant slopes (dashed lines) indicate no change for solute over the range of experimental osmolalities examined represents the absence of brain volume regulation (i.e., ideal osmometer). Significant slopes (solid line) indicate that volume regulation was present in the cerebrum in each group.
Regional brain water decreased with increasing plasma osmolality in all age groups (data not shown). Figures 3–5 show the scattergrams of the osmotically active solute (VC) plotted against plasma osmolality (C). The absence of brain volume regulation (i.e., ideal osmometer) is illustrated by a nonsignificant slope (dashed line) indicating no change in the quantity of solute in the brain region over the range of osmolalities examined. The osmotically active solute (mosmol/g dry wt) values demonstrated direct linear correlations with plasma osmolality (mosmol/kg H2O) in the cerebral cortex (Fig. 3) of the fetuses at 60% of gestation ($r = 0.72, n = 24, P = 0.0001$), premature lambs ($r = 0.58, n = 22, P = 0.005$), newborn lambs ($r = 0.57, n = 24, P = 0.004$), and adult sheep ($r = 0.70, n = 18, P = 0.001$). These positive slopes indicate that volume regulation was present in the cerebrum in each group.

Fig. 4. Osmotically active solute plotted against plasma osmolality in the cerebellum for fetuses at 60% of gestation (A), preterm lambs at 90% of gestation (B), newborn lambs at 1–5 days of age (C), and adult sheep (D). Descriptions of symbols and axes are as in the legend of Fig. 3. Fetuses at 60% of gestation: $r = 0.44, n = 24, P = 0.03$. Preterm lambs at 90% of gestation: $r = 0.75, n = 22, P = 0.00006$. Newborn lambs at 1–5 days of age: $r = 0.75, n = 24, P = 0.00003$. Adult sheep: $r = 0.46, n = 18, P = 0.05$. A nonsignificant slope (dashed lines), indicating no change for solute over the range of experimental osmolalities examined, represents the absence of brain volume regulation (i.e., ideal osmometer). A significant slope (solid lines) indicates that volume regulation was present in the cerebellum in each group.

Fig. 5. Osmotically active solute plotted against plasma osmolality in medulla for fetuses at 60% of gestation (A), preterm lambs at 90% of gestation (B), newborn lambs at 1–5 days of age (C), and adult sheep (D). Descriptions of symbols and axes are as in the legend of Fig. 3. Fetuses at 60% of gestation: $r = 0.42, n = 24, P = 0.04$. Preterm lambs at 90% of gestation: $r = 0.58, n = 22, P = 0.004$. Newborn lambs at 1–5 days of age: $r = 0.49, n = 24, P = 0.014$. Adult sheep $r = 0.85, n = 18, P = 0.000009$. A nonsignificant slope (dashed lines), indicating no change for solute over the range of experimental osmolalities examined, represents the absence of brain volume regulation (i.e., ideal osmometer). A significant slope (solid lines) indicates that volume regulation was present in the medulla in each group.
premature lambs (r = 0.42, n = 24, P = 0.03), premature lambs (r = 0.75, n = 22, P = 0.00006), newborn lambs (r = 0.75, n = 24, P = 0.00003), and adult sheep (r = 0.46, n = 18, P = 0.05) indicate that volume regulation was present in the cerebellum. Similarly, the direct linear correlations with plasma osmolality were shown in the medulla (Fig. 5) of the fetuses at 60% of gestation (r = 0.42, n = 24, P = 0.04), premature lambs (r = 0.58, n = 22, P = 0.004), newborn lambs (r = 0.49, n = 24, P = 0.014), and adult sheep (r = 0.85, n = 18, P = 0.0001) indicate that volume regulation was also present in this brain region.

The actual water loss in the brain regions as a percentage of water loss expected under ideal osmotic behavior is summarized in Table 3. These values represent the actual percentage of water loss of that predicted on the basis of an ideal osmotic behavior in response to acute hyperosmolality. In this table, volume regulation is more effective when the actual water loss is as a percent of expected under ideal osmotic behavior is lower. Therefore, it appears that the volume regulation might be more effective in the adult than the immature sheep.

Sodium and potassium values in the brain regions did not demonstrate significant correlations with plasma osmolality (mosmol/kgH2O) in any of the age groups of the sheep (data not shown). The plasma sodium, potassium, and chloride concentrations in the glucose- and placebo-infused groups at 60 min of study are summarized in Table 4.

**DISCUSSION**

The purpose of our study was to examine the ability of the immature sheep brain to exhibit volume regulation in response to a glucose-induced acute hyperosmotic stress. We examined the effects of glucose on the regulation of brain volume because perturbations in systemic glucose homeostasis are common in immature subjects (9, 11, 17, 22, 31). In our laboratory’s earlier work, we used mannitol, a sugar that is not transported into or metabolized by the brain (25). The major findings of the present study were that volume regulation was present in the cerebrum, cerebellum, and medulla of the fetuses at 60% of gestation, premature lambs, newborn lambs, and adult sheep during glucose-induced hyperosmolality.

The design of our study was the same as in our laboratory’s previous work (25) to produce both a wide range of plasma osmolalities within each group and a steady-state increase in plasma osmolality within each subject for the duration of the study. This was achieved by a combination of a rapid injection of glucose plus NaCl followed by a continuous infusion. In the case of the fetuses at 60% of gestation, we found it necessary to administer the infusions to both the fetuses and ewes to achieve stable elevations in plasma osmolality over the 1-h study presumably because of fluid shifts among the fetus, placenta, amniotic fluid, fetal membranes, and ewes (2). Although there were differences among the curves for plasma osmolality (Fig. 1) in the fetuses at 60% of gestation and adult sheep compared with the newborn lambs, it is important to emphasize that volume regulation was examined separately within each group and each of the four groups of sheep exhibited effective volume regulation (Figs. 3–5, Table 3). The presence or absence of volume regulation was determined by whether all the points of total solute vs. plasma osmolality yield a slope of zero or statistically significant positive slope. Therefore, the differences among the stimulus, i.e., plasma osmolality curves (Fig. 1) and plasma glucose concentrations (Fig. 2), most likely did not affect the ability of each age group to exhibit volume regulation.

The capillary endothelium that forms the blood-brain barrier is impermeable to solutes but is permeable to water (3). Osmotic forces dominate water flux across the blood-brain barrier, and brain volume is determined by the osmolyte content of the tissue (3). In the adult rat during acute hyperosmolality, water loss from the brain is only about one-third of that predicted on the basis of ideal osmotic behavior revealing the presence of volume regulatory mechanisms (5). We have

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Actual Water Loss as a Percentage of Expected Water Loss Under Ideal Osmotic Behavior*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td></td>
</tr>
<tr>
<td>Fetuses at 60% of gestation</td>
<td>64</td>
</tr>
<tr>
<td>Preterm lambs at 90% gestation</td>
<td>74</td>
</tr>
<tr>
<td>Lambs at 1–5 days of age</td>
<td>88</td>
</tr>
<tr>
<td>Adult sheep at 4 yr of age</td>
<td>53</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
</tr>
<tr>
<td>Fetuses at 60% of gestation</td>
<td>71</td>
</tr>
<tr>
<td>Preterm lambs at 90% of gestation</td>
<td>70</td>
</tr>
<tr>
<td>Lambs at 1–5 days of age</td>
<td>75</td>
</tr>
<tr>
<td>Adult sheep at 4 yr of age</td>
<td>66</td>
</tr>
<tr>
<td>Medulla</td>
<td></td>
</tr>
<tr>
<td>Fetuses at 60% of gestation</td>
<td>82</td>
</tr>
<tr>
<td>Preterm lambs at 90% of gestation</td>
<td>77</td>
</tr>
<tr>
<td>Lambs at 1–5 days of age</td>
<td>82</td>
</tr>
<tr>
<td>Adult sheep at 4 yr of age</td>
<td>60</td>
</tr>
</tbody>
</table>

*Lower values indicate more efficient volume regulation.

Table 4. **Plasma electrolyte concentrations at 60 min of study by group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose Infused</th>
<th>Placebo Infused</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium</td>
<td>Potassium</td>
</tr>
<tr>
<td>Fetuses at 60% of gestation</td>
<td>152±3</td>
<td>3.86±0.12</td>
</tr>
<tr>
<td>Preterm lambs at 90%</td>
<td>151±3</td>
<td>3.43±0.13</td>
</tr>
<tr>
<td>of gestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lambs at 1–5 days of age</td>
<td>144±2</td>
<td>3.78±0.12</td>
</tr>
<tr>
<td>Adult sheep</td>
<td>135±4</td>
<td>3.52±0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE; given in meq/l. Glucose infused: fetuses at 60% of gestation, n = 17; preterm lambs at 90% of gestation, n = 14; lambs at 3–5 days of age, n = 19; adult sheep at 4 yr of age, n = 9. Placebo infused: fetuses at 60% of gestation, n = 7; preterm lambs at 90% of gestation, n = 8; lambs at 1–5 days of age, n = 6; adult sheep at 4 yr of age, n = 9. *P < 0.05 vs. glucose infused.
shown that, during acute mannitol-induced hyperosmolality in the adult sheep, water loss from the cerebral cortex is about three-quarters of that predicted on the basis of ideal osmotic behavior. Therefore, although the volume-regulatory response is present in the sheep cortex, it may be less effective than in the adult rat brain (25). In this study, during glucose-induced hyperosmolality, water loss from the cerebral cortex in the adult sheep was about one-half of that predicted on the basis of ideal osmotic behavior (Table 3). These findings suggest that the volume regulatory response in the cerebral cortex of adult sheep appeared more efficient during glucose- than mannitol-induced hyperosmolality and closer to that found in the adult rat (5).

The response of the immature brain to glucose-induced hyperosmolality is important in premature and newborn subjects because their smaller size and large surface area render them susceptible to dehydration and hyperosmolality (19). Furthermore, hyperglycemia is a common complication in premature and full-term infants (9, 11, 13, 17, 18). The ability of the brain to exhibit volume regulation had not been examined during development before our laboratory’s recent work (25). We have demonstrated that during mannitol-induced hyperosmolality, brain water loss is maximal and brain volume regulation impaired in most brain regions of fetuses at 60 and 90% of gestation and premature lambs; water loss is limited and volume regulation present in the brain regions of young lambs and adult sheep; and that the ability of the brain to exhibit volume regulation develops in a region and age-related fashion (25). In this and our former study (25), we determined the presence or absence of brain volume regulation by the presence of a significant slope indicating changes (solid lines in Figs. 3–5) or a nonsignificant slope indicating no change (dashed lines) in the quantity of the brain solute over the range of plasma osmolalities examined. This approach has the advantage that we do not have to correct for the higher water content in the immature animals (14, 25, 29) and examines the presence or absence of volume regulation separately within each age group. During glucose-induced hyperosmolality, volume regulation was present, because a significant increase in the amount of osmotically active solute indicated nonideal osmotic behavior in the cerebrum, cerebellum, and medulla of the fetuses at 60% of gestation, premature lambs, newborn lambs, and adult sheep. Although we cannot determine statistically whether volume regulation is more efficient in a particular age group during glucose-induced hyperosmolality, volume regulation might have been more efficient in the adult than immature sheep, because the actual water loss as a percentage of that expected under ideal osmotic behavior is lower in this group (Table 3). Consistent with our findings in sheep, Puri et al. (21) demonstrated that lateral ventricle volume decreased after glucose ingestion in adult humans and suggested that brain volume regulation was present in these subjects.

Although we cannot be certain of the reason that brain volume regulation was more efficient in the immature subjects (fetuses at 60% of gestation and premature lambs) during glucose- than mannitol-induced hyperosmolality, the range of plasma osmolalities were similar between the two studies (25). However, mannitol is not metabolized, whereas glucose is transported into the brain and metabolized (8). Therefore, we speculate that glucose transport into the brain and its metabolism to other organic solutes such as glycolytic intermediates increase the osmolyte concentration in brain tissue. This phenomenon enhances the volume regulatory response and limits water loss during a systemic glucose-induced osmotic stress.

The regulatory response was present at all stages of development and in all brain regions because each region exhibited significant increases in the amount of osmotically active brain solute over the range of osmolalities examined (Figs. 3–5) and the actual water loss as a percentage of the expected water loss under ideal osmotic behavior was similar among regions within each group (Table 3). The reason for the lack of differences in the volume regulatory responses among brain regions during glucose-induced hyperosmolality in contrast to our laboratory’s previous work (25) was most likely because the glucose-related increases in tissue osmolyte content was similar among regions.

In contrast to our laboratory’s findings with mannitol (25), the premature lambs exhibited effective volume regulation in all brain regions. We had attempted to enroll fetuses at 90% of gestation similar to our laboratory’s former study (25), but we found that the fetuses at this gestational age were not able to tolerate the glucose infusions and died after onset of the infusions. The reason that we were able to complete the studies in the fetuses at 60 and not 90% of gestation is not certain. However, maternal hyperglycemia has been shown to decrease umbilical blood flow to the placenta and result in fetal acidemia in late gestation fetal sheep (4). We speculate that the tolerance of the early-gestation fetuses to the glucose infusions may be because the placental vasculature might exhibit less vasoconstriction to hyperglycemia at this early time in gestation.

In the adult rat, brain volume is regulated during acute hyperosmolar states, in part on the basis of tissue electrolyte gain (6, 7). In our laboratory’s previous work in sheep (25), brain volume regulation could not be accounted for on the basis of tissue electrolyte gain. Similarly, in this study, electrolyte gain into the brain did not account for brain volume regulation. Inorganic ions do not account for the entire volume regulatory response of the brain during hyperosmotic stress. Other solutes such as amino acids, methyl amines, and polyols also serve to offset water loss from the brain (12, 16, 28). In adult rats, free amino acids, including taurine and glutamate, have been shown to increase after chronic exposure to hyperglycemic hyperosmolality (1). In young rats, taurine appears to be an important organic osmolyte component of the volume regulatory response (29). However, we cannot comment on the relative roles that these organic osmoles might have played in the volume regulatory response in our sheep, because they were not measured in our study. Nonetheless, we speculate that glycolytic intermediates and other organic osmoles such as amino acids contributed to brain volume regulatory response during hyperglycemic hyperosmolality in our sheep (1, 29).

In summary, during acute glucose-induced hyperosmolality, the brain shrinks less than predicted on the basis of an ideal osmometer and exhibits volume regulation in fetuses at 60% of gestation, premature lambs, newborn lambs, and adult sheep.

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REFERENCES


