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 μm microspheres, labelled with 85Sr, 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 (H1 and H2 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.

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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.18 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,9 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 Ifa-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 μm in diameter, labelled with 85Sr) were injected into the left internal carotid artery as previously described.8 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 H2O/100 g, fresh weight) was determined by difference after drying at 95° C to a
constant weight. Sodium and potassium determinations (mEq/kg, dry weight) were made in the dry hemisphere using a flame photometer.

### Treatment Schedules

Dexamethasone (Merck Sharp, & Dohme), 1 mg/kg i.p., was given every 6 h, the first injection 15 min before embolization. Parachlorophenylalanine (AB Biotec), 100 mg/kg i.p. was given daily for 4 days before embolization and the last injection 30 min before embolization.10 Metiamide (Smith Kline & French) + mepyramine (May and Baker), 5 mg/kg i.p. each was given every 6 h, with the first injection 15 min before embolization. Aminophylline (Millot), 100 mg/kg i.p., was given every 6 h, the first injection 15 min before embolization and the last injection 30 min before embolization. Parachlorophenylalanine (Sigma, 300 mg/kg, i.p.) and gamma-butyrolactone (300 mg/kg, i.p.) was administered i.p., 30 min before embolization. Aminophylline (100 mg/kg i.p.) 30 min before embolization.

In the group given gamma-butyrolactone, the rats were anesthetized with a mixture of chloral hydrate (180 mg/kg, i.p.) and gamma-butyrolactone (Sigma, 300 mg/kg, i.p.). Gamma-butyrolactone (300 mg/kg, i.p.) was then injected 2 h after embolization and every 2 h until sacrifice.

For each treatment, one group of control rats received an equivalent volume of saline and was embolized with the same microsphere suspension. Means were determined with their standard errors and the Student's t-test was used for statistical comparison.

### Results

On an average, after injection of 4,000 microspheres into the left internal carotid artery, 550 to 650 microspheres were found in the left hemisphere and 50 to 100 in the right hemisphere. In each series of experiments, the numbers of microspheres in the brain were not significantly different in control and treated rats.

As seen in table 1, treatments with dexamethasone, parachlorophenylalanine and metiamide-mepyramine did not change significantly the water content of the cerebral hemispheres evaluated 24 h after embolization.

Table 2 shows the levels of water, sodium and potassium in the non-embolized and embolized cerebral hemispheres of rats receiving saline or aminophylline. In non-embolized rats, aminophylline treatment (100 mg/kg) influenced slightly, but significantly, the water level which was reduced (−0.21%) and the sodium level which was enhanced (+8.7 mEq/kg). In rats with emboli aminophylline did not change significantly the water and electrolyte concentrations evaluated 24 h after embolization. As in non-embolized rats, aminophylline led to slight but not significant decrease in water content (about −0.3%) in both hemispheres. Furthermore, in this series of experiments, the mortality 24 h after embolization was 10% in saline treated and reached 50% in aminophylline treated rats.

Gamma-butyrolactone administered in a hypnotic dose (300 mg/kg) markedly inhibited the formation of cerebral edema (table 3). Twelve hours after embolization, the increase in water content of the left hemisphere was 1.35% in saline treated rats and only 0.72% in the gamma-butyrolactone treated group (p < 0.02). The sodium level showed similar variations. In the left hemisphere, it increased by 69.1

### Table 1: Effect of Dexamethasone, Parachlorophenylalanine and Mepyramine-metiamide on Water Content of Cerebral Hemispheres in Embolized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of microspheres</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left hemisphere</td>
<td>Right hemisphere</td>
</tr>
<tr>
<td>Saline (n = 12)</td>
<td>549 ± 43</td>
<td>70 ± 20</td>
</tr>
<tr>
<td>Dexamethasone (n = 14)</td>
<td>547 ± 32</td>
<td>74 ± 14</td>
</tr>
<tr>
<td>Saline (n = 10)</td>
<td>618 ± 41</td>
<td>97 ± 19</td>
</tr>
<tr>
<td>p-Chlorophenylalanine</td>
<td>582 ± 29</td>
<td>102 ± 21</td>
</tr>
<tr>
<td>Saline (n = 12)</td>
<td>558 ± 48</td>
<td>51 ± 15</td>
</tr>
<tr>
<td>Mepyramine-metiamide</td>
<td>588 ± 54</td>
<td>61 ± 16</td>
</tr>
</tbody>
</table>

Table 2: Effect of Aminophylline on Water, Sodium and Potassium Content of Cerebral Hemispheres in Control and Embolized Rats

<table>
<thead>
<tr>
<th>Determination</th>
<th>Non-embolized</th>
<th>Aminophylline</th>
<th>Embolized</th>
<th>Aminophylline</th>
<th>Embolized</th>
<th>Aminophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left + right hemisphere</td>
<td>Left hemisphere</td>
<td>Left hemisphere</td>
<td>Right hemisphere</td>
<td>Left hemisphere</td>
<td>Right hemisphere</td>
</tr>
<tr>
<td>Number of microspheres</td>
<td>606 ± 58</td>
<td>606 ± 58</td>
<td>116 ± 26</td>
<td>87 ± 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2O %</td>
<td>79.05 ± 0.03</td>
<td>78.84 ± 0.04***</td>
<td>81.99 ± 0.32</td>
<td>81.72 ± 0.32</td>
<td>79.33 ± 0.17</td>
<td>79.02 ± 0.17</td>
</tr>
<tr>
<td>Na mEq/kg, dry weight</td>
<td>229.1 ± 0.3</td>
<td>237.8 ± 2.5**</td>
<td>374.2 ± 14.5</td>
<td>397.1 ± 19.9</td>
<td>245.2 ± 4.4</td>
<td>247.8 ± 4.9</td>
</tr>
<tr>
<td>K mEq/kg, dry weight</td>
<td>454.0 ± 3.9</td>
<td>442.0 ± 5.1</td>
<td>407.1 ± 15.0</td>
<td>404.8 ± 14.8</td>
<td>447.4 ± 8.9</td>
<td>444.6 ± 5.0</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.\(^{12}\) reported that dexamethasone had no effect, whereas aminophylline, a histamine H\(_1\)-receptor antagonist, was effective in reducing the amount of brain edema in the ischemic hemisphere.\(^{13,14}\) 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.\(^{15}\) This detrimental effect may be related to the observation that aminophylline increases the cerebral metabolic rate.\(^{16}\) 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.\(^{17,18}\)

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 anesthetic\(^{19}\) and is believed to act through formation of gamma-hydroxybutyrate.\(^{20}\) 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.\(^{21}\) It reduces markedly cerebral energy metabolism\(^{22}\) and protects the brain from ischemia.22,23

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### TABLE 3  Effect of Gamma-Butyrolactone on Water, Sodium and Potassium Content of Cerebral Hemispheres in Embolized Rats

<table>
<thead>
<tr>
<th>Determination</th>
<th>Non-embolized</th>
<th>Embolized Left hemispheres</th>
<th>Embolized Right hemispheres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left + right hemispheres (n = 12)</td>
<td>Saline (n = 16)</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>H(_2)O %</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>253.0 ± 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>453.6 ± 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.

definition of the degree of embolization and the cant. In the weakly embolized right hemisphere, the variation in water, sodium and potassium content seen after gamma-butyrolactone were never statistically significant.

**Discussion**

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.\(^{12}\) reported that dexamethasone had no beneficial effect, which is confirmed in the present work.

It has been suggested that an accelerated release of 5-hydroxytryptamine from ischemic brain is involved in the pathogenesis of progressive cerebral ischemia.\(^{12,14}\) In the gerbil with unilateral carotid artery occlusion, the incidence of stroke was found to be reduced after pretreatment with a tryptophan hydroxylase inhibitor, parachlorophenylalanine, but the development of edema in the ischemic hemisphere was not significantly altered.\(^{15}\) Our results show also that this treatment has no significant effect on the development of brain edema following microembolism.

The regulation of permeability in brain capillaries may be mediated by cyclic AMP. It has been reported that histamine activates adenylate cyclase in a subcellular fraction enriched by brain capillaries.\(^{14}\) Treatment with metiamide, a histamine H\(_2\)-receptor antagonist, was effective in reducing the amount of brain edema following the cerebral implantation of yttrium 90.\(^{17}\) In our experimental model, no beneficial effect was observed after treatment by metiamide and mepyramine, a histamine H\(_1\)-receptor antagonist. The lack of effect of another H\(_1\)-receptor antagonist, chlorpheniramine, on edema following a cryogenic brain lesion, has been previously reported.\(^{18}\)

Kogure et al.\(^{19}\) reported that aminophylline treatment (100 mg/kg, i.p., 30 min before injection of 35 \(\mu\) microspheres into the internal carotid artery of the rat) 24 h after embolization, had a protective effect shown by a diminution of mortality and by a decrease in the water content of the cerebral hemispheres. Our results do not support these findings. Aminophylline treatment in the same dose did not change significantly the amount of brain edema and was associated with a higher mortality. The differences between the Kogure et al. study and this work appear to ufnoo be: 1) the number and size of the injected microspheres which led to more ischemia in Kogure's model suggested by the high mortality (56% within 24 h); 2) the determination of the number of emboli in the present study. An increase in mortality following aminophylline treatment (100 mg/kg) 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.\(^{22,23}\)
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.30-33 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.34 Moreover, in the present investigation, gamma-butyrolactone was given before cerebral ventilation.35-38 It is premature to postulate that gamma-butyrolactone would be lethal or require artificial ventilation.


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References

Influence of various agents on the development of brain edema in the rat following microembolism. Protective effect of gamma-butyrolactone.

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