Alterations of Cyclic AMP in Cerebral Ischemia

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SUMMARY Cyclic AMP levels were measured in brains of 21 cats after occlusion of a middle cerebral artery by a trans-orbital approach. Brain samples for determination of cAMP by a protein binding radioassay were obtained from the ischemic and contralateral temporal lobes before sacrifice 1, 3, and 24 hours after occlusion of the right middle cerebral artery. At 1 hour, no difference between the 2 sides was observed; a mean of 17.4 pmoles/mg protein was observed from the side of the occlusion and 18.9 pmoles/mg protein from the contralateral side. At 3 hours, a mean value of 8.9 pmoles/mg protein on the ischemic side and a level of 21.7 pmoles/mg protein on the contralateral side were observed. At 24 hours, the cAMP level on the ischemic side remained below the nonischemic side; the values obtained were 13.7 pmoles/mg protein compared to 27.9 pmoles/mg protein. These changes in cAMP as early as 3 hours after the onset of ischemia, when such a lesion is reversible, may indicate that an initial step in the alteration of cellular metabolism following ischemia is due to membrane perturbation and altered production of this second messenger.

MANY biochemical changes in glucose and energy metabolism following cerebral ischemia have been studied, but a direct relationship between these pathways and cyclic nucleotide levels has not yet been firmly established in the central nervous system. Our own interest in a connection between cyclic 3', 5'-adenosine monophosphate (cAMP) and cerebral ischemia began with the observation that patients treated with aminophylline and isoproterenol for cerebral vasospasm following subarachnoid hemorrhage often showed neurologic improvement that was out of proportion to the change in vasospasm seen on their angiograms. These agents are known to increase the production and retard the degradation of cAMP within vascular smooth muscle. The question arose whether these drugs might be producing a direct effect on cAMP in ischemic areas of the brain, in addition to affecting the vascular smooth muscle of the vessels in spasm. Since the resultant pathology of vasospasm is infarction, we decided to evaluate cAMP in an animal model of focal cerebral ischemia.

Materials and Methods

Twenty-one mature cats were anesthetized with sodium pentobarbital, 25 mg/kg. Femoral arterial and venous catheters were inserted, and arterial blood pressure and gases monitored continuously. Cats were kept at constant body temperature (37 ± 1°) with a heating pad.

The right middle cerebral artery (MCA) was exposed by a transorbital microsurgical approach, and either coagulated with bipolar forceps and divided, or occluded with a microclip. The wound was closed and the animal monitored until time of sacrifice. The animals observed at 24 hours had a left hemiparesis. When attempting to walk, they would circle to the right. The majority of these animals failed to perceive a visual stimulus from the left side. In addition, placing reflexes were decreased or absent on the left.

Results

Neurologic Status

The animals studied at 1 and 3 hours after right MCA occlusion were still anesthetized at the time of sacrifice. The animals observed at 24 hours had a left hemiparesis. When attempting to walk, they would circle to the right. The majority of these animals failed to perceive a visual stimulus from the left side. In addition, placing reflexes were decreased or absent on the left.
Histologic Findings

No evidence of cerebral ischemia was noted on microscopic examination of specimens obtained 1 hour after MCA occlusion. At 3 hours, astrocytic swelling was occasionally noted, but typical ischemic neuronal changes were not seen. In the specimens obtained 24 hours after MCA occlusion, definite infarction, as well as areas of necrosis, were noted on the side of the occlusion. There was a considerable amount of swelling and edema in the right hemispheres, which were grossly larger than the left. The control sides were histologically normal in all 3 groups.

cAMP Concentrations in the Brain

The concentration of cAMP in brain tissue at 1, 3, and 24 hours following right MCA occlusion are shown in table 1. The changes are expressed as percent difference of the control (left) side. After 1 hour, no difference was observed in the concentration of cAMP in the specimens obtained from the right (17.4 pmoles/mg protein) and the left (18.9 pmoles/mg protein) sides of the brain.

At 3 hours, a significant reduction in the concentration of cAMP on the occluded side was noted. The right side had a mean of 8.9 pmoles/mg protein, compared with 21.7 pmoles/mg protein on the left. This represents a difference of 58.9% and is significant at the 0.001 level. At 24 hours, the infarcted side still had a lower cAMP content than the control: 13.7 pmoles/mg protein on the right, and 27.9 pmoles/mg protein on the left. This difference of 50.9% is significant at the 0.015 level. Although some differences were also noted in cAMP concentrations on the non-occluded side in the 3 groups of cats, these variations were not statistically significant.

Discussion

This model provides an easy method of producing focal cerebral ischemia without the manipulation of the brain. It has been used in many laboratories and the response is predictable in terms of the neurologic and pathologic changes. Animals with MCA occlusions of less than 3 hours recover without deficit when blood flow is re-established. Furthermore, histologic changes are not seen until after 4 to 6 hours of occlusion. The present decrease in cAMP was observed after 3 hours of occlusion when the lesion is reversible and histologic changes have not appeared. This finding raises the question of whether a decrease in cAMP as a result of cerebral ischemia is causally related to the subsequent non-reversible events that develop if blood flow is not re-established. Does the decrease in this intracellular regulator or second messenger reflect a response to extracellular events of ischemia? If so, does this offer an approach to treatment by manipulating the cyclic nucleotide pathways?

Previous studies have reported a rise in brain cAMP levels several minutes after cerebral trauma and ischemia. These studies produced anoxia by decapitating mice and measuring cAMP at different times after death, or by embolization of the carotid artery. Watanabe noted a rise in cerebral cAMP in the first 10 minutes after a stab wound to the brain of mice. Thereafter, no differences from controls were noted. All of these papers measured cAMP in the first few minutes after the onset of ischemia. Although the initial change was an increase, values returned to control levels or below by 30 minutes. The present study cannot be compared with these results since the specimens were obtained as biopsies from intact animals with only focal cerebral ischemia.

A recent paper by Sokoll and Stullken studied the alterations in cortical evoked potentials following brain anoxia. The administration of dibutyryl cAMP was noted to hasten the return of the evoked potentials and improve the overall survival of animals that received this drug. Although cAMP levels were not measured, it does suggest that replacement of cAMP by its dibutyryl analogue reversed a biochemical deficit.

Several clinical studies have noted changes in concentration of cAMP in the cerebrospinal fluid (CSF) following ischemia. Rudman et al. noted a decrease in CSF cAMP that could be correlated with the patient's neurologic status. They studied 6 patients who had sustained head injury or intracranial hemorrhage; they found that the levels of cAMP in CSF were lowest in the patients with the poorest neurologic condition. In contrast, Welch et al. noted an increase in CSF content of cAMP in a group of patients with cerebral infarction. In neither of these studies were cerebral cAMP concentrations determined.

Although high levels of adenylate cyclase and cAMP in the brain have been noted for many years, their specific role has yet to be defined. A role for cAMP in glycogenolysis and glycolysis has been suggested in liver and muscle, but the exact role in the central nervous system is as yet unclear. Although brain glycogen is present in limited amounts, it does represent an energy reserve. The initiation of glycogenolysis and utilization of glycogen by the brain requires the activation of cAMP-dependent protein

### Table 1 Effect of Middle Cerebral Artery (MCA) Occlusion on the Concentration of cAMP in the Brain of Cats

<table>
<thead>
<tr>
<th>Right MCA occlusion (hrs)</th>
<th>Number of animals</th>
<th>cAMP (pmoles/mg protein)*</th>
<th>% Change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Left (Control)</td>
<td>Right (Occluded)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>18.85 ± 3.56</td>
<td>17.35 ± 5.62</td>
<td>- 8.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>21.74 ± 3.07</td>
<td>8.93 ± 0.81</td>
<td>- 58.9</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>27.96 ± 6.50</td>
<td>13.72 ± 3.72</td>
<td>- 50.9</td>
</tr>
</tbody>
</table>

*Mean ± SEM.
kinase to activate phosphorylase kinase.\textsuperscript{18-22} The latter enzyme catalyzes the conversion of inactive phosphorylase B to active phosphorylase A which is necessary to initiate glycogenolysis. A decrease in available cAMP may interfere with this needed alternate energy pathway in cerebral ischemia.

Other steps in glycolysis that have been shown to be influenced by cAMP and which conceivably could be impaired by ischemia and a resultant decrease in cAMP are the phosphorylation of glucose to glucose-6-phosphate by hexokinase, and fructose-6-phosphate to fructose-1,6-diphosphate by phosphofructokinase.\textsuperscript{18–21}

At present, no evidence is available to explain the decrease noted in cAMP prior to cell death. One possible explanation may be a change in the functional status of the membrane-bound enzyme adenylyl cyclase. It has been suggested that ischemia may initiate a series of molecular events which lead to membrane perturbation and damage by free radical peroxidation of phospholipids.\textsuperscript{23,24} The activity of many membrane-bound enzymes, such as adenylyl cyclase and Na-K ATPase, has been shown to be altered by changes in neighboring phospholipids.\textsuperscript{25,26} These enzymes have an obligatory requirement for certain lipids in a non-substrate role. It is this type of molecular change that may be produced by altering oxygen supply and the uncoupling of normally occurring radical moieties of the electron transport chain.\textsuperscript{24} In support of this, we have observed a large decrease in the normally occurring antioxidant, ascorbic acid, as shortly as 1 hour after MCA occlusion.\textsuperscript{24} The consumption of tissue antioxidants is a valid indication that radical processes may be operative and may explain changes in biochemical parameters prior to any tissue destruction being evident histologically.

Other explanations of the decrease in cAMP following cerebral ischemia might be a lack of available ATP, increased phosphodiesterase (PDE), or PDE activator activity.\textsuperscript{28,29} Another mechanism might be the decrease in the neurotransmitters norepinephrine and dopamine in ischemic brain. This was noted by Zervas et al. and Kogure et al. in gerbils and rats.\textsuperscript{30,31} Kogure also noted a rapid rise in cAMP which fell within the first 10 minutes even though the decrease in NE persisted for 4 hours.\textsuperscript{18}

If the decrease in cAMP concentration represents an early, significant change following cerebral ischemia, it might offer a rational method for protection by the use of drugs which either increase the production, or retard the degradation, of this nucleotide. This would have implications both for temporary surgical occlusions during cerebral revascularization, as well as for the overall treatment of occlusive cerebrovascular disease.

References

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