Enzymological Studies on the Choroid Plexus Following Bilateral Carotid Ligation

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INTRODUCTION

Secretory function of the choroid plexus was first demonstrated by Dandy in 1919,[7] by the observation that dilatation of the lateral ventricle did not occur but it entirely collapsed when the choroid plexus was completly removed on the animal whose foramen of Monro had been occluded Despite this observation was later controverted by Bering,[4] the choroid plexus is generally regarded as a source of the cerebrospinal fluid, at least of a portion of it.

Although the precise mechanism of the production of cerebrospinal fluid is not clear, morphological[24] as well as biochemical[8] evidences support the secretion of the fluid by the choroid plexus.

Moreover, observations on the strict regulation mechanism for transport of the composition of cerebrospinal fluid across the plexus[1][15] is interpreted as a metabolic property of the choroid plexus which may be the storong support to the secretion theory.

As regards metabolic studies of the choroid plexus, Stiehler and Flexner[34] showed that the activity of indophenol oxidase was high in the epithelial cells of the plexus, and later Friedenwald[11] found the high activities of some respiratory enzymes such as lactic, maleic, and succinic dehydrogenases in the choroid plexus. Recent studies on the choroid plexus revealed that this tissue was found to contain various enzymes, such as alkaline[9][16][18][29] and acid phosphatases,[3][6][16][18] carbonic anbydrase,[9][13] some dehydrogenases,[9][16] cholin esterase,[9] cytochrome oxidase,[9][16] and adenosine triphosphatase (ATPase),[5][16][36][39] and there were many obvious evidences that some of these enzymes were concerned with the production of cerebrospinal fluid.[3][6][13][14][21][37][38][39]

On the other hand, since Levine[19] investigated the experimental anoxic ischemic encephalopathy in rat, there has been many histochemical studies related to anoxic ischemic encephalopathy,[2][22][31][32][34][41] while little is known about the metabolic activities of the choroid plexus in the anoxic-ischemic condition.[33]

The purpose of this study is to investigate biochemically changes of enzymic activity in the choroid plexus in correlation with the production of cerebrospinal

fluid, such as alkaline and acid phosphatases, ATPase, and carbonic anhydrase, following bilateral carotid ligation.

MATERIAL AND METHOD

Seventy five adult rabbits of both sexes weighting between 1.5 to 2.5 kg were used. The animals were anesthetized with sodium thiobarbiturate intravenously with an initial dose of about 20 mg per kg body weight and small supplemental doses if necessary. In those animals the bilateral common carotid arteries were ligated for the complete cessation of carotid blood flow. During the experiments femoral arterial pressure, internal jugural venous pressure, and cisternal pressure were recorded with strain gauge electronic manometer (Nihon Kohden, Type MP-4) in every a half hour.

At the period of 1, 2, and 24 hours following the bilateral carotid ligation, the animal was sacrified by an overdose of sodium thiobarbiturate. Then the brain was removed rapidly. Then the choroid plexus of the lateral ventricles and the brain tissue in the frontal lobe were dissected and weighed. Immediately before the enzymic assay these tissues were homogenized in distilled water maintained at a temperature less than 5°C with a cold all-glass homogenizer.

The activity of acid and alkaline phosphatases, ATPase, and carbonic anhydrase was determined biochemically by means of the following methods in the homogenized tissues.

Determination of the concentration of hemoglobin in the tissue homogenate was made by the cyanhaematin method.[42]

Enzyme assay. Acid and alkaline phosphatases were measured by the Kind-King's modification of the phenylphosphate method,[17] using 0.1 ml of 1 % w/v choroid plexus homogenate or 10 % w/v brain homogenate.

ATPase was studied according to the method described by Bonting et al:[5] 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, 1/5M 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. medium 2; 10^{-4}M ouabain, which is the inhibitor of Na-K ATPase, added to the medium 1.

After incubation and adding of 10 % w/v trichloroacetic acid, the free inorganic phosphate in supernatant was determined colorimetrically as the description by Fiske and Subbarow.[10] Na-K activated ATPase (Na-K ATPase) activity was measured from the difference between the activities in medium 1 and 2.

Carbonic anhydrase activity was measured with the method of Maren[20] modified by Nishimura,[23] using 0.1 % (for choroid plexus) or 0.5 % (for brain tissue) homogenate.

Except for carbonic anhydrase, enzyme activities were expressed as micro moles of their final products 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.

Changes in blood flow rate of the vertebral artery after bilateral carotid ligation. Dogs were chosen for the experimental animal, because the vertebral artery of rabbit was too slender to put on a probe of the electromagnetic flowmeter in use.

Adult dogs (about 10 kg body weight) were anesthetized with about 35 mg per kg body weight intravenous sodium thiobarbiturate. Systemic blood pressure was monitored in every experiments at the femoral artery using electronic manometer. The rate of vertebral blood flow was measured with an electromagnetic flowmeter (Nihon Kohden Type M F-2) at the most proximal portion of the operatively exposed right vertebral artery before and after the complete ligation of both common carotid arteries at the period of every 15 minutes untill 2 or 3 hours following the carotid interruption.

RESULTS

Enzymic studies on the choroid plexus and the brain tissue

It was shown that, in the normal animals, the activity of alkaline phosphatase of the choroid plexus was appeared to be 1220.0 ± 48.8 μM phenol/hour/gm. tissue wt. in average, about ten times higher than that of the brain tissue in the frontal lobe; 97.4 ± 3.4 μM phenol/hour/gm. tissue wt. in average. After the bilateral carotid ligation alkaline phosphatase activity changed markedly both in the choroid plexus and the frontal lobe. The activity in the choroid plexus remained unchanged at an hour after the carotid ligation, while it increased to 1812.6 ± 142.0 μM phenol/hour/gm. tissue wt. in average after 2 hours, one and a half times higher than the control level, and then decreased to the control level at 24 hours after the carotid ligation.

Changes of the enzyme activity in the frontal lobe appeared to be different from that in the choroid plexus. The activity in the frontal lobe decreased gradually untill 2 hours after the carotid ligation at which time the activity showed about 30 per cent decrease from the initial level, 63.3 ± 7.3 μM phenol/hour/gm. tissue wt. in average, and then regained its normal activity after 24 hours (Table 1).

Table 1. Alkaline phosphatase activity

	choroid plexus	frontal lobe
control	1220.0 ± 48.8 (8)	97.4 ± 3.4 (5)
1 hour	1230.3 ± 125.5 (8)	84.1 ± 7.5 (5)
2 hours	1812.6 ± 142.0 (8) $p<0.005$	63.3 ± 7.3 (5) $p<0.005$
24 hours	1285.6 ± 57.0 (7)	94.4 ± 6.3 (6)

Numerals represent mean values with standard errors. Number of animals is indicated in parentheses. Significant changes from control values are indicated with the corresponding p values. Enzyme activity units are expressed as μM phenol/hr./gm. tissue wt.

Acid phosphatase activity of the control level in the choroid plexus was measured as 65.7 ± 3.1 μM phenol/hour/gm. tissue wt. in average, double of that in the frontal lobe; 25.2 ± 2.1 μM phenol/hour/gm. tissue wt. in average. Remarkable changes of the activity after the carotid ligation was observed on acid phosphatase as likely as alkaline phosphatase. The activity in the choroid plexus showed a remarkable increase throughout the experimental period; up to 89.3 ± 5.9 μM phenol/hour/gm. tissue wt. in average, about 1.5 times as higher as the control level at 1 and 2 hour period after the carotid ligation and it still remained in higher level than the controls even after 24 hours. In the frontal lobe, however, there appeared to be 25 per cent loss of activity at an hour after the carotid ligation; 19.6 ± 2.5 μM phenol/hour/gm. tissue wt. in average, and then it increased to the control level after 24 hours (Table 2).

Table 2. Acid phosphatase activity

	choroid plexus	frontal lobe
control	65.7 ± 3.1 (8)	25.2 ± 2.1 (5)
1 hour	84.4 ± 4.2 (8) $p<0.005$	19.6 ± 2.5 (6)
2 hours	89.3 ± 5.9 (7) $p<0.005$	22.0 ± 4.6 (5)
24 hours	80.9 ± 5.5 (7) $p<0.02$	26.3 ± 3.7 (6)

Numerals represent mean values with standard errors. Number of animals is indicated in parentheses. Significant changes from control values are indicated with the corresponding p values. Enzyme activity units are expressed as μM phenol/hr./gm. tissue wt.

In contrast to the above two enzymes, remarkable change was not seen in the activity of both total and Na-K ATPase in the choroid plexus throughout the experiment. 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 frontal lobe, however, total ATPase activity decreased gradually as time passed and similar tendency of the change of the activity was also observed in Na-K ATPase activity without any statistical significance (Table 3).

Table 3. Total and Na-K ATPase activity

Total ATPase

	choroid plexus	frontal lobe
control	450.7 ± 22.7 (8)	316.5 ± 15.9 (5)
1 hour	442.3 ± 26.8 (8)	332.6 ± 16.7 (6)
2 hours	487.9 ± 35.4 (7)	291.4 ± 33.2 (5)
24 hours	417.8 ± 37.0 (7)	235.2 ± 35.3 (6) $p<0.05$

Na-K ATPase

	choroid plexus	frontal lobe
control	85.9 ± 18.6 (8)	133.7 ± 11.8 (5)
1 hour	77.9 ± 10.8 (8)	137.4 ± 11.3 (6)
2 hours	95.8 ± 19.2 (7)	116.0 ± 23.6 (5)
24 hours	71.7 ± 14.0 (7)	108.2 ± 12.7 (6)

Numerals represent mean values with standard errors. Number of animals is indicated in parentheses. Significant changes from control values are indicated with the corresponding p values. Enzyme activity units are expressed as μM P split/hr./gm. tissue wt.

The most conspicuous change was observed in the carbonic anhydrase activity of the choroid plexus. While the control level of the activity in the choroid plexus was as same level as that in the frontal lobe tissue (choroid plexus; 84.0 ± 9.0 unit/100mg tissue wt. in average, frontal lobe; 73.9 ± 3.7 unit/100mg tissue wt in average), the activity in the choroid plexus increased rapidly and reached to the double of the control level after 2 hours; 161.0 ± 8.2 unit/100mg tissue wt. in average. And even after 24 hours the activity was still elevated above the control level; 105.0 ± 6,5 unit/100mg tissue wt. in average. Otherwise, the changes of the activity of this enzyme in the frontal lobe tissue

appeared to be sharply contrasted to the choroid plexus; it steadily fell throughout the experimental period and reached to two thirds of the control level at 24 hours after the carotid ligation; 49.9 ± 6.8 unit/100mg tissue wt. in average (Table 4).

Table 4. Carbonic anhydrase activity

	choroid plexus	frontal lobe
control	84.0 ± 9.0 (5)	73.9 ± 3.7 (7)
1 hour	143.8 ± 13.2 (6) $p<0.01$	51.9 ± 9.8 (6) $p<0.05$
2 hours	161.0 ± 8.2 (6) $p<0.005$	54.8 ± 1.5 (5) $p<0.005$
24 hours	105.0 ± 6.4 (6)	49.9 ± 6.8 (6) $p<0.01$

Numerals represent mean values with standard errors. Number of animals is indicated in parentheses. Significant change from control values are indicated with the corresponding p values. Enzyme activity units are expresed as activity unit/100mg. tissue wt.

Hemoglobin concentration in the choroid plexus is shown in Table 5. Although the hemoglobin concentration increased to about double of the control value at the period of 24 hours after the carotid ligation, no changes were observed after 1, and 2 hours. Therfore, the blood volume in the choroid plexus might not altered until 2 hours after the carotid ligation. In the frontal lobe, hemoglobin volume was too little to determine colorimetorically (Table 5).

Table 5. Hemoglobin concentration in the choroid plexus

control	10.9 ± 1.7 (6)
1 hour	13.6 ± 1.3 (5)
2 hours	11.4 ± 2.8 (5)
24 hours	23.3 ± 4.1 (5)

Numerals represent mean values with standard errors.
Number of animals is indicated in parentheses.
Concentration units are expressed as hemoglobin mg/gm. tissue wt.

Table 6 shows the data on the systemic blood pressure, vonous pressure, and cisternal pressure before and during 2 hours after the carotid ligation. No change of the systemic blood pressure was observed during the experimental period, while venous pressure showed some fluctuations throughout the experiment. Cisternal pressure increased gradually as time passed, and reached at the level of 1.2 times higher than the control pressure, probably due to the cerebral edema following the carotid ligation (Table 6).

Table 6. Femoral arterial pressure, internal jugural pressure and cisternal pressure during experiments

	F.A.P. (mm Hg)	J.V.P. (mm H_2O)	C.P. (mm H_2O)
control	70.1 ± 3.8 (8)	−55.2 ± 1.9 (8)	21.9 ± 1.3 (9)
carotid ligation	71.0 ± 6.9 (5)	−53.6 ± 5.1 (8)	22.7 ± 1.7 (6)
30 min.	66.8 ± 6.6 (5)	−55.7 ± 10.3 (6)	24.0 ± 2.8 (6)
60 min.	71.5 ± 8.9 (5)	−63.7 ± 10.7 (6)	24.5 ± 2.1 (6)
90 min.	70.2 ± 6.7 (5)	−51.2 ± 7.5 (6)	25.1 ± 1.5 (6)
120min.	67.2 ± 4.8 (5)	−53.9 ± 11.7 (6)	26.7 ± 1.7 (6)

Numerals represent mean values with standard errors.
Number of animals is indicated in parentheses.
F.A.P.; femoral arterial pressure. J.V.P.; internal jugral pressure. G.P.; cisternal pressure.

Changes in blood flow rate of the vertebral artery before and after the carotid ligation

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 was held the normal blood circulation even after the bilateral carotid ligation.

Vertebral blood flow rate was measured as between 25 and 44 ml/min. in three anesthetized dogs. It elevated rapidly and reached to the steady state level, 2.5 times higher than the control level, within 15 minutes after the carotid ligation. The flow rate was maintained at the constant level after the steady state was reached during the experimental period until 2 or 3 hours after the carotid ligation (Fig. 1).

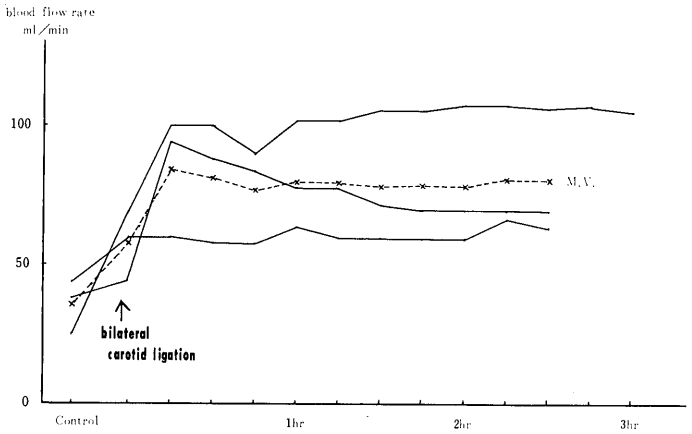

Fig. 1. Vertebral blood flow rate before and affer carotid ligation (Dog)

DISCUSSION

Histochemical studies in anoxic-ischemic condition of the brain have been made by many invastigators,[2)22)31)32)33)41)] while little has been known on the changes of enzyme activity in the choroid plexus under such pathological conditoin.[33)] Although the changes of enzyme activity in the localized portion of the brain such as the frontal lobe has not been observed previously, the activities on the cerebral tissue of the frontal lobe might be parallel to other regions in the brain. In this study, considerable decrease in activities of alkaline and acid phosphatase, carbonic anhydrase and ATPase in the frontal lobe were verified after the carotid ligation.

On the other hand, high activities of these four enzymes in the choroid plexus have been reported.[5)6)9)16)18)36)39)]

Histochemical studies revealed that alkaline phosphatase was confined mainly to the wall of blood vessel in the brain and in the choroid plexus, and no activity was demonstrable in the parenchyma of any other region of the brain.[9)12)29)39)] It is also reported that alkaline phosphatase is concerned with the permeability of the blood vessel[14)] as well as the blood brain barrier.[6)28)39)] According to Samorajski,[28)] damaged blood vessel in the brain contained an increased level of alkaline phosphatase activity, and he suggested that an increased level of alkaline phosphatase activity in the vascular endothelium could be interpreted as singifying an increased transmitting function across the barrier.

Acid phosphatase is localized entirely intracellulary in the neurones and in the choroid plexus.[16)18)] This enzyme is also thought to be concerned with formation of cerebrospinal fluid in relation to the metabolism within the choroid plexus cells. Becker[3)] 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 acid phosphatase might correlate to the increased formation of cerebrospinal fluid.

Carbonic anhydrase is also thought to be concerned with the formation of cerebrospinal fluid. It is demonstrated that the inhibition of cerebrospinal fluid formation occurs by administration of carbonic anhydrase inhibitor; acetazolamide.[21)37)40)]

According to Giacobini,[13)] carbonic anhydrase is selectively concentrated in the glial and choroid plexus cell and this specific localization supports the view that carbonic anhydrase is implicated in a mechanism for active transport of chloride from the capillaries to the interstitial and cerebrospinal fluid.

In the present experiment, the activities of above three enzymes were apparently increased in the choroid plexus following the carotid ligation, whereas those in the frontal lobe brain tissue were depressed considerably as a result of anoxic-ischemic condition of the brain as Yap[41)] demonstrated the same results in his 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 cerebrospinal fluid, may be enhanced following the carotid ligation, at least for certain transient period, in contrast to the anoxic or ischemic changes in the brain tissues.

Nevertheless, considering the fact that some of these enzymes show their high activity in the blood, a question arises as to whether the observed changes of the enzymes in such vascular tissue would be the result of the change of 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 interruption.

Consequently, no significant changes of hemoglobin concentration were observed as long as the period of 1 to 2 hours after the bilateral carotid ligation, so that the concept that increased activities of the enzymes might be depend upon the increase of blood volume of the choroid plexus was unlikely.

Na-K ATPase is considered to be involved in the active linked transport of sodium and potassium across the cell membrane, while in vitro as well as in vivo studies revealed that this enzyme was inhibited completely with certain concentration of ouabain.[25)30)] Vates[38)] showed that administration of ouabain in high concentration caused almost complete inhibition of cerebrospinal fluid formation and concluded that choroid plexus Na-K ATPase activity had an effect on the formation of cerebrospinal fluid. This observation suggests that Na-K ATPase in the choroid plexus has an important role in the production of cerebrospinal fluid.

In this study, however, no significant changes were observed in Na-K ATPase

activity either of its absolute value or of its proportion to total ATPase.

This discrepancy of the changes in activities among these enzymes in the choroid plexus is unable to explain on the basis of the present experiment. Only probable explanation may be proposed from the basis of the different susceptibility in each enzyme to sudden mechanical or chemical stress within the plexus.

In histochemical studies it is demonstrated that various enzyme activities in the brain tissues are decreased in anoxic-ischemie condition within 24 hours, while it thereafter gradually increase.[41]

The same tendency was seen in the present experiment concerning some of the enzyme activities in the frontal lobe tissue (alkaline and acid phosphatase), whereas the others still kept its lower activity even 24 hours after the carotid ligation. In as much as the anatomical fact that the choroid plexus is supplied from both anterior and posterior choroidal arteries, it is conceivable that the complete cessation of blood supply may not occur in the choroid plexus even if the carotid arteries are bilaterally occluded. In other words, when the blood supply from the anterior choroidal artery is interrupted by the carotid ligation, the blood flow through the posterior choroidal artery via the vertebral basiler system seems to compensate the total flow within the plexus.

Symon[35] reported that total cephalic blood flow was kept at 70 per cent of the normal after the bilateral carotid ligation. In order to maintain such level, he described, vertebral blood flow increased above double of the control level after the carotid ligation.

The present observation in dogs quite agreed with above concept, i.e. vertebral blood flow rate increased to 2.3 times higher than the cohtrol level after the carotid ligation. From above results, it is assumed that the blood supply of the choroid plexus may be less affected by the carotid ligation.

It is known that certain physical forces exert marked effects on the production of cerebrospinal fluid. Any changes of blood cerebrospinal fluid osmotic pressure ratio would result in an immediate shift of flow of the fluid in either direction to maintain the balance in both phases of the barrier. Therefore, cerebrospinal fluid shift may occur with a physical manner to reform any change of cerebral blood volume.[26][27]

Considering the correlation of the observed enzymes with secretory function of the choroid plexus, it is reasonable to suggest that the reflectoric hyperfunction of this tissue may take place at least in a transient period following sudden interruption of the carotid blood flow, probably as a part of homeostatic mechanism involved.

However, whether such metabolic hyperfunction occured within the choroid plexus tissue would directly result in increase in the production of cerebrospinal fluid or not is a matter of question. Further studies in this problem should be

made in future.

SUMMARY

The activities of four enzymes which were thought to be correlating with the production of cerebrospinal fluid, such as alkaline and acid phosphatases, carbonic anhydrase and ATPase were determined biochemically in the choroid plexus as well as the frontal lobe tissue in adult rabbits.

The activities of alkaline and acid phosphatases and carbonic anhydrase were transiently enhanced in the choroid plexus after bilateral ligation of the carotid arteries, in spite of consistent decrease of their activities observed in the frontal lobe tissue under such condition. As to the ATPase, both total and Na-K activated ATPase, no significant change was observed in its activity in the choroid plexus, while in the frontal lobe tissue it decreased as similar tendency as the others.

Cisternal fluid pressure elevated gradually after the carotid ligation, but arterial and venous blood pressures were little affected by this procedure.

Vertebral blood flow rate was measured in doges, in order to estimate the change of blood flow within the choroid plexus under the complete interruption of the carotid blood flow. As a result, the flow rate of the vertebral artery was abruptly increased as double as the normal level following the bilateral carotid ligation.

Considering the correlation of examined enzymes with secretory function of the choroid plexus, it is suggested that the reflectoric hyperfunction of the choroid plexus may take place under the cessation of the carotid blood flow for at least a transient period.

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