Comparison of the Effects of Hypertonic Glycerol and Urea on Brain Edema, Energy Metabolism and Blood Flow Following Cerebral Microembolism in the Rat. Deleterious Effect of Glycerol Treatment

JEAN BRALET, PH.D., PAULETTE BELEY, M.SC., ANNE-MARIE BRALET, PH.D., AND ALAIN BELEY, PH.D.

SUMMARY

Cerebral microembolism was performed in rats by injecting radioactive calibrated 50 μm microspheres into the left internal carotid artery. The use of radioactive microspheres as embolic agents enabled the number of microspheres to be determined in each cerebral hemisphere. Edema was assessed 24 h after embolization by measuring brain water, sodium, and potassium content. Equimolar doses (40 mmol/kg) of glycerol or urea were injected i.p. at various times before sacrifice. Both treatments caused similar changes in water and electrolyte content, brain dehydration being maximal 30 min after urea and 2 h after glycerol injection. Cerebral energy metabolism and regional blood flow were evaluated at the times of maximal brain dehydration. Urea treatment resulted in an improvement of the cerebral circulation whereas glycerol treatment led to a deterioration of cerebral blood flow which cannot be explained by failure to reduce edema and the consequent microcirculatory impairment. Urea treatment had no marked effect on cerebral energy metabolism whereas glycerol injection resulted in an important increase in brain lactate level which may be relevant to the impairment of cerebral reperfusion. These results point out that administration of a metabolized solute like glycerol may exert deleterious effects on the ischemic brain.

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Regional cerebral blood flow and brain energy metabolism were evaluated at the times of maximal brain dehydration.

Methods

The experiments were performed on male Sprague-Dawley rats (Ifna Credo) weighing 280–320 g.

Production and Evaluation of Embolization

Cerebral microembolism was produced by injecting 4,000 carbonized microspheres (50 μm diameter, labelled with 85Sr, suspended in 20% polyvinylpyrrolidone) into the left internal carotid artery as previously described. The mean radioactivity present in one microsphere was determined by simultaneous counting of the microsphere suspension in a hemocytometer and a scintillation crystal well counter in order to calculate the number of microspheres contained in the cerebral hemispheres.

Brain Edema and BBB Permeability

Brain edema was evaluated by the content of water, sodium and potassium. The rats were killed by decapitation. The brain was removed, the cerebellum and the brain stem were discarded. Brain water (g H2O/100 g of wet weight) was determined in each hemisphere after drying at 95°C to a constant weight. Sodium and potassium determinations were made in the dry hemisphere using flame photometer. The values were calculated per kg wet weight and per kg dry weight to eliminate change due to dilution.

BBB permeability was studied by estimation of the transfer of 125I-albumin from blood into brain. Rats received i.v. injection of 5 μCi of 125I-albumin (Centre National de Transfusion Sanguine, Paris) immediately after embolization. After decapitation, the 125I radioac-
tivity in plasma and cerebral hemispheres was measured and the uptake of $^{125}$I-albumin was calculated by the ratio:

$$\frac{^{125}\text{I cpm/g cerebral tissue}}{^{125}\text{I cpm/g plasma}} \times 100$$

**Brain Energy Metabolism and Cerebral Blood Flow**

The rats were initially anesthetized by inhalation of ether and tracheotomized. Polyethylene catheters were inserted in the femoral arteries for continuous blood pressure recording and anaerobic blood sampling. Thereafter the animals were paralyzed with tubocurarine chloride (1 mg/kg, i.v.) and connected to a respirator which delivered 70% N2O and 30% O2. Arterial P02, PCO2, and pH were measured using direct reading electrodes. Body temperature was kept close to 37°C by external heating.

For brain metabolite measurements, a skin incision was made to fit a plastic funnel over the skull bone for freezing the brain in situ by pouring liquid nitrogen into the plastic funnel during 3 min. Thereafter the animal was submersed in liquid nitrogen for another 5 min. The brain was then chiselled out in the frozen state, the hemispheres were separated in the midline and prepared for analysis in a refrigerated box (−25°C) according to the technique of Folbergrova et al. The frozen hemispheres were weighed, homogenized in methanol-HCl at −25°C and extracted twice with 0.3 N HCl, at 0°C. The perchloric acid extracts were centrifuged and the neutralized supernatants were assayed for their metabolite contents by the enzymatic methods of Lowry and Passoneau. The centrifugation methods of Lowry and Passoneau. The centrifugation methods of Lowry and Passoneau.

Regional cerebral blood flow was measured by the tissue sampling technique using $^{14}$C-iodoantipyrine as indicator. The tracer (iodoantipyrine, 4-N-methyl-$^{14}$C, New England Nuclear, specific activity 53.4 Ci/mmole) was dissolved in isotonic saline and 5 μCi were infused via the femoral vein over a period of 30 seconds at a rate of 0.6 ml/min. During the infusion, arterial blood samples (50 μl) were collected in glass capillaries every third second. Immediately after the end of the infusion period, the rats were decapitated and the cerebral hemispheres were dissected into 5 parts: corpus striatum, frontal cortex, parieto-temporal cortex, occipital cortex and rest of the hemisphere. Brain samples were dissolved with 1 ml Soluene (Packard) and blood samples were solubilized by means of 1 ml Soluene/isopropanol (1:1) and decolorized with 0.2 ml 110 vol. H2O2. Ten ml Instagel (Packard) / 0.5 N HCl (9:1) were then added and the $^{14}$C radioactivity was measured in a liquid scintillation spectrometer. Flow was calculated using a partition coefficient of 0.8.

**Glycerol and Urea Treatments**

The rats received a single i.p. injection of a 2M solution of urea or glycerol in isotonic saline. The volume injected, 2 ml/100 g of body weight, was equivalent, for the two substances, to a dose of 40 mmol/kg (3.68 g/kg of glycerol and 2.40 g/kg of urea). The hypertonic solutions were injected to non-embolized or embolized rats at selected times before sacrifice (0.5, 1 or 2 h after urea and 1, 2, 4 or 6 h after glycerol injection). The embolized animals were always sacrificed 24 h after embolization. Control rats received an equivalent volume of saline. Plasma osmolality was measured with an osmometer on samples obtained 0.5 and 2 h after glycerol or urea injection.

Means were determined with their standard errors and the Student’s t test was used for statistical comparison.

**Results**

**Plasma**

Plasma osmolality (mOsm/l) was found equal to 301 ± 4 in control rats (n = 5). Thirty minutes after the injection of hypertonic solutions, it reached 346 ± 7 for urea (n = 6) and 335 ± 6 for glycerol (n = 5). Two hours after the injection, the values were 326 ± 5 for urea and 330 ± 4 for glycerol. The differences between the two treatments were not significant.

By gross inspection of plasma, there was no apparent hemolysis 0.5 and 2 h after urea injection. After glycerol, the plasma samples showed a marked hemolysis at time 0.5 h and only a slight hemolysis at time 2 h.

**Brain Water and Electrolytes**

The effect of hypertonic solutions on brain water, sodium and potassium was evaluated in the cerebral hemispheres of intact and embolized rats which were sacrificed at selected times after glycerol (1, 2, 4 or 6 h) or urea (0.5, 1 or 2 h) injection.

**Intact Rats**

In intact animals (table 1), both treatments caused significant decreases in brain water concentration. Maximal decrease was seen 2 h after glycerol (−0.76%) and 1 h after urea (−0.84%) injection. Brain water returned to the original value 4 h after glycerol and 2 h after urea injection. The loss of water resulted in significant increments in concentrations of sodium (expressed on wet weight basis) which were maximal 2 h after glycerol and 1 h after urea injection. At these times, the sodium contents, expressed on dry weight basis, were significantly increased by 7% for glycerol and 6% for urea. In glycerol-treated animals, the sodium content had returned to the original value 4 h after injection and was then reduced (−5%) 6 h after injection.

**Embolized Rats**

All the embolized animals were sacrificed 24 h after embolization. The degree of embolization through the number of microspheres in the hemispheres was sys-
EFFECTS OF HYPTERTONIC GLYCEROL AND UREA

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TABLE 1  Effect of Glycerol and Urea on Water, Sodium and Potassium Content of Cerebral Hemispheres in Intact Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H₂O %</th>
<th>Sodium mEq/kg</th>
<th>Potassium mEq/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>78.99 ± 0.03</td>
<td>48.01 ± 0.40</td>
<td>228 ± 2</td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h (n = 6)</td>
<td>78.89 ± 0.29</td>
<td>51.82 ± 0.76†</td>
<td>247 ± 2‡</td>
</tr>
<tr>
<td>2 h (n = 6)</td>
<td>78.23 ± 0.18‡</td>
<td>53.23 ± 0.55‡</td>
<td>244 ± 3†</td>
</tr>
<tr>
<td>4 h (n = 6)</td>
<td>78.85 ± 0.14</td>
<td>44.94 ± 0.64†</td>
<td>224 ± 3</td>
</tr>
<tr>
<td>6 h (n = 6)</td>
<td>79.13 ± 0.08</td>
<td>44.96 ± 0.59†</td>
<td>216 ± 3†</td>
</tr>
<tr>
<td>Urea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 h (n = 6)</td>
<td>78.59 ± 0.15*</td>
<td>49.52 ± 0.68</td>
<td>232 ± 3</td>
</tr>
<tr>
<td>1 h (n = 6)</td>
<td>78.15 ± 0.07‡</td>
<td>52.46 ± 0.33‡</td>
<td>241 ± 2‡</td>
</tr>
<tr>
<td>2 h (n = 6)</td>
<td>78.81 ± 0.15</td>
<td>50.15 ± 0.71*</td>
<td>236 ± 2*</td>
</tr>
</tbody>
</table>

Glycerol (3.68 g/kg, i.p.) was injected 1, 2, 4 or 6 h and urea (2.40 g/kg, i.p.) 0.5, 1 or 2 h before sacrifice.

*p < 0.05, †p < 0.01, ‡p < 0.001 between control and treated rats.

n = number of animals. Values are means ± standard error.

TABLE 2  Effect of Glycerol and Urea on Water Content of Cerebral Hemispheres in Embolized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of microspheres</th>
<th>H₂O %</th>
<th>Number of microspheres</th>
<th>H₂O %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>609 ± 28</td>
<td>82.58 ± 0.32</td>
<td>99 ± 22</td>
<td>79.31 ± 0.06</td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h (n = 9)</td>
<td>629 ± 60</td>
<td>80.82 ± 0.39†</td>
<td>87 ± 18</td>
<td>78.18 ± 0.31†</td>
</tr>
<tr>
<td>2 h (n = 10)</td>
<td>628 ± 36</td>
<td>80.22 ± 0.29‡</td>
<td>104 ± 15</td>
<td>77.46 ± 0.19‡</td>
</tr>
<tr>
<td>4 h (n = 10)</td>
<td>556 ± 28</td>
<td>81.50 ± 0.23*</td>
<td>90 ± 17</td>
<td>79.10 ± 0.19</td>
</tr>
<tr>
<td>6 h (n = 9)</td>
<td>572 ± 38</td>
<td>82.11 ± 0.35</td>
<td>88 ± 11</td>
<td>79.59 ± 0.14</td>
</tr>
<tr>
<td>Urea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 h (n = 8)</td>
<td>594 ± 50</td>
<td>80.11 ± 0.41‡</td>
<td>91 ± 23</td>
<td>77.75 ± 0.23‡</td>
</tr>
<tr>
<td>1 h (n = 8)</td>
<td>594 ± 45</td>
<td>80.70 ± 0.30‡</td>
<td>116 ± 28</td>
<td>77.99 ± 0.14‡</td>
</tr>
<tr>
<td>2 h (n = 8)</td>
<td>617 ± 72</td>
<td>82.54 ± 0.36</td>
<td>113 ± 24</td>
<td>79.68 ± 0.21</td>
</tr>
</tbody>
</table>

Embolicization was performed by injecting 4000 microspheres into the left internal carotid artery and the animals were sacrificed 24 h later. Glycerol (3.68 g/kg, i.p.) was injected 1, 2, 4 or 6 h and urea (2.40 g/kg, i.p.) 0.5, 1 or 2 h before sacrifice.

*p < 0.05, †p < 0.01, ‡p < 0.001 between control and treated rats.

n = number of animals. Values are means ± standard error.
Cerebral Blood Flow

Table 5 gives the physiological parameters of the animals subjected to the measurement of cerebral blood flow. There were no consistent differences in the parameters between the experimental groups. Twenty-four hours after embolization, the blood flow was significantly reduced in both hemispheres (table 6), the magnitude of decrease being most pronounced in the left hemisphere. In this hemisphere, blood flow was decreased by about 70% in the corpus striatum and the cortical regions and by 51% in the rest of the hemisphere. In the right hemisphere, it was decreased by 27% in the corpus striatum, 30% in the occipital cortex, 37% in the rest of the hemisphere, 48% in the parieto-temporal cortex and 58% in the frontal cortex.

The effect of hypertonic solutions on regional cerebral blood flow of embolized rats was evaluated at the times of maximal brain dehydration i.e., 2 h after glycerol and 0.5 h after urea injection. Following glycerol treatment, there was a further decline of flow in both hemispheres. In the left hemisphere, the decreases reached about 30% in the corpus striatum, the frontal and the parieto-temporal cortex (not significant) and 43% in the occipital cortex ($p < 0.05$). In the right hemisphere, significant decreases were seen in the corpus striatum ($-40\%$), the parieto-temporal ($-47\%$) and the occipital cortex ($-55\%$). By contrast, urea treatment caused a spectacular improvement of the brain circulation. In the left hemisphere, blood flow was significantly increased in all the brain regions ($+41\%$ in the frontal cortex, $+77\%$ in the parieto-temporal cortex, $+86\%$ in the corpus striatum, $+123\%$ in the occipital cortex and $+65\%$ in the rest of the hemisphere). In the right hemisphere, blood flow returned to normal values which did not differ significantly from those which were seen in non-embolized rats (table 6).

Brain Energy Metabolism

In the animals subjected to brain energy metabolism determination, there were no significant differences in any of the physiological parameters ($pH$, $PaO_2$, $PaCO_2$, body temperature, mean arterial blood pressure) between the experimental groups (results not shown).

Embolization caused marked changes in cerebral energy metabolites (table 7). The left hemisphere showed decreases of phosphocreatine ($-24\%$), ATP ($-41\%$), ADP ($-27\%$), glucose ($-32\%$) and increases of pyruvate ($+89\%$) and lactate ($+222\%$). The right hemisphere was less affected showing only decreases of ATP ($-10\%$) and ADP ($-23\%$) and increase of lactate ($+72\%$).

Following glycerol treatment (table 7), lactate level increased from 6.92 to 15.19 mmol/kg in the left hemisphere and to 3.70 to 7.91 mmol/kg in the right hemisphere. Glucose level increased significantly only in the left hemisphere (from 2.78 to 4.38 mmol/kg). These increases in metabolite levels were not due to dehydration of the brain by glycerol. The urea-treated
The importance of edema which developed in the left hemisphere.

The value of hypertonic solutions as brain dehydrating agents is predicated on the assumption that the brain is relatively impermeable to the solutes. An osmotic gradient between brain and blood must be produced to result in a water shift. Consequently, the integrity of the BBB may be an important factor in the activity. Our results show that brain dehydration was of the same importance after injection of equiosmolar doses of glycerol and urea but that the time of maximal dehydration and the time required for return to control level were shorter after administration of urea than after glycerol. In spite of an alteration of BBB permeability in embolized rats, reduction in brain water concentration was more important than in intact rats. At the times of maximal dehydration, the fall in water concentration averaged 0.8% in intact rats whereas it reached 2.4% in the left hemisphere and 1.8% in the right hemisphere of embolized rats. In intact animals, as previously reported, the brain responded to the osmotic load by a removal of electrolyte-free water as evidenced by increases in sodium and potassium concentrations. In the left hemisphere of embolized rats, potassium level increased whereas sodium level decreased abruptly following injection of the hypertonic solutions. Thus the water osmotically removed must have been accompanied by some sodium, the sodium content being reduced by about 20%.

These data differ from those reported on the cryo-

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### Table 4: Effect of Glycerol and Urea on Potassium Content of Cerebral Hemispheres in Embolized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wet weight</th>
<th>Potassium mEq/kg</th>
<th>Dry weight</th>
<th>Potassium mEq/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>60.35 ± 3.05</td>
<td>356 ± 12</td>
<td>91.39 ± 1.34</td>
<td>446 ± 5</td>
</tr>
<tr>
<td>Glycerol 1 h (n = 9)</td>
<td>78.84 ± 3.12‡</td>
<td>408 ± 8†</td>
<td>101.21 ± 1.78‡</td>
<td>453 ± 4</td>
</tr>
<tr>
<td>Glycerol 2 h (n = 10)</td>
<td>72.99 ± 3.10*</td>
<td>367 ± 11</td>
<td>99.46 ± 1.33‡</td>
<td>449 ± 6</td>
</tr>
<tr>
<td>Glycerol 4 h (n = 10)</td>
<td>70.18 ± 2.36*</td>
<td>387 ± 9*</td>
<td>89.42 ± 1.00</td>
<td>434 ± 4</td>
</tr>
<tr>
<td>Glycerol 6 h (n = 9)</td>
<td>72.26 ± 2.83*</td>
<td>410 ± 10*</td>
<td>90.55 ± 0.98</td>
<td>438 ± 6</td>
</tr>
<tr>
<td>Urea 0.5 h (n = 8)</td>
<td>81.36 ± 4.12‡</td>
<td>405 ± 17*</td>
<td>97.08 ± 1.74*</td>
<td>450 ± 16</td>
</tr>
<tr>
<td>Urea 1 h (n = 8)</td>
<td>74.36 ± 3.39†</td>
<td>383 ± 11</td>
<td>94.47 ± 1.38</td>
<td>427 ± 8</td>
</tr>
<tr>
<td>Urea 2 h (n = 8)</td>
<td>67.93 ± 4.55</td>
<td>386 ± 17</td>
<td>89.84 ± 1.99</td>
<td>426 ± 9</td>
</tr>
</tbody>
</table>

The animals were sacrificed 24 h after embolization (same animals as in table 2). Glycerol (3.68 g/kg, i.p.) was injected 1, 2, 4 or 6 h and urea (2.40 g/kg, i.p.) 0.5, 1 or 2 h before sacrifice.

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### Table 5: Physiological Parameters in Animals Subjected to Measurement of Regional Cerebral Blood Flow

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body temp. (°C)</th>
<th>MABP (mm Hg)</th>
<th>Pa CO₂ (mm Hg)</th>
<th>Pa O₂ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-embolized (n = 7)</td>
<td>37.2 ± 0.2</td>
<td>113 ± 4</td>
<td>38.1 ± 0.7</td>
<td>130 ± 5</td>
<td>7.43 ± 0.01</td>
</tr>
<tr>
<td>Embolized (n = 7)</td>
<td>36.9 ± 0.3</td>
<td>110 ± 4</td>
<td>38.4 ± 1.6</td>
<td>148 ± 6</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td>Embolized + glycerol (n = 7)</td>
<td>37.6 ± 0.3</td>
<td>106 ± 4</td>
<td>38.9 ± 2.2</td>
<td>145 ± 6</td>
<td>7.35 ± 0.02</td>
</tr>
<tr>
<td>Embolized + urea (n = 7)</td>
<td>37.7 ± 0.2</td>
<td>111 ± 7</td>
<td>37.4 ± 1.2</td>
<td>127 ± 8</td>
<td>7.36 ± 0.01</td>
</tr>
</tbody>
</table>

n = number of animals. Values are means ± standard error.
Effects of Embolization and of Treatments with Glycerol and Urea on Brain Energy Metabolism

### Table 6 Effects of Embolization and of Treatments with Glycerol and Urea on Regional Cerebral Blood Flow (ml/100g/min)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Corpus striatum</th>
<th>Frontal cortex</th>
<th>Parieto-temporal cortex</th>
<th>Occipital cortex</th>
<th>Rest of the hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-embolized (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>left side</td>
<td>109.8±11.8</td>
<td>144.3±14.7</td>
<td>139.2±14.8</td>
<td>123.6±13.7</td>
<td>94.3±8.8</td>
</tr>
<tr>
<td>right side</td>
<td>107.4±11.4</td>
<td>141.9±16.2</td>
<td>134.5±16.4</td>
<td>108.7±11.7</td>
<td>99.2±8.0</td>
</tr>
<tr>
<td>Embolized (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>left side</td>
<td>29.9±6.5a†</td>
<td>45.7±8.6a‡</td>
<td>37.3±5.2a‡</td>
<td>38.2±6.6a‡</td>
<td>46.3±4.2a‡</td>
</tr>
<tr>
<td>right side</td>
<td>78.8±5.0a*</td>
<td>59.9±14.3a†</td>
<td>69.8±3.5a†</td>
<td>75.8±3.1a*</td>
<td>62.3±7.0a†</td>
</tr>
<tr>
<td>Embolized + glycerol (n = 7)</td>
<td>55.6±9.6b*</td>
<td>64.5±12.5b*</td>
<td>65.9±10.1b*</td>
<td>85.4±16.2b*</td>
<td>76.6±9.6b*</td>
</tr>
<tr>
<td>Embolized + urea (n = 7)</td>
<td>112.6±13.8b*</td>
<td>112.2±18.4b*</td>
<td>124.9±16.4b*</td>
<td>120.0±17.0b*</td>
<td>97.3±11.7b*</td>
</tr>
</tbody>
</table>

The embolized animals were sacrificed 24 h after microsphere injection. Glycerol (3.68 g/kg, i.p.) was injected 2 h and urea (2.40 g/kg, i.p.) 0.5 h before sacrifice.

a: comparison between non-embolized and embolized rats.
b: comparison between embolized and treated embolized rats.

### Table 7 Effects of Embolization and of Treatments with Glycerol and Urea on Brain Energy Metabolism

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Non-embolized</th>
<th>Embolized (n = 7)</th>
<th>Embolized + glycerol (n = 7)</th>
<th>Embolized + urea (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>left + right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>hemispheres</td>
<td>hemisphere</td>
<td>hemisphere</td>
<td>hemisphere</td>
</tr>
<tr>
<td>PCr</td>
<td>4.06±0.22</td>
<td>3.09±0.15a†</td>
<td>4.09±0.08</td>
<td>3.37±0.32</td>
</tr>
<tr>
<td>ATP</td>
<td>2.73±0.07</td>
<td>1.62±0.07a‡</td>
<td>2.45±0.06a§</td>
<td>1.67±0.15</td>
</tr>
<tr>
<td>ADP</td>
<td>0.319±0.028</td>
<td>0.233±0.023a§</td>
<td>0.246±0.014a§</td>
<td>0.335±0.048</td>
</tr>
<tr>
<td>AMP</td>
<td>0.033±0.004</td>
<td>0.032±0.002</td>
<td>0.026±0.003</td>
<td>0.028±0.005</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.08±0.22</td>
<td>2.78±0.30a†</td>
<td>4.27±0.35</td>
<td>4.38±0.47b*</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.15±0.18</td>
<td>6.92±0.55a‡</td>
<td>3.70±0.70a*</td>
<td>15.19±1.78b‡</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.162±0.013</td>
<td>0.306±0.036a†</td>
<td>0.191±0.026</td>
<td>0.361±0.037</td>
</tr>
</tbody>
</table>

The embolized animals were sacrificed 24 h after microsphere injection. Number of microspheres in the left hemisphere: 536 ± 60 in the untreated group, 513 ± 53 in the group receiving glycerol and 546 ± 66 in the group receiving urea. The same values for the right hemisphere were: 139 ± 48, 139 ± 40 and 133 ± 20. Glycerol (3.68 g/kg, i.p.) was injected 2 h and urea (2.40 g/kg, i.p.) 0.5 h before sacrifice. Values are means ± standard error. n = number of animals.
a: comparison between non-embolized and embolized rats.
b: comparison between embolized and treated embolized rats.

*p < 0.05, †p < 0.01, ‡p < 0.001.
bral circulation by reducing intracranial pressure. The present results indicate that despite a similar brain dehydration after glycerol or urea injection, the consequence on cerebral blood flow was completely different. Whereas urea treatment resulted in an improvement of the cerebral circulation, glycerol treatment led to a deterioration of cerebral flow which cannot be explained by failure to reduce edema and the consequent microcirculatory impairment. In our experimental conditions, glycerol injection caused hemolysis which might lead to microvascular obstruction and partly account for the flow reduction which is observed after glycerol treatment.

Beneficial effects of hypertonic urea have been previously reported. In rats subjected to anoxic-ischemic encephalopathy, urea injection (2 g/kg) led to a decrease in the mortality and to a reduction of the severity of hemiplegia in survivors. After occlusion of the middle cerebral artery in cats, Sundt et al. found a significant reduction in the size of the infarcted areas in animals treated with urea (1 g/kg, 6 h after occlusion). Beneficial effects of glycerol have been found following transient ischemia. In the monkey subjected to temporary occlusion of the middle cerebral artery (30 min to 4 h), injection of glycerol (0.08–0.8 g/kg) immediately after the occlusion led to a reduction of the ultrastructural cerebral alterations. Following temporary ischemia induced in baboons by occluding the carotid and vertebral arteries bilaterally, glycerol injection (1 g/kg) was found to be effective in reducing intracranial pressure and increasing cerebral blood flow. In patients with cerebral infarction, numerous studies have evidenced the effectiveness of glycerol in reducing cerebral edema and increasing cerebral blood flow. Other studies failed to observe any beneficial effects of glycerol, especially in patients with severe disability.

It has been suggested that glycerol achieved its beneficial effects through its direct action as a dehydrating agent as well as through its metabolic effects. In patients with recent cerebral infarction, glycerol infusion led to a decrease in cerebral oxygen consumption and carbon dioxide production whereas glucose consumption, pyruvate and lactate production by brain were found to be unchanged. It was hypothesized that glycerol may cause a reversal of uncoupled oxidative phosphorylation.

Brain can utilize glycerol as a metabolic substrate but, in intact animals, it appears that the rate-limiting step in cerebral utilization would be the penetration into the brain. However, the entry of glycerol into brain may be enhanced in case of an alteration of the BBB. Present results show that the main metabolic effect of glycerol in embolized rats consisted in important increase in brain lactate level. Thurston et al. found that injection of glycerol in mice (2 g/kg) increased the brain levels of glucose, lactate, glycolytic and citric acid cycle intermediates, suggesting that glycerol may have increased the metabolic rate for glucose. The enhanced brain glucose concentration observed after glycerol may be the result of de novo glucose synthesis from glycerol. The limiting enzymes of gluconeogenesis have been detected in brain but they are present at low rates of activity. The increased brain glucose content may be also attributed to an enhanced penetration of glucose from the blood. Important increase in brain lactate (145%) was observed in intact rats following a high dose of glycerol (4 i.p. injections of 7.36 g/kg at 15 min intervals) whereas an equivalent dose of mannitol increased brain lactate by only 40%. Glycerol had also striking effects on brain amino acids metabolism that were not observed with mannitol.

Recent studies have shown that nutritional state of animals subjected to reversible brain ischemia influenced the neurological recovery as well as cerebral blood flow and metabolism. Increase in cerebral metabolism by administration of glucose to animals resulted in excess amounts of brain lactate and markedly altered the capacity for recovery. In this way, the high brain lactate level that was observed after glycerol injection in embolized rats may be relevant to the impairment of cerebral reperfusion.

Although the high dose of glycerol (3.68 g/kg) used in the present work limits its clinical implication, these results point out that administration of a metabolized solute like glycerol may exert deleterious effects on the ischemic brain. In this connection, a number of papers have described the beneficial effects of hypertonic mannitol, a metabolically inert solute, in the treatment of brain ischemia.

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