Beneficial Effect of 1,3-Butanediol on Cerebral Energy Metabolism and Edema Following Brain Embolization in Rats

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We assessed the effect of 1,3-butanediol on cerebral energy metabolism and edema after inducing multifocal brain infarcts in 108 rats by the intracarotid injection of 50-µm carbonized microspheres. An ethanol dimer that induces systemic ketosis, 25 mmol/kg i.p. butanediol was injected every 3 hours to produce a sustained increase in the plasma level of β-hydroxybutyrate. Treatment significantly attenuated ischemia-induced metabolic changes by increasing the concentrations of phosphocreatine, adenosine triphosphate, and glycogen and by reducing the concentrations of pyruvate and lactate. Lactate concentration 2, 6, and 12 hours after embolization decreased by 13%, 44%, and 46%, respectively. Brain water content increased from 78.63% in six unembolized rats to 80.93% in 12 saline-treated and 79.57% in seven butanediol-treated rats 12 hours after embolization. (p<0.05). The decrease in water content was associated with significant decreases in the concentrations of sodium and chloride. The antiedema effect of butanediol could not be explained by an osmotic mechanism since equimolar doses of urea or ethanol were ineffective. Our results support the hypothesis that the beneficial effect of butanediol is mediated through cerebral utilization of ketone bodies arising from butanediol metabolism, reducing the rate of glycolysis and the deleterious accumulation of lactic acid during ischemia. (Stroke 1990;21:1458-1463)

Previous studies have shown that treatment with 1,3-butanediol, an ethanol dimer (CH₃-CHOH-CH₂CH₂OH), has beneficial effects in models of hypoxia and ischemia. Butanediol prolongs the survival time in mice exposed to 4-5% O₂; it extends the time to isoelectric electroencephalogram and reduces the neurologic deficit in ischemic-hypoxic Levine rats²; and it attenuates neurologic deficit, histologic damage, and cerebral metabolic alterations induced by four-vessel occlusion in rats.³,⁴ Butanediol is converted in the body to β-hydroxybutyrate,⁶ and it has been proposed¹ that damage is attenuated through a cerebral protective effect mediated by ketone metabolism.

We examined the effect of butanediol in a rat model of multifocal brain infarction produced by the intracarotid injection of calibrated microspheres.⁷ We studied the effect of butanediol over 12 hours by measuring cerebral energy metabolism and edema. In addition, to test whether a direct effect or an osmotic effect contributed to the beneficial action of butanediol, we compared its effect with those of ethanol and urea administered at equimolar doses.

Materials and Methods

We performed experiments on 154 male Sprague-Dawley rats weighing 280–320 g that were allowed free access to water and food.

Cerebral microembolism was produced in 108 rats under ether anesthesia by injecting approximately 4,000 carbonized microspheres 50 µm in diameter labeled with strontium-85 and suspended in 20% polyvinylpyrrolidone (3M, Minneapolis) into the left internal carotid artery as previously described.⁷ Simultaneous counting of the microsphere suspension in a hemocytometer and a scintillation crystal well counter allowed us to calculate the number of microspheres contained in the left cerebral hemisphere.

For the measurement of cerebral energy metabolite concentrations, 90 rats were anesthetized by inhaling ether and tracheotomized. Polyethylene catheters were inserted into the femoral arteries for continuous recording of blood pressure and anacrobic sampling of blood. An incision was made in the scalp to fit a plastic funnel over the skull for later in situ freezing of the brain.⁸ The administration of ether was discontinued after the end of the surgery.
and the rats were paralyzed with 1 mg/kg i.v. d-
tubocurarine hydrochloride and connected to a res-
pirator that delivered 70% N₂ and 30% O₂. Pao₂,
Paco₂, and arterial pH were measured in 100-μl
blood samples using direct-reading electrodes. Body
temperature was kept close to 37°C by external
heating. The rats were kept in a respiratory steady
state for at least 15 minutes, and the brains were
frozen in situ by pouring liquid nitrogen into the
funnel for 3 minutes. Thereafter, the rats were
submerged in liquid nitrogen for another 5 minutes.
The brains were chiseled out of the skulls in the
frozen state during intermittent irrigation with liquid
nitrogen, and the left cerebral hemispheres were
separated and stored in a liquid nitrogen freezer for
subsequent metabolite assay.

The frozen hemispheres were weighed, homoge-


einized in methanol-HCl at -25°C, and extracted
twice with 0.3N perchloric acid at 0°C.9 The perchlo-
ric extracts were centrifuged at 0°C, and the neutral-
ized supernatants were assayed for phosphocreatine
(PCr), adenosine triphosphate (ATP), glucose, pyru-
vate, and lactate using enzymatic methods.10 The
centrifugation pellets, which contained the radioac-
tive microspheres, were subjected to scintillation
counting. The pellets were then prepared and
assayed for glycogen.11

For the evaluation of cerebral edema, 18 nonem-
bolized and 34 embolized rats were decapitated, the
left cerebral hemispheres were removed from the
skulls, and the radioactivity was measured. Brain
water content (grams water per 100 g wet weight) was
determined after drying at 95°C to a constant weight.
Sodium and potassium concentrations were mea-
sured using a flame photometer, and chloride con-
centration was determined potentiometrically.
The values were calculated as milliequivalents per kilo-
gram dry weight.

Plasma concentrations of glucose and β-hydroxy-
butyrate were determined in 12 awake nonembolized
rats. Blood from the tail artery was immediately
centrifuged at 0°C. The plasma was deproteinized by
adding 35% perchloric acid (weight/volume), and the
supernatant was used for the enzymatic measure-
ment of the concentrations of glucose10 and D-(-)
β-hydroxybutyrate.12

Butanediol (Prolabo, Paris), urea, or ethanol (Pro-
labo) were dissolved in distilled water and given
intraperitoneally (25 mmol/kg, 0.9 ml/100 g). Control
rats received equivalent volumes of saline. The rats
were divided into five groups. Group 0 was used to
determine plasma concentrations of glucose and β-
hydroxybutyrate in nonembolized rats. They received
either no treatment (n=6) or four injections of
butanediol (n=6) but were not decapitated. Group 1
was used to determine the effect of butanediol on
cerebral energy metabolism in nonembolized rats.
They were decapitated 0.5 hour after the injection of
saline (n=8) or butanediol (n=8). Group 2 rats were
decapitated 2 hours after embolization and used for
cerebral energy metabolite determination. They
received one injection of saline (n=8) or butanediol
(n=8) 0.5 hour before embolization. Group 3 rats
were decapitated 6 hours after embolization and
used for cerebral energy metabolite determination.
They received two injections of saline (n=9) or
butanediol (n=9), the first injection 0.5 hour before
and the second 2.5 hours after embolization. Group 4
rats were decapitated 12 hours after embolization.
They received four injections of saline, butanediol,
urea, or ethanol 0.5 hour before and 2.5, 5.5, and 8.5
hours after embolization and were used for either
cerebral energy metabolite determination (saline:
n=20, butanediol: n=1, urea: n=9) or evaluation of
edema (saline: n=12, butanediol: n=7, urea: n=9,
ethanol: n=6).

The means and their standard errors were deter-
mined and compared by using one-way analysis of
variance followed by the Newman-Keuls test. The
level of significance was set at p<0.05.

Results

The effect of butanediol treatment on plasma
levels of β-hydroxybutyrate and glucose is shown in
Table 1. Repeated injections of 25 mmol/kg butane-
diol every 3 hours led to a significant and sustained
increase in the plasma level of β-hydroxybutyrate,
which was associated with a sustained 20% decrease
in the plasma level of glucose.

Mean arterial blood pressure, body temperature,
Pao₂, and Paco₂ were not significantly modified by
treatment with butanediol or urea (data not shown).
The only significant difference between the saline-
treated and butanediol-treated groups was a decrease in arterial pH from 0.06 to 0.11 unit in the
butanediol-treated rats (not shown), probably as a
consequence of the increase in the level of ketone
bodies in the blood.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Time of blood sampling (hours)</th>
<th>β-Hydroxybutyrate (mmol/l)</th>
<th>Glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6</td>
<td>...</td>
<td>0.08±0.01</td>
<td>8.5±0.3</td>
</tr>
<tr>
<td>Butanediol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One injection at time 0 hours</td>
<td>6</td>
<td>2.5</td>
<td>1.79±0.08*</td>
<td>6.6±0.2*</td>
</tr>
<tr>
<td>Two injections at times 0 and 3 hours</td>
<td>6</td>
<td>6.5</td>
<td>1.5±0.13*</td>
<td>6.5±0.3*</td>
</tr>
<tr>
<td>Four injections at times 0, 3, 6, and 9 hours</td>
<td>5</td>
<td>12.5</td>
<td>1.73±0.27*</td>
<td>6.7±0.4*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *p<0.05 different from untreated by one-way analysis of variance.
TABLE 2. Effect of Butanediol on Concentrations of Cerebral Energy Metabolites in Nonembolized Rats

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Saline (n=8)</th>
<th>Butanediol (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphocreatine</td>
<td>4.08±0.19</td>
<td>4.38±0.17</td>
</tr>
<tr>
<td>ATP</td>
<td>2.79±0.10</td>
<td>2.95±0.10</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.137±0.007</td>
<td>0.115±0.004*</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.96±0.14</td>
<td>2.33±0.10*</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.50±0.18</td>
<td>3.74±0.16</td>
</tr>
<tr>
<td>Glycogen</td>
<td>2.37±0.06</td>
<td>2.63±0.07*</td>
</tr>
</tbody>
</table>

Values are mean±SEM μmol/g wet tissue.
*p<0.05 different from saline by one-way analysis of variance.

Table 2 shows the effect of butanediol treatment on the concentrations of cerebral energy metabolites in Group 1. Treatment did not significantly change the concentrations of PCr, ATP, or glucose but decreased the concentrations of pyruvate (−16%) and lactate (−21%) and increased the concentration of glycogen (11%).

Embolization caused marked changes in the concentrations of cerebral energy metabolites (Figure 1): decreases in PCr (−30% after 6 and 12 hours) and ATP (−10% after 2 hours, −19% after 6 and 12 hours), increases in pyruvate (80% after 2, 6, and 12 hours) and lactate (108% after 2 hours, 251% after 6 hours, and 156% after 12 hours). The glucose level was not significantly affected, whereas the glycogen level progressively increased with time, reaching 268% of the control value after 12 hours. The degree of embolization was determined for each rat by measuring the number of microspheres present in the embolized hemisphere. For all rats used for the measurement of energy metabolites the mean±SEM number of microspheres in the hemisphere was 698±21, and there were no significant differences among groups (data not shown).

Butanediol treatment attenuated the metabolic changes induced by embolization (Figure 1). Butanediol-treated rats exhibited better preservation of high-energy phosphate compounds and lower levels of pyruvate (−20% at 6 and 12 hours) and lactate (−13% at 2 hours, −44% at 6 hours, and −46% at 12 hours) than saline-treated rats. Butanediol treatment significantly increased the glucose level at 2 hours (28%) and the glycogen level at 2 (22%) and 12 (34%) hours relative to saline treatment.

Considering the osmotic load produced by repeated injections of butanediol and the possible beneficial effect of osmotic agents in brain infarction, cerebral energy metabolites were measured after

FIGURE 1. Graph of mean±SEM concentrations of cerebral energy metabolites in rats given saline (○) or butanediol (●). Brain samples were taken before (time 0) and 2, 6, or 12 hours after embolization. Number of rats at each time (n=8–11) is given in “Materials and Methods”. *p<0.05 different from butanediol at that time by one-way analysis of variance.
injections of equimolar doses of urea. The results (not shown) indicate that urea treatment does not significantly change the concentrations of cerebral energy metabolites 12 hours after embolization.

Table 3 shows the effect of treatment with butanediol or urea on brain water content in embolized and nonembolized rats. In the latter, neither treatment had a dehydrating effect relative to saline treatment. Embolization led to the development of significant edema in saline-treated rats. Butanediol-treated embolized rats showed a significant reduction in brain water content relative to saline-treated embolized rats, whereas urea treatment had no significant effect. Likewise, ethanol injected at equimolar doses had no significant effect on brain water content. The antiedema effect of butanediol was associated with significant decreases in the sodium and chloride concentrations, but the potassium content was not significantly modified by butanediol treatment (Table 4).

Discussion

Intracarotid injection of 50-μm microspheres produces multiple infarcts in cortical and subcortical cerebral structures.13,14 Reliability of this method is strengthened by the use of radioactive microspheres as embolic agents, enabling the degree of embolization to be determined and allowing comparisons among experiments. Microscopic examination of embolized hemispheres14 show infarcted regions surrounded by normal tissue. Though we did not precisely measure infarct volume, it was estimated to be approximately 20% of the hemispheric volume. This estimation agrees with data from biochemical studies showing that a 20–30% depletion of cellular constituents such as high-energy phosphates, phospholipids,13 and catecholamines15 occurs in the embolized hemisphere 24 hours after microsphere injection.

Many studies16,17 have identified a topographic gradient of ischemic perturbations associated with vascular occlusion, the ischemic core being surrounded by a penumbral region that is potentially salvageable and exhibits a large accumulation of lactate in the presence of a near-normal ATP concentration. The existence of such penumbral regions following embolization is suggested by the relatively small decreases in the concentrations of high-energy phosphates with a relatively large increase in the concentration of lactate. Lactate accumulation is an important determinant of ischemic brain damage (see Reference 18), and by minimizing lactacidosis in the penumbral regions, butanediol treatment may prolong tissue survivability and delay irreversible cell damage.

The metabolic effects of butanediol were seen in nonembolized rats, which exhibited slight but significant decreases in the concentrations of pyruvate and lactate and an increase in the concentration of glycogen. A decrease in the concentration of pyruvate has been reported in the brains of butanediol-treated mice.19 These effects suggest a reduction of glycolytic metabolism, which may be influenced by the systemic acidosis that develops after butanediol injection. Indeed, chronic metabolic acidosis induced by

### Table 3. Brain Water Content in Nonembolized and Embolized Rats Administered Saline, Butanediol, Urea, or Ethanol

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Microspheres (number)</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonembolized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>6</td>
<td>...</td>
<td>78.63±0.07</td>
</tr>
<tr>
<td>Butanediol</td>
<td>6</td>
<td>...</td>
<td>78.71±0.07</td>
</tr>
<tr>
<td>Urea</td>
<td>6</td>
<td>...</td>
<td>78.83±0.07</td>
</tr>
<tr>
<td>Embolized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>12</td>
<td>668±29</td>
<td>80.93±0.24</td>
</tr>
<tr>
<td>Butanediol</td>
<td>7</td>
<td>690±56</td>
<td>79.57±0.27*</td>
</tr>
<tr>
<td>Urea</td>
<td>9</td>
<td>757±33</td>
<td>80.49±0.33</td>
</tr>
<tr>
<td>Ethanol</td>
<td>6</td>
<td>655±33</td>
<td>80.91±0.28</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*p<0.05 different from saline, urea, and ethanol by one-way analysis of variance.
ammonium chloride in rats reduces cerebral glucose utilization and blood flow.20

The protective effect of butanediol was probably not secondary to an osmotic effect because equimolar doses of urea were ineffective. The protective effect of butanediol may be due to the alcohol itself or it may be mediated through the formation of β-hydroxybutyrate. Butanediol is a lipophilic molecule that reaches the brain by passively diffusing across the blood–brain barrier,21 and doses of 20–40 mmol/kg exert depressant effects on central nervous system function in ways similar to that of ethanol.22 Depressant agents are known to exert protective effects in hypoxic and ischemic situations by reducing the cerebral metabolic rate, and ethanol has been reported to prolong survival in hypoxic mice.23 However, our results indicate that ethanol, unlike butanediol, does not prevent the development of edema secondary to embolization and are consistent with those of other studies that reported no beneficial effect of ethanol in various models of brain injury. Pretreatment with 47 mmol/kg ethanol, unlike pretreatment with butanediol, did not increase survival time in the Levine rat model,24 and high doses of ethanol (87 mmol/kg) potentiated traumatic spinal cord and cerebral hemisphere damage in cats.25,26 Ethanol (100 mmol/kg) had no significant effect on the concentrations of cerebral energy metabolites in intact or ischemic rats,27 which contrasts with the metabolic effects of butanediol that we report.

Butanediol is converted in the liver to β-hydroxybutyrate by alcohol and aldehyde dehydrogenases,6 and several reports have suggested that the antihypoxic properties of butanediol are linked to its ketogenic effect. Ketosis induced by a variety of methods (such as fasting, diabetes, or a ketogenic diet) increases hypoxic survival time in rats28 and mice.29,30 Ketone bodies can be used as an alternate substrate for brain metabolism, and their rate of utilization seems to be regulated by their blood concentration and the activity of their blood–brain transport system.31–35 Following butanediol administration, a supplementary source of ketone would be the conversion of butanediol to β-hydroxybutyrate in the brain, which contains enzymes for this transformation.36,37 Intracerebral production of β-hydroxybutyrate seems to be an important factor in the protective action of butanediol. Lundy et al34 reported that acute elevation of blood ketone levels of β-hydroxybutyrate infusion was not sufficient to provide cerebral protection in the Levine rat model.

In normoxic brains, an increase in ketone body oxidation leads to a reduced glycolytic rate, probably due to inhibition of phosphofructokinase activity.33,38,39 Though it is uncertain to what extent these mechanisms operate during ischemia, studies performed on brain slice preparations40 have shown an increased utilization of ketone bodies instead of glucose under hypoxic conditions. Taken together, these data agree with the hypothesis that preferential metabolism of ketones rather than glucose as an energy substrate may reduce the deleterious accumulation of brain lactic acid during ischemia.

Acknowledgments

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**KEY WORDS** • brain edema • embolism • metabolism • rats
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