Distribution of Amino Acid Activating Enzymes in Rabbit's Brain

In order to understand the brain function, it is considered to be necessary to study the regional pattern of enzymes and many compounds. Recently, the localization of protein metabolism in various brain areas has been studied by several investigators[1-3]; however, little work has been done to study regional distribution of individual enzymes participating in protein metabolism of the brain. LAJTHA[4] reported the distribution of proteinase activity in various brain areas. The present report describes some results on the distribution of amino acid activating enzymes in various brain areas, as one of our reports on the protein metabolism of brain tissue[5-8].

Rabbits weighing about 2.5 kg were killed by decapitation. The brains were removed immediately and cerebral cortex, cerebral white matter, hippocampus, hypothalamus, midbrain, cerebellum, medulla-pons and spinal cord were isolated. A portion of each tissue was transferred to a glass homogenizer tube and homogenized in one volume of medium A[9]. The homogenates were separated into a soluble fraction and a centrifugal residue by centrifugation for 90 min at 105000 g in a Spinco L centrifuge. A soluble fraction was used for the assay of amino acid activating enzymes. The assay procedures for amino acid activating enzymes were carried out using PP^{32}–ATP exchange method. 1 ml of incubation mixture for the PP^{32}–ATP exchange contained 3 μmoles ATP, 10 μmoles $MgCl_2$, 3 μmoles PP^{32} containing about 100000 counts/min, 100 μmoles Tris buffer, pH 7.8, an amino acid mixture containing 3 μmoles of each of 20 amino acids and the enzyme fraction. Incubation was carried out at 37° for 15 min. After incubation, the reaction was stopped with cold TCA (final concentration 5%) and PP and ATP were separated by charcoal absorption according to the method of CRANE and LIPMANN[10]. The concentration and radioactivities of ATP and pp were estimated as orthophosphate after these compounds had been hydrolysed in 1 N H_2SO_4 for 15 min at 100°C. The determination of inorganic phosphate was that of FISKE and SUBBAROW[11]. The radioactivity was determined with a RIDL Model 21 gas flow counter. The data were expressed as percent exchange according to HOAGLAND et al.[12]. P^{32} was obtained from Radiochemical Centre, Amersham, England. P^{32} labelled inorganic pyrophosphate (PP^{32}) was prepared by the method of KORNBERG and PRICER[13] and purified according to the modified procedure of COHN and CARTER[14]. Radiochemical analysis of the products showed that the contamination of P^{32} orthophosphate was less than 1%.

PP^{32}–ATP exchange reaction of the cell sap of rabbit brain areas was observed to be slight in the absence of amino acids. PP^{32}–ATP exchange reaction of the spinal cord in absence of the amino acid mixture was rather high; among various brain areas, however, little significant differences between various areas were observed (Table).

A rapid PP^{32}–ATP exchange reaction of the cell sap of various brain areas was observed in the presence of the amino acid mixture. While PP^{32}–ATP exchange of cerebral cortex and medulla-pons was more active, that of white matter, hippocampus and cerebellum was less active.

Increase in PP^{32}–ATP% exchange in the presence of an amino acid mixture was also shown in the Table. This increase may be regarded as the true activity of the amino acid activating enzymes. The value of cerebral cortex was highest, while that of cerebral white matter was lowest. Cerebellum, medulla-pons, midbrain and hippocampus showed intermediate values, and spinal cord a rather low value. The experimental number of hypothalamus was low.

The presence of amino acid activating enzymes in brain cell sap was first observed using hydroxamate method by LIPMANN[15] and general characteristics of these enzymes in brain were studied by our group[16]. When a comparison of the distribution of amino acid activating enzymes in various brain areas of rabbit was made with the results of the incorporation of radioactive amino acid

PP^{32}-ATP exchange in cell sap of different areas of rabbit's brain

Areas[a]	PP^{32}-ATP % exchange[b] Amino acid mixture (+)	Amino acid mixture (–)	Δ[c]	% Variation from cerebral cortex
Cerebral cortex	33.2	8.1	25.1	100
Cerebral white matter	25.5	10.3	15.2	61
Cerebellum	27.6	8.2	19.4	77
Medulla-Pons	33.5	11.3	22.2	88
Midbrain	30.7	10.1	20.6	82
Hypothalamus	27.2	7.5	19.7	79
Hippocampus	26.1	8.4	17.7	71
Spinal cord	29.9	12.5	17.4	70

[a] The values are means of 4–5 experiments except for hypothalamus (2 experiments). [b] PP^{32}-ATP % exchange/mg protein. [c] Increase due to addition of the amino acid mixture.

into the protein of various brain areas by various authors, including our group[17], some parallelism exists in that the high incorporation is in the cerebral cortex and the amino acid activating enzymes are also more active. In cerebral white matter and spinal cord, there is low incorporation and low amino acid activating enzyme activities. However, hippocampus showed high incorporation and inter-

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mediate amino acid activating enzymes activities. The amino acid activating enzymes of cerebellum, medulla, and midbrain did not show differences as significant as the incorporation results of the protein of these areas. It seems possible that the results with amino acid activating enzymes represent differences in the enzymes content or variation in the stability of these enzymes. It is not surprising that parallelism does not generally exist between the incorporation results and amino acid activating enzymes activities of various brain areas. Amino acid activating enzymes may not be the limiting factor in protein biosynthesis and may be present in excess to incorporate a radioactive amino acid into proteins of the various brain areas. More definite differences may be in the activity and content of ribosome and transfer enzymes in various brain areas[18].

Zusammenfassung. Untersuchungen an Aminosäure-aktivierenden Fermenten in den diversen Kaninchenhirnregionen, haben gezeigt, dass die Aktivität der Grosshirnrinde am höchsten, die der weissen Substanz des Grosshirns am niedrigsten ist, während sie in den übrigen Hirnteilen in der Mitte liegt.

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5-Hydroxytryptamine Content of Glioma

It is not known whether 5-HT is located in excitable neural tissue or in other types of cell in the central nervous system. The results of experiments in which C^{14} labelled 5-hydroxytryptophan (5-HTP) was injected into animals and its subsequent distribution on autoradiographs examined[1] have suggested that 5-HT may be contained in the glial cells and that its chief action is on the blood vessels around which glial cells cluster.

Information relevant to the location of 5-HT in the central nervous system may also be sought in the 5-HT content of cerebral neoplasms of different cell types and the present study compares estimates of 5-HT in human tissues classified as 'normal' cerebral cortex, 'glioma' and 'meningioma'.

Neoplastic tissues were obtained from two female and five male patients at the time of operation. Their ages ranged from 19 to 71 years. The neoplastic tissues were all from cerebral cortical areas except in case No. 4 where the tumour involved the basal ganglia. The tumours were classified by histological examination of a piece of abnormal tissue adjoining that used for the pharmacological examination. Samples of normal cerebral cortical tissue were also obtained when their removal was necessary for access to the tumour.

The tissues on which 5-HT estimations were to be carried out were collected directly from the operating theatre in containers at 0°C. Pieces of approximately 1 g in weight were extracted with four volumes of acetone according to CORREALE[2] and within 30 min of removal from the body. The brain extracts were taken up in distilled water and assayed biologically on the rat stomach by the method of VANE[3]. The potency of each individual extract was estimated at least four times. Hyoscine 10^{-7} was present in the bathing solution and the active substance assayed was identified as 5-HT by the action of BOL 10^{-7} at the end of each experiment. The methods used have already been fully described[4].

It was necessary to confirm that 5-HT loss after surgical removal occurs at the same rate in normal and neoplastic tissues. Normal tissue left at room temperature (20°C) lost 19% of its 5-hydroxytryptamine content in 24 h whereas the glioma lost 27%. These values are of the same order as those previously reported for cerebral cortical tissues[4].

The results in the Table show the mean 5-HT concentration of extracts from several tissue samples from the same patient. With one exception (case No. 4) and regardless of the nature of the tumour, 5-HT concentrations were found to be lower in the neoplasm than in the adjacent cerebral cortex. The one neoplasm which showed a higher concentration of 5-HT differed from the others in that the tumour involved the basal ganglia. This patient had also previously received high doses of antibiotic (penicillin and streptomycin) and although the oral administration of antibiotics has been found to cause an increase in platelet 5-HT[5] but not of brain 5-HT, the possibility must be considered that if a rise in platelet 5-HT does occur, the concentration of 5-HT may consequently increase in an area such as a neoplasm where the blood brain barrier has suffered a local breakdown. The most pronounced behavioural disturbance was also seen in this patient (No. 4)

5-Hydroxytryptamine (5-HT) concentrations in normal and pathological tissues

Patient No.	Age	Sex	Tumour	5-HT mean ng/g wet weight ± SE (n) Normal frontal lobe	Temporal lobe	Tumour
1	67	F	Meningioma	36.5±3.5 (2)		18.5±3.5 (2)
2	59	M	Meningioma	38.0 (1)		23.5±1.7 (4)
3	23	F	Glioma	54.5±7.5 (2)		20.5±3.5 (2)
4	71	M	Glioma	30.0 (1)		84.3±5.5 (3)
5	27	M	Glioma	59.3±8.1 (4)		31.1±2.8 (5)
6	39	M	Glioma		47.5±3.5 (2)	29.7±0.5 (3)
7	19	M	Oligodendroglioma			16.0±1.0 (2)

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