Influence of Various Agents on the Development of Brain Edema in the Rat Following Microembolism

Protective Effect of Gamma-Butyrolactone

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

SUMMARY  Brain edema was induced in rats by injecting $50 \, \mu \text{m}$ microspheres, labelled with $^{85}\text{Sr}$, into the 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 12 or 24 h after embolization by measuring brain water content and, in some experiments, sodium and potassium.

Pretreatments with dexamethasone, parachlorophenylalanine (an inhibitor of 5-hydroxytryptamine synthesis), mepyramine and metiamide (H, and H$_2$ histamine receptor antagonists) or aminophylline did not influence significantly the development of brain edema evaluated 24 h after embolization. Aminophylline treatment (100 mg/kg) markedly increased mortality following embolization.

Gamma-butyrolactone (300 mg/kg, every 2 h) inhibited significantly the development of brain edema evaluated 12 hours after embolization. Increases in water and sodium in the embolized cerebral hemisphere were reduced by about 50%. This protective effect may be related to the known depressant action on brain metabolism.

BRAIN EDEMA is an important clinical problem and a number of experimental models have been described for producing brain edema with infarction. Among them, embolic methods have been proposed which consist of injection of microemboli into the carotid arterial system. These methods have not been used to study the effect of anti-edema therapy, perhaps due to the difficulty of obtaining reproducible embolization.

In previous work, we described a brain edema model using radioactive calibrated microspheres as the embolic agent permitting determination of the degree of brain embolization. It was demonstrated that the amount of brain edema and degree of increase in the blood-brain barrier permeability was proportional to the number of microspheres present in a cerebral hemisphere. In the present investigation, this model has been used to study the effect of various agents on brain edema development.

Methods

Male Iffa-Credo rats (220–300 g) were anesthetized with chloral hydrate (360 mg/kg, i.p.). About 4,000 carbonized microspheres (3M, 50.6 ± 2.5 $\mu$ in diameter, labelled with $^{85}\text{Sr}$) were injected into the left internal carotid artery as previously described. Rats were decapitated 12 or 24 h after embolization. The brain was removed, the cerebellum and the brain stem were discarded, both hemispheres were placed into tared vials and their radioactivity was determined in a scintillation crystal well counter (Nuclear Chicago). In order to calculate the number of microspheres contained in each sample, the mean radioactivity in one microsphere (about 3–6 cpm) was determined by simultaneous counting of a measured microsphere suspension in a hemocytometer. Animals which showed an abnormal distribution of microspheres were discarded.

Water content (g H$_2$O/100 g, fresh weight) was determined by difference after drying at 95$^\circ$C to a
TABLE 2

Effect of Aminophylline on Water, Sodium and Potassium Content of Cerebral Hemispheres in Control and Embolized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Non-embolized</th>
<th>Embolized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left + right</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>hemisphere</td>
<td>hemisphere</td>
</tr>
<tr>
<td>Saline (n = 10)</td>
<td>—</td>
<td>657 ± 38</td>
</tr>
<tr>
<td>Aminophylline (n = 10)</td>
<td>79.05 ± 0.03</td>
<td>78.84 ± 0.04</td>
</tr>
<tr>
<td>H2O%</td>
<td>79.05 ± 0.03</td>
<td>78.84 ± 0.04</td>
</tr>
<tr>
<td>Na mEq/kg, dry weight</td>
<td>229.1 ± 0.3</td>
<td>237.8 ± 2.5</td>
</tr>
<tr>
<td>K mEq/kg, dry weight</td>
<td>454.0 ± 3.9</td>
<td>442.0 ± 5.1</td>
</tr>
</tbody>
</table>

The embolized rats were sacrificed 24 h after the microspheres injection and received aminophylline (100 mg/kg, i.p.) 30 min before embolization. The non-embolized rats received aminophylline 24.5 h before sacrifice. **p < 0.01, ***p < 0.001.

n = number of animals.
The use of radioactive microspheres allows the determination of the degree of embolization and the distribution of emboli between the 2 cerebral hemispheres. Ten to twenty percent of the rats have an abnormal distribution of microspheres, making it necessary to assess the amount of embolization if comparisons are to be made between control and treated animals.

The possible anti-edema properties of steroids have been studied with variable results (see reference 11). In a brain edema model induced by microembolism, Siegel et al.15 reported that dexamethasone had no beneficial effect, which is confirmed in the present study. An increase in mortality following aminophylline treatment (100 mg/kg, i.p.) has also been reported in gerbils with unilateral carotid ligation.20 This detrimental effect may be related to the observation that aminophylline increases the cerebral metabolic rate.21 As the supply of oxygen is limited during ischemia, an increased metabolic rate would accelerate cell damage. Conversely, situations in which energy demands are reduced, as in hypothermia or with barbiturate anesthesia, have been reported to protect the brain from ischemia.22-24

The present results show that gamma-butyrolactone administered in an hypnotic dose markedly inhibited the development of brain edema following microembolism. The usual increases in water and sodium in the ischemic cerebral hemisphere were reduced by about 50% 12 h after induction of brain injury. Gamma-butyrolactone is hypnotic and anesthetic25 and is believed to act through formation of gamma-hydroxybutyrate.26 Some particular effects differentiate it from other depressant drugs. It produces alterations of the electroencephalogram which suggest a state of generalized non-convulsive epilepsy and causes increases in the brain levels of dopamine and acetylcholine by inhibiting the neuronal activity.27 It reduces markedly cerebral energy metabolism28 and glucose consumption.29 In man, gamma-hydroxybutyrate reduces the rate of cerebral oxygen consumption and has been proposed for the management of brain injuries.30

<table>
<thead>
<tr>
<th>Determination</th>
<th>Non-embolized</th>
<th>Embolized</th>
<th>Embolized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left + right hemispheres</td>
<td>Saline (n = 12)</td>
<td>Butyrolactone (n = 16)</td>
</tr>
<tr>
<td>Number of microspheres</td>
<td>—</td>
<td>655 ± 38</td>
<td>605 ± 38</td>
</tr>
<tr>
<td>H2O %</td>
<td>79.23 ± 0.05</td>
<td>80.58 ± 0.14</td>
<td>79.95 ± 0.19*</td>
</tr>
<tr>
<td>Na mEq/kg, dry weight</td>
<td>222.2 ± 5.2</td>
<td>291.3 ± 8.4</td>
<td>253.9 ± 7.2**</td>
</tr>
<tr>
<td>K mEq/kg, dry weight</td>
<td>454.1 ± 7.6</td>
<td>426.7 ± 6.1</td>
<td>433.0 ± 5.3</td>
</tr>
</tbody>
</table>

Rats were sacrificed 12 h after embolization. Gamma-butyrolactone (300 mg/kg, i.p.) was injected every 2 h (first injection 15 min before embolization). *p < 0.02, **p < 0.01. n = number of animals.
Gamma-butyrolactone seems to be more active than barbiturates in its inhibitory action on cerebral metabolism. Doses of barbiturates necessary to inhibit cerebral glucose utilization to the same degree seen with butyrolactone would be lethal or require artificial ventilation. It is premature to postulate that gamma-butyrolactone may be of greater interest than barbiturates in the management of acute stroke. It seems that barbiturates exert their protective effect on the ischemic brain not only by their action on brain metabolism and cerebral blood flow but also by another mechanism which has not been clearly elucidated. Moreover, in the present investigation, gamma-butyrolactone was given before cerebral edema had occurred remains to be demonstrated.

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