Changes in Enzyme Activities of the Choroid Plexus and Brain Tissue following Bilateral Carotid Ligation in Correlation with the Formation of Cerebrospinal Fluid

Kenichiro HIGASHI, Mitsunori HATANO and Yasuo FUKUDA*

Department of Neurosurgery, Yamaguchi University School of Medicine, Ube, Yamaguchi, 755 Japan

Summary

The activity of four enzymes which are thought to be correlated to the production of CSF, such as acid and alkaline phosphatase, carbonic anhydrase, and Na-K ATPase, were determined biochemically in the choroid plexus and the frontal lobe brain tissue in adult rabbits. The activities of these enzymes, except Na-K ATPase, were transiently enhanced in the choroid plexus after bilateral ligation of the common carotid arteries, although considerable decrease was found in the brain tissue. Since the above results had suggested that enhancement of secretory activity of the choroid plexus followed bilateral carotid ligation, the rate of CSF formation was measured by means of the ventriculocisternal perfusion method in the same species of animals before and after bilateral carotid ligation. However, no significant changes were observed in the rate of CSF formation until 2 hours after carotid ligation. After bilateral occlusion of the common carotid arteries, vertebral blood flow, measured electromagnetically, immediately increased to twice its normal rate. Also, resin casts made of the choroid plexus after bilateral carotid ligation, confirmed that there was an adequate blood supply to the entire choroid plexus through the vertebral-posterior choroidal artery system. Considering the dual source of CSF production, it is likely that the secretory activity of the choroid plexus not only is unaffected by a sudden change of cerebral blood flow but also may compensate for the reduced production of CSF in the brain for at least a temporary period. Therefore, in face of ischemic-anoxic changes in the brain this could lead to the maintenance of a normal rate of CSF production by a homeostatic control mechanism.

Key words: Cerebrospinal fluid formation, carotid ligation, choroid plexus, enzyme activities

Introduction

Since Faibre12) first suggested the secretory nature of the choroid plexus cells, many investigators have regarded this particular tissue within the cerebral ventricles as a major site of elaboration of cerebrospinal fluid (CSF). Dandy8) emphasized the role of the choroid plexus as a source of CSF by his observation that dilatation of the lateral ventricle did not occur but rather entirely collapsed when the choroid plexus was completely removed in the animal whose foramen of Monro had been occluded. Although his observation was later controverted by Bering5) and Milhorat,26) the choroid plexus is generally regarded as a source of at least a portion of CSF.

Recent ultrastructural studies of the choroid plexus10) as well as physicochemical studies on the blood-CSF barrier9) have been quite consistent with the concept of the secretory property of the choroid plexus. Furthermore, biochemical and histochemical analysis of the choroid plexus reveals that this tissue contains various enzymes such as acid2,5,20,23) and alkaline phosphatases,13,20,23,34) carbonic anhydrase,13,16) some
dehydrogenases, choline esterase, cytochrome oxidase, and adenosine triphosphatase (ATPase). There is also considerable evidence that some of these enzymes are concerned with the production of CSF. This study was designed to investigate the changes in activity of acid and alkaline phosphatase, carbonic anhydrase and ATPase, and any subsequent effect on the rate of CSF formation, following acute ischemic changes of the brain and choroid plexus by means of bilateral carotid ligation.

Materials and Methods

Adult albino rabbits weighing between 1.5 to 3 kg were used. The animals were anesthetized with sodium thiobarbiturate intravenously with an initial dose of 20 mg per kg body weight. Small supplemental doses were added when necessary. In these animals, the common carotid arteries were ligated bilaterally for the complete cessation of carotid blood flow. Femoral arterial pressure, internal jugular venous pressure and cisternal fluid pressure were monitored by a strain gauge electric manometer (Nihon Kohden, Type MP-4) throughout the experiment.

Enzymatic Study

At 1, 2 and 24 hours following the bilateral carotid ligation, the animals were sacrificed by an overdose of sodium thiobarbiturate. The brains were removed rapidly, and the choroid plexus of the lateral ventricles and the brain tissue in the frontal lobe were dissected and weighed. Immediately before the enzymatic assay, these tissues were homogenized in distilled water which was maintained at a temperature less than 5°C with a cold all-glass homogenizer. Determination of the concentration of hemoglobin in the tissue homogenate was made by the cyanohematin method.

Enzyme Assay

Acid and alkaline phosphatases were measured by Kind and King's modification of the phenylphosphate method, using 0.1 ml of 1%W/V choroid plexus homogenate or 10% W/V brain tissue homogenate. Carbonic anhydrase activity was measured by the method of Maren modified by Nishimura, using 0.1% (for choroid plexus) or 0.5% (for brain tissue) homogenate, ATPase was analyzed according to the method described by Bonting et al.; 0.1 ml of 5% (for choroid plexus) or 10% (for brain tissue) homogenate was added to 0.8 ml of each substrate media and 0.1 ml of pH 7.5, 1/5 M Tris-buffer. The composition of the media (in mM final concentration) was as follows, medium 1: ATP Na-salt 2, Mg+ 1, K+ 5, Na+ 58, CN- 10, EDTA 0.1; and medium 2: 10-4 ouabain, which is an inhibitor of Na-K ATPase, was added to the medium 1. After incubation and addition of 10% W/V trichloroacetic acid, the free inorganic phosphate in the supernatant was determined colorimetrically as described by Fiske and Subbarow. Activity of Na-K activated ATPase (Na-K ATPase) was measured from the difference between the activities in medium 1 and 2. Except for carbonic anhydrase, enzyme activities were expressed as micro moles of their final product per gram tissue weight per hour. Carbonic anhydrase was shown by the activity unit per 100 mg tissue weight according to the description by Nishimura.

Ventriculocisternal Perfusion

In order to estimate the rate of formation of CSF, ventriculocisternal perfusion was carried out in 16 rabbits. Eight animals were subjected to bilateral carotid ligation after a steady state of perfusion was reached. The remaining 8 animals served as controls. The method of ventriculocisternal perfusion was previously described by us in detail. Perfusion fluid consisted of mock CSF to which was added 80 mg/100 ml inulin. The rate of perfusion was 47 ml/min. Collection of samples from the outflow tube was carried out at 15-minute intervals over a 3 hour perfusion period. Since the steady-state condition was reached an hour after the beginning of the perfusion, the first four successive samples were discarded. If blood appeared in the effluent at any time, the experiment was terminated. The quantity of each sample was measured gravimetrically.

Measurement of Vertebral Blood Flow

Blood flow rate of the vertebral artery was measured before and after bilateral carotid ligation in 4 rabbits. Animals were anesthetized with intravenous sodium thiobarbiturate, and
the right vertebral artery was exposed just after ramifying from the brachiocephalic trunk. The rate of vertebral blood flow was measured with an electromagnetic flow meter (Nihon Kohden, Type MF 26) for 2 hours following the carotid interruption.

**Observation of Blood Supply to the Choroid Plexus**

Bilateral carotid arteries and one vertebral artery were exposed in anesthetized rabbits. Polyethylene catheters were inserted into the lumina of one carotid and one vertebral artery, and the jugular veins were opened bilaterally. After flushing of cerebral blood vessels with 200 ml of saline, colored synthetic resin (Epolak N-6800, Japan Catalyst Chemical Industry Co.) was injected by manual pressure simultaneously into the carotid and vertebral arteries. Red resin was injected into the carotid artery and blue resin was injected into the vertebral artery. After an hour, when the resins had polymerized, the brain was removed and the choroid plexus was dissected preserving its feeding vessels.

**Results**

**Enzyme Activities in the Choroid Plexus and the Brain Tissues (Table 1)**

**Acid Phosphatase:** The measured acid phosphatase activity of the control level in the choroid plexus averaged 65.7 µM phenol/hour/gm tissue wt. Double of that in the brain tissue in the frontal lobe (25.2 µM phenol/hour/gm tissue wt). The activity in the choroid plexus showed a remarkable increase throughout the experimental period. It was about 1.5 times higher than the control level at 1 and 2 hours after the carotid ligation, and it remained higher than the control even after 24 hours. In the brain tissue, however, there appeared to be a 25% loss of activity at one hour and then it increased to the control level after 24 hours.

**Alkaline Phosphatase:** In normal rabbits, the averaged activities of alkaline phosphatase of the choroid plexus and brain tissue were 1,220 and 97.4 µM phenol/hour/gm tissue wt, respectively. The activity was approximately ten times higher in the former than in the latter. The activity in the choroid plexus remained unchanged at one hour after the carotid ligation, while it increased by 50% after 2 hours, and then decreased to the control level at 24 hours. Changes in the enzyme activity in brain tissue appeared to be different from those in the choroid plexus. The activity decreased gradually until 2 hours (up to 30% decrease from the initial level) and then regained its normal value after 24 hours.

**Carbonic Anhydrase:** The most conspicuous change was observed in the carbonic anhydrase activity of the choroid plexus. While the control level of the activity was similar to the level in brain tissue (84.0 unit/100 mg tissue wt in the choroid plexus and 73.9 unit/100 mg tissue wt in the brain tissue), the activity in the choroid plexus increased rapidly following the carotid ligation and reached double the control after 2 hours. Even after 24 hours from the carotid interruption, it was still higher than the control level. On the other hand, in brain tissue the activity of this enzyme appeared to be sharply different from that in the choroid plexus. It steadily fell throughout the experimental period and dropped to two thirds of the control level at 24 hours after the carotid ligation.

**ATPase:** In contrast to the other enzymes, remarkable change was not observed in the activity of total and Na-K ATPase in the choroid plexus throughout the experimental period. Though a slight decrease of the activity was observed at 24 hours after the carotid ligation, there was no statistical significance among these values. In the brain tissue, however, total ATPase activity decreased gradually as time elapsed and a similar tendency was also seen in Na-K ATPase activity without any statistical significance.

**Hemoglobin Content in the Choroid Plexus:** Hemoglobin concentration in the choroid plexus was as shown in Table 2. Although the hemoglobin concentration increased to approximately twice the control value 24 hours after the carotid ligation, no changes were observed after 1 and 2 hours. Therefore, the blood volume in the choroid plexus might not be altered until 2 hours after the carotid ligation. In the brain tissue, the hemoglobin volume was too small to be determined colorimetrically.

**Rate of CSF Formation:** The control value of steady state CSF formation rate before the carotid ligation averaged 11.8 ± 0.5 µl/min in 8 rabbits. Changes of the formation rate of CSF following bilateral carotid ligation were plotted...
Table 1  Enzyme activities in the choroid plexus and brain tissue before and after the bilateral carotid ligation. Numerals represent mean values with standard errors. Number of experiments is indicated in parentheses. Significant changes from control values are indicated with the corresponding p values (* p <0.01; ** p <0.05). Enzyme activity units are expressed as μM phenol/hr/gm in acid and alkaline phosphatases, unit/100mg tissue wt in carbonic anhydrase, and μM P split/hr/gm tissue wt. in total and Na–K ATPases.

<table>
<thead>
<tr>
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<th>Acid Phosphatase</th>
<th>Alkaline Phosphatase</th>
<th>Carbonic Anhydrase</th>
<th>Total ATPase</th>
<th>Na–K ATPase</th>
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<tr>
<td><strong>Choroid Plexus</strong></td>
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<tr>
<td>Control</td>
<td>65.7 ± 3.1 (8)</td>
<td>1220.0 ± 48.8 (8)</td>
<td>84.0 ± 9.0 (5)</td>
<td>450.7 ± 22.7 (8)</td>
<td>85.9 ± 18.6 (8)</td>
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<td>1 hour</td>
<td>84.4 ± 4.2* (8)</td>
<td>1230.3 ± 125.5 (8)</td>
<td>143.8 ± 13.2* (6)</td>
<td>442.3 ± 26.8 (8)</td>
<td>77.9 ± 10.8 (8)</td>
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<td>2 hours</td>
<td>89.3 ± 5.9* (7)</td>
<td>1812.6 ± 142.0* (8)</td>
<td>161.0 ± 8.2* (6)</td>
<td>487.9 ± 35.4 (7)</td>
<td>95.8 ± 19.2 (7)</td>
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<td>24 hours</td>
<td>80.9 ± 5.5* (7)</td>
<td>1285.6 ± 57.0 (7)</td>
<td>105.0 ± 6.4 (6)</td>
<td>417.8 ± 37.0 (7)</td>
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<tr>
<td>Control</td>
<td>25.2 ± 2.1 (5)</td>
<td>97.4 ± 3.4 (5)</td>
<td>73.9 ± 3.7 (7)</td>
<td>316.5 ± 15.9 (5)</td>
<td>133.7 ± 11.8 (5)</td>
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<td>1 hour</td>
<td>19.6 ± 2.5 (6)</td>
<td>84.1 ± 7.5 (5)</td>
<td>51.9 ± 9.8** (6)</td>
<td>332.6 ± 16.7 (6)</td>
<td>137.4 ± 11.3 (6)</td>
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<td>2 hours</td>
<td>22.0 ± 4.6 (5)</td>
<td>63.3 ± 7.3* (5)</td>
<td>54.8 ± 1.5* (5)</td>
<td>291.4 ± 33.2 (5)</td>
<td>116.0 ± 23.6 (5)</td>
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<td>24 hours</td>
<td>26.3 ± 3.7 (6)</td>
<td>94.4 ± 6.3 (6)</td>
<td>49.9 ± 6.8* (6)</td>
<td>235.2 ± 35.3** (6)</td>
<td>108.2 ± 12.7 (6)</td>
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in Fig. 1 at 15-minute intervals up to 2 hours. The mean values of the rate gradually decreased only slightly as time passed. At the period of 45–60 min and 105–120 min after the carotid ligation, the rate of formation decreased by 8% and 16%, respectively. However, when this declining curve of the formation rate was superimposed on that of the control experiment, no difference was observed between these two groups.

Changes in Arterial, Venous and Cisternal Pressure: Fig. 2 shows the changes in mean arterial blood pressure, jugular venous pressure and cisternal fluid pressure before and after the bilateral carotid ligation. Systemic blood pressure elevated immediately after the carotid ligation and a level of 10–20% higher than the control was maintained throughout the experimental period. Jugular venous pressure showed some fluctuations but no remarkable changes were observed throughout the experiment. Cisternal pressure tended to increase gradually as time passed, although statistically it was not significant.

Vertebral Blood Flow Rate: The purpose of this experiment was to investigate the change of vertebral blood flow rate before and after the carotid ligation, and to prove that the choroid plexus maintained normal blood circulation even after the bilateral carotid ligation.

The blood flow rate of the one carotid artery in the control period varied from 2 to 12 ml/min, averaging 6.3 ml/min in 4 rabbits. Immediately after the bilateral carotid ligation, the flow rate of the vertebral artery increased rapidly and maintained a level 2.5–3 times higher than the control value for up to 2 hours. (Fig. 3).

Observation of the Blood Supply to the Choroid Plexus with Intravascular Resin Cast: The choroid plexus of the lateral ventricle received its blood supply from two sources. One is the anterior choroidal artery which is a branch of the internal carotid artery. This artery enters the choroid fissure after branching from the internal carotid artery and supplies the posterior half of the choroid plexus. The other is the posterior choroidal artery which is given off from the posterior cerebral artery and supplies the anterior half of the choroid plexus in the lateral ventricle.

When red resin was injected into the internal
carotid artery and blue resin was injected into the vertebral artery, the anterior and posterior choroidal arteries became red and blue, respectively. Accordingly, the anterior part of the choroid plexus was dyed blue and the posterior part of it was stained red (Fig. 4). If the common carotid arteries were ligated bilaterally, and blue resin was injected into the vertebral artery, both internal carotid and vertebrobasilar systems were stained blue due to the flow through the posterior communicating artery (Fig. 5). In this case, the entire choroid plexus was stained blue. Such observations indicate that the blood supply of the choroid plexus within the lateral ventricle is easily exchanged in either direction of flow on the basis of the dual source of blood supply.

**Discussion**

Histochemical studies revealed that acid phosphatase is located entirely intracellularly in the neurones and the choroid plexus. This enzyme is thought to be concerned with the formation of CSF in relation to the metabolism within the choroid plexus cells. Becker showed that acid phosphatase activity was increased in the choroid plexus under the condition of hypervitaminosis A which led to hydrocephalus in experimental animals. Thus, he suggested that increased activity of this enzyme might correlate to the increased formation of CSF.

On the other hand, alkaline phosphatase was confined mainly to the wall of blood vessels in the brain and the choroid plexus and no activity was found in the parenchyma of any other region of the brain. It is also reported that alkaline phosphatase is concerned with the
permeability of the blood vessel\textsuperscript{17} as well as the blood-brain barrier.\textsuperscript{5,32,41} According to Samorajski and McClaus,\textsuperscript{32} damaged blood vessels in the brain contained an increased level of alkaline phosphatase activity, and they suggested that an increased activity of this enzyme in the vascular endothelium could be interpreted as signifying an increased transmitting function across the barrier.

Carbonic anhydrase is also thought to be concerned with the formation of CSF. It has been demonstrated that acetazolamide, an inhibitor of carbonic anhydrase, potentially inhibits the rate of CSF formation.\textsuperscript{25,38,40,42} According to Giacobini,\textsuperscript{16} carbonic anhydrase is selectively concentrated in the glial and choroid plexus cell, and this enzyme is implicated in a mechanism for active transport of chloride from the capillaries to the interstitial fluid and CSF.

Na-K ATPase is considered to be involved in the active linked transport of sodium and potassium across the cell membrane, while \textit{in vitro} as well as \textit{in vivo} studies have revealed that this enzyme was inhibited completely with certain concentration of ouabain.\textsuperscript{39,35} This drug is also reported to inhibit the rate of CSF formation in a manner similar to acetazolamide.\textsuperscript{1,39,40} This observation suggests that Na-K ATPase in the choroid plexus has an important role in the production of CSF.

In the present study, the activities of three of the above four enzymes were apparently increased in the choroid plexus tissue following the carotid ligation, whereas those in the frontal brain tissue were depressed considerably as a result of the anoxic-ischemic condition of the brain, as Yap and Spector\textsuperscript{44} have demonstrated in their histochemical studies. From these results, it is likely that the function of the choroid plexus, in relation to the permeability of the vessels as well as the production of CSF, may be enhanced following carotid ligation, at least for a certain transient period. This is in contrast to the depressed function observed with anoxic-ischemic changes in the brain tissue.

Nevertheless, considering the fact that some of these enzymes show high activity in the blood, a question arises as to whether or not the observed changes of the enzyme in such vascular tissue would result from the change of the blood volume within it. In order to elucidate this problem, gross estimation of the blood volume in the choroid plexus was made by a quantitative analysis of hemoglobin under the condition of carotid ligation. Consequently, no significant changes of hemoglobin concentration were observed for periods of 1 to 2 hours after the bilateral carotid ligation. Therefore, the concept that increased activities of the enzyme might be due to the increase of blood volume of the choroid plexus seems unlikely.

Among the four enzymes tested, only Na-K ATPase showed no significant changes of activity in both tissues, either of its absolute value or of its proportion to the total ATPase following bilateral carotid ligation. This discrepancy of the changes in activities of these enzymes in the face of an anoxic-ischemic condition is difficult to explain on the basis of the present experiment. The only probable explanation concerns the different susceptibility of each enzyme to sudden mechanical or chemical stress within the plexus or the brain tissue. In this context, Wright's observation\textsuperscript{43} that sodium transport across the choroidal epithelium was not influenced by hypoxia may provide a valuable reference in interpreting this problem, on the basis of the fact that the energy for Na\textsuperscript{+} transport is derived from ATPase.

In the histochemical studies it has been demonstrated that various enzyme activities in the brain tissue decrease under anoxic-ischemic conditions within 24 hours, followed by a gradual increase thereafter.\textsuperscript{44} The same tendency was seen in the present study concerning some of the enzyme activities in the frontal lobe tissue.

Although systemic blood pressure elevates after bilateral carotid ligation,\textsuperscript{15,22} as noted in the present study, cerebral blood flow decreases. This was confirmed not only totally by means of the \textsuperscript{133}Xe method in baboons\textsuperscript{33} but also regionally in the frontoparietal region of rats using antipyrine-\textsuperscript{14}C.\textsuperscript{11}

In the choroid plexus, however, there is a dual source of blood supply as mentioned above, so that it is conceivable that the complete cessation of blood supply may not occur in this tissue even if the carotid arteries are bilaterally occluded. Symon et al.\textsuperscript{36} reported that total cephalic blood flow was kept at 70% of normal after bilateral carotid ligation. In order to maintain such a level, they reported that vertebral blood flow more than doubles that of the control
level after bilateral carotid ligation. The present observation of direct measurement of vertebral blood flow after the bilateral carotid ligation was quite consistent with the above result, i.e. vertebral blood flow rate increased to 2.5 to 3 times higher than the control level after the carotid ligation.

It is known that certain physical forces exert their marked effects upon the production rate of CSF. We observed that changes of the osmotic pressure gradient between both sides of the choroidal epithelium resulted in an immediate shift of flow of the fluid in either direction. The rate of CSF formation is also affected by the change of cerebral blood flow. Particularly, blood flow through the choroid plexus may be a rate limiting factor in CSF production.

Considering the correlation of the observed enzymes with secretory function of the choroid plexus, it is reasonable to postulate that the reflectoric hyperfunction of this tissue may take place at least in a transient period following sudden interruption of the carotid blood flow. In contrast to our expectation, however, ventriculocisternal perfusion did not yield any change in the rate of CSF formation after bilateral carotid ligation.

In order to make clear the reason for this discrepancy in the results from the enzyme study and the perfusion study, the blood flow rate within the choroid plexus and the brain separately during an acute phase following the bilateral carotid ligation should be known. Moreover, it should also be known how efficient the alternative blood supply to the choroid plexus from the vertebral arterial system is when the choroid source of supply is interrupted in this species.

Since the measurement of the choroidal blood flow is technically difficult, an attempt was made to examine the efficacy of compensated blood supply to the choroid plexus on the anatomical basis using the injection with colored plastic materials in rabbits. It was revealed that the choroid plexus received a reasonable amount of blood through the increased flow of the vertebral-posterior choroidal arterial system as well as alternative blood flow into the anterior choroidal artery via the posterior communicating artery even if the internal carotid route was occluded. From the combination of the results from direct measurement of the vertebral blood flow and from the resin cast study, it is conceivable that the choroid plexus blood flow is less affected by the sudden occlusion of the carotid arterial supply.

However, these observations do not explain why the activities of some enzymes in the choroid plexus tissue were enhanced transiently after bilateral carotid ligation. Therefore, experiments for the direct measurement of the choroidal blood flow rate using the hydrogen clearance method are in progress.

Recent evidence suggests that a significant proportion of CSF derives from extrachoroidal sites, although the exact location of these sites and their proportion of the rate of fluid formation in comparison with that of the choroid plexus have not been determined accurately. Recent evidence suggests that extrachoroidal CSF originates from the brain. If the concept that the secretory function of the choroid plexus cells is stimulated transiently after bilateral carotid ligation on the basis of the result from enzyme study is correct, stimulated secretory activity might reflect the overproduction of CSF from the choroid plexus while the production of CSF within the brain may more or less decrease. Thus the rate of CSF formation in total might not be altered even in face of such an abrupt change of cerebral blood flow.

In conclusion, it is suggested that delicate homeostatic regulation of the volume flow may be involved in the mechanism of CSF production against sudden change of cerebral blood flow for at least a transient period in order to maintain normal water metabolism within the central nervous system.

References


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