Experimental Prevention of Cerebral Vasculature Obstruction Produced by Ischemia*

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Marked increases in vascular resistance following ischemia have been demonstrated in the kidney, heart, adrenal gland, and hindlimb. In the brain, this impairment of circulation develops much earlier, becoming first apparent after only 5 to 7.5 min of ischemia and involving more than 50% of the brain within 15 min. It is of particular importance in the brain as it may constitute the first irreversible change in the animal and lead directly to death. Previously reported investigations of this phenomenon, including light and electron microscopic studies of the vasculature, have indicated that the circulatory impairment results from shifts of water and electrolytes from plasma to perivascular cells with a resultant increase in blood viscosity and narrowing of vascular lumen.

Our experiments were undertaken to study factors that might modify this reaction and particularly to investigate measures that might prevent it. The effects of increasing serum osmolarity were investigated in detail. Other variables studied included production of cerebral vasodilation or vasoconstriction prior to ischemia by hypercapnia or hypocapnia; prevention of the presumed fall in pH during ischemia by increasing the pH and buffer capacity of the blood; prophylactic administration of glucocorticoids; and variation of arterial pressure in the post-ischemic period.

Significant protection against the vascular lesion was obtained in experiments in which serum osmolarity was increased with mannitol or glucose and in experiments in which the cerebral vasculature was dilated prior to the ischemia by CO₂ inhalation.

Received for publication March 11, 1968.

* Supported by research grants from the National Institute of Neurological Diseases and Blindness (NB-04512) and the National Institute of Mental Health (K3-MH-3769).

Methods

The experiments were performed on New Zealand white rabbits weighing 2 to 3 kg. They were anesthetized with intravenous sodium pentobarbital, 25 mg/kg. A tracheostomy tube was inserted; following intravenous anecine, they were respired with a Harvard respirator using room air. Respiratory rate was adjusted on the basis of measurements of pH, pCO₂, and PO₂ made on samples of femoral arterial blood. The thorax was opened by splitting the sternum, and the ascending and descending portions of the aorta were exposed. Ligatures were placed loosely around the ascending aorta just above the aortic ring and around the descending aorta (Fig. 1).

Cerebral ischemia was produced by tightening the ligature around the ascending
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aorta. During the ischemic period, the ascending aorta was incised just distal to the ligature but well proximal to the innominate artery, and a cannula of about the same size as the aorta was inserted and secured with a second ligature. This provided the means for perfusing the cerebral vasculature with a suspension of carbon in order to determine its degree of patency. The descending aorta was ligated and ligatures were placed at the base of each ear to reduce the portion of the vascular tree to be perfused with carbon. At the end of the period of ischemia, the rostral portion of the rabbit (excluding the ears) was perfused with a carbon suspension for 30 sec at 110 mm Hg pressure. The carbon suspension, the same as that used in previous studies, consisted of 10% soot, 9.5% gelatin, and 1.3% phenol (prepared by Pelikan Werke, Hannover, W. Germany).

At the end of the carbon perfusion, the animals were sacrificed. The brain was removed, fixed in 10% formalin, and cut into six coronal sections as follows: 1) mid-frontal lobe; 2) optic chiasm; 3) anterior edge of mammillary body; 4) posterior edge of mammillary body; 5) inferior colliculus; and 6) mid-cerebellum. The sections were examined under a dissecting microscope and scored for the percentage of the surface not perfused with carbon. The percentage figures thus obtained for each of the six sections were then averaged to obtain a total figure for the extent of vascular obstruction in the entire brain. These total figures were then used to compare results obtained under different experimental conditions.

Measurements were made on a total of 66 experimental animals divided into 12 groups as follows:

Group 1 (4 rabbits). Controls for the ischemia. The brains of these animals were examined after periods of ischemia of less than 5 min.

Group 2 (6 rabbits). Controls for the effects of experimental changes introduced before or after a 15-min ischemic period. These animals were subjected to no additional experimental variables.

All of the animals in the following groups were also subjected to 15 min of ischemia:

Group 3 (6 rabbits). Serum osmolarity increased with Mannitol. A solution of 20% Mannitol was infused into the left ventricle immediately before the aorta was occluded. Serum osmolarity of jugular venous blood, sampled at the end of the infusion, was between 404 and 497 mOsm/liter.

Group 4 (6 rabbits). Serum osmolarity increased with glucose. In one animal, 20% glucose was infused into the ventricle (as above). In five animals, 75 ml of 20% glucose (three with 40 units of insulin and three without) was administered intravenously over a 15-min period preceding the ischemia. Blood glucose at the time of ischemia was between 785 and 1700 mg%, and serum osmolarity was between 347 and 380 mOsm/liter.

Group 5 (2 rabbits). As a control for the hemodilution (about 12%) produced in Groups 3 and 4 above, isotonic saline was infused into the left ventricle at the same rate as the Mannitol or glucose infusions.

Group 6 (4 rabbits). Hypoglycemia. Regular insulin (20 units/kg) was administered intravenously 30 min before ischemia. Blood glucose levels were between 20 and 40 mg% when ischemia was produced.

Group 7 (6 rabbits). Hypercapnia. The rabbits were respired with a mixture of 20% CO₂, 20% O₂, 60% N₂ for 10 min before ischemia. Arterial blood analyses demonstrated the pCO₂ between 100 and 125 mm Hg and pO₂ between 98 and 120 mm Hg.

Group 8 (4 rabbits). Hypocapnia. The animals were hyperventilated for 10 min before ischemia. Arterial blood analyses demonstrated the pCO₂ between 15 and 20 mm Hg and pO₂ between 105 and 307 mm Hg.

Group 9 (3 rabbits). Hypoxia. The animals were respired at normal rates for 6 min before ischemia with 100% N₂. Arterial blood analyses demonstrated the pO₂ between 5 and 15 mm Hg.

Group 10 (7 rabbits). Glucosteroids. Methylprednisolone, 40 mg, was administered intravenously from 45 min to 2 hours before ischemia.

Group 11 (3 rabbits). Prevention of acidosis. Isotonic tris acetate, 50 ml at pH
7.64, was infused into the left ventricle just before ischemia.  

**Group 12 (15 rabbits).** Variation of perfusion pressure after ischemia. The carbon perfusion was carried out at 190 mm Hg in four animals, at 160 mm Hg in four animals, at 80 mm Hg in four animals, and at 50 mm Hg in three animals.

**Results**

Cerebral perfusion was complete when the period of ischemia was less than 5 min (Group 1); but after 15 min of ischemia under control conditions (Group 2) there was a consistent and marked impairment of reperfusion involving an average of 51% of the brain substance (Table 1).

The vascular obstruction developing during ischemia was almost completely prevented by increasing the serum osmolarity with either glucose or Mannitol before ischemia (Groups 3 and 4); this effect was statistically highly significant (Table 1). Glucose was somewhat more effective than Mannitol in preventing the obstruction in spite of the fact that the average serum osmolarity obtained with glucose (355 mOsm/liter) was appreciably less than that obtained with Mannitol (459 mOsm/liter). There was no difference between the animals treated with glucose and insulin and those treated with glucose alone. The animals receiving infusions of isotonic saline (Group 5) did not differ from the ischemic controls (Table 2), indicating that the hemodilution of about 12%, associated with administration of the hypertonic agents, did not, in itself, account for the beneficial effects. Hypercapnia, produced before the ischemia with insulin (Group 6), did not increase the degree of vascular obstruction over that observed in the ischemic controls (Group 2).

Hypercapnia produced before the ischemia (Group 7) afforded significant protection (Table 1), although less than that provided by increasing serum osmolarity. Hypercapnia (Group 5) did not increase the amount of obstruction (Table 2). With hypoxia the degree of obstruction was less than that in ischemic controls, but the difference in four animals was barely significant (p = <0.05). No effect was observed with glucocorticoid administration (Group 10) or from infusing tris acetate (Group 11) to reduce the presumed fall in pH occurring in the ischemic brain. Varying the pressure at which the carbon suspension was perfused at the end of the ischemia (Group 12) had no significant effect between 190 and 80 mm Hg, but a significant increase in the perfusion defect occurred when the pressure was further reduced to 50 mm Hg.

**Discussion**

The results obtained on those brains made ischemic under control conditions are quite consistent with those previously reported, although quite different methods were used for producing the ischemia and for perfusing the post-ischemic brain with a suspension of carbon. The portion of the brain involved in the vascular obstruction after 15 min of ischemia was somewhat less in our present

| TABLE 1 |
|---|---|---|---|---|
| **Experimental conditions causing significant reduction in vascular obstruction after ischemia** |

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of rabbits</th>
<th>Serum osmolarity</th>
<th>% of brain not perfused</th>
<th>SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 5 min of ischemia (Group 1)</td>
<td>4</td>
<td>—</td>
<td>0</td>
<td>±0</td>
</tr>
<tr>
<td>15 min of ischemia: controls (Group 2)</td>
<td>6</td>
<td>309</td>
<td>51</td>
<td>±5</td>
</tr>
<tr>
<td>hypertonic Mannitol (Group 3)</td>
<td>6</td>
<td>459</td>
<td>8†</td>
<td>±2</td>
</tr>
<tr>
<td>hypertonic glucose (Group 4)</td>
<td>6</td>
<td>355</td>
<td>1.5†</td>
<td>±0.6</td>
</tr>
<tr>
<td>hypercapnia (Group 7)</td>
<td>6</td>
<td>—</td>
<td>17†</td>
<td>±5</td>
</tr>
</tbody>
</table>

* SE = standard error of the mean.  
† Significantly less than in the 15 min ischemic controls; p < .001.
overhydration and swelling of the perivascular cells; and prior dilation of the vasculature with CO₂ would be expected to prevent its constriction, during ischemia, to the critical point that prohibits passage of the red blood cells.

Besides increasing serum osmolarity, the glucose infusions provided additional exogenous substrate; indeed, the additional substrate may even have been more important than the osmotic increase in preventing vascular obstruction. This would explain why the glucose infusions were somewhat more effective than the Mannitol infusions despite the fact that the serum osmolarity attained with glucose was considerably less than that attained with Mannitol. Calculations show that, even with the high concentrations of serum glucose attained (about 1000 mg%), the amount present in cerebral blood at the onset of the ischemia was small compared with the normal glucose consumption by the brain over a 15-min period. It seems likely, therefore, that the concentration of glucose within the microvasculature fell rapidly during the ischemia, due to anaerobic glycolysis. The extra glucose present in the blood may have been enough to maintain the perivascular cells in a sufficiently normal metabolic state to prevent their swelling. It is also possible that considerably larger amounts of intracellular glucose had accumulated in the brain as a result of the high serum levels achieved during the pre-ischemic period. Since the amount of glucose required for an appreciable osmotic effect is greater than that needed for a substrate effect (about 3 mM), removal by glycolysis would eliminate its osmotic role first.

Vascular obstruction first appears after 5 to 7½ minutes of ischemia. It has been proposed that this represents the first irreversible change and that the parenchymal cells may remain viable for a significant period after the vascular lesion has developed to prevent their reperfusion.² If this is the case, prevention of the vascular obstruction with Mannitol, glucose, or hypocapnia may increase appreciably the period of ischemia that can be reversibly sustained.

Summary

In rabbits, 15 minutes of total ischemia caused cerebrovascular obstruction such that

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**TABLE 2**

*Experimental conditions causing no significant change (p. 0.01) in vascular obstruction after ischemia*

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of rabbits</th>
<th>% of brain not perfused</th>
<th>SE *</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min of ischemia: controls (Group 2)</td>
<td>6</td>
<td>51</td>
<td>±5</td>
</tr>
<tr>
<td>isotonic saline (Group 5)</td>
<td>2</td>
<td>53</td>
<td>—</td>
</tr>
<tr>
<td>hypoglycemia (Group 6)</td>
<td>4</td>
<td>47</td>
<td>±5</td>
</tr>
<tr>
<td>hypoxia (Group 8)</td>
<td>4</td>
<td>46</td>
<td>±7</td>
</tr>
<tr>
<td>glucocorticoids (Group 9)</td>
<td>4</td>
<td>31</td>
<td>±6</td>
</tr>
<tr>
<td>glucocorticoids (Group 10)</td>
<td>7</td>
<td>42</td>
<td>±7</td>
</tr>
<tr>
<td>tris buffer (Group 11)</td>
<td>3</td>
<td>43</td>
<td>±15</td>
</tr>
<tr>
<td>perfusion pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>190 mm Hg (Group 12)</td>
<td>4</td>
<td>42</td>
<td>±5</td>
</tr>
<tr>
<td>160 mm Hg</td>
<td>4</td>
<td>40</td>
<td>±8</td>
</tr>
<tr>
<td>80 mm Hg</td>
<td>4</td>
<td>58</td>
<td>±3</td>
</tr>
</tbody>
</table>

* SE = standard error of the mean.

study (51% vs 70%) but the results were more reproducible. This is probably because we performed the carbon perfusion through a large cannula in the ascending aorta rather than through small needles in the carotid arteries, which may have resulted in somewhat lower and more variable perfusion pressure.

Dramatic protection against the ischemia-induced vascular obstruction was obtained by increasing serum osmolarity with Mannitol or glucose before ischemia. Significant protection was also afforded by making the animal hypercapnic before producing the ischemia; this was probably due to the dilatation of the cerebral vasculature produced by the increased pCO₂. Both of these results are consistent with, and provide support for, the previous studies,¹² which indicated that obstruction resulted from a narrowing of the lumen of the microvasculature due to a migration of water and electrolytes from the serum into the endothelial cells and perivascular astrocytes. The increase in serum osmolarity with glucose or Mannitol would be expected to counteract changes leading to
51% of the brain could no longer be perfused at an arterial pressure of 110 mm Hg. Hypercapnia, instated before the ischemia, reduced the obstructed regions to 17%, probably by dilating the vasculature before the blood flow was stopped. High levels of Mannitol or glucose in the plasma almost completely prevented obstruction, the former probably acting osmotically and the latter both osmotically and as substrate. Other variables were tested that had no significant effect on the obstruction.

References