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ACTIVE TRANSPORT OF 5-HYDROXYINDOLEACETIC ACID BY THE RABBIT CHOROID PLEXUS *IN VITRO*

BLOCKADE BY PROBENECID AND METABOLIC INHIBITORS

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Abstract—When rabbit choroid plexuses were incubated at 37° with Krebs–Ringer solutions of [^{14}C]5-hydroxyindoleacetic acid (5-HIAA), the tissue took up the acid by a process showing all the characteristics of active transport. Uptake against a concentration gradient occurred by a saturable process that was inhibited by several metabolic inhibitors. The transport of 5-HIAA was also inhibited by probenecid and para-aminohippuric acid, known inhibitors of organic acid transport systems in many organs. 5-HIAA was found to bind to homogenates of choroid plexus, but the binding would not account for the bulk of acid accumulation seen in the intact tissue. Moreover, the transport of 5-HIAA in rabbit brain cortex slices was not affected by probenecid.

AFTER probenecid administration the concentration of 5-hydroxyindoleacetic acid (5-HIAA) in brain and cerebrospinal fluid (CSF) rises.[1] The rate of increase in 5-HIAA levels after probenecid is almost identical to that at which the acid is formed from serotonin (5-HT), and it has been concluded that probenecid blocks the mechanism that transports 5-HIAA from brain into the circulation.[2,3] This property of probenecid has been used to measure the turnover rate of serotonin synthesis,[2] and to measure brain monoamineoxidase activity *in vivo*[4]. However, little work has been done to localize and to determine the mechanism of transport of 5-HIAA in brain.

The present studies show that in the rabbit choroid plexus there is an active transport of 5-HIAA from the CSF into the blood, and that this transport is blocked by probenecid and by several metabolic inhibitors. Furthermore, in brain slices the transfer of 5-HIAA out of the neurons is not blocked by probenecid, suggesting that the active transport of 5-HIAA in brain is limited to the choroid plexus and similar vascular structures.

METHODS AND MATERIALS

Experiments with choroid plexus. Male New Zealand white rabbits weighing 1·8–2·2 kg were killed by an intravenous injection of air, and the brain was quickly exposed. The choroid plexuses of both lateral ventricles were excised, weighed and placed in a petri dish with Krebs–Ringer bicarbonate solution which was gassed with 95% O_2–5% CO_2 and consisted of 119 mM NaCl; 4·8 mM KCl; 25 mM $NaHCO_3$; 1·2 mM KH_2PO_4; 2·6 mM $CaCl_2$; 2·4 mM $MgSO_4$ and 0·1% glucose.

Each choroid plexus was placed in a glass counting vial containing 5 ml of Krebs–Ringer bicarbonate solution and the different drugs were immediately added in small volumes to give the final desired concentrations. Five min later [^{14}C]5-HIAA was

added, the vials gassed with 95% O_2–5% CO_2, capped and incubated in a Dubnoff metabolic shaker at 37° for various times. After the incubation period the tissue was removed from the vial, blotted on filter paper and weighed. For the estimation of [^{14}C]5-HIAA the tissue was digested in 1 N NaOH and counted. Results were expressed as a (wet weight) tissue to medium concentration ratio of [^{14}C]5-HIAA. Because the volume of the medium was much larger than that of the tissue the concentration of compounds in the medium remained virtually constant during an experimental period.

Experiments with brain slices. Brain cortex slices of 0·3-mm thickness were prepared with a McIlwain tissue chopper and distributed in counting vials containing 5 ml of Krebs–Ringer bicarbonate solution. The vials were preincubated at 37° for 5 min, and then 10 μc of randomly labeled [^{3}H]*l*-tryptophan were added to give a final concentration of 10^{-4} M. The vials were gassed, capped and incubated at 37° for 1 hr. Cold tryptophan and radioactive tryptophan and 5-HIAA were measured in the incubation medium and in the slices as described elsewhere.[5]

Chromatographic identification of 5-HIAA taken up by the choroid plexus. To determine whether 5-HIAA is taken up by the choroid plexus without undergoing metabolic alteration, choroid plexus that had been incubated with [^{14}C]5-HIAA for 2 hr were homogenized in 2 ml of 80% methanol. The homogenate was centrifuged and the resultant supernatant fluid evaporated under vacuum. The residuum was dissolved in a small volume of 80% methanol and applied to Silica Gel G thin layer plates (250 μ). For control, pure [^{14}C]5-HIAA was added to a methanol extract of tissue and the same procedure was followed. The chromatograms were developed ascendingly with the two following solvent systems: A, methylacetate–isopropanol–25% NH_3 (45:35:20, by vol.); B, ethanol–acetic acid (96:4, v/v). 5-HIAA did not undergo metabolic change in these experiments, since the radioactivity extracted from the tissue (80–85 per cent recovery) was chromatographically identical with that of the control.

Materials. [^{14}C]5-Hydroxyindoleacetic-carboxyl acid in acetonitrile solution (specific activity 5 mc/mM) was obtained from New England Nuclear. Acetonitrile was evaporated under vacuum and the radioactive compound dissolved in 0·01 N HCl. [^{3}H]*l*-tryptophan (G) (3·4 c/mM) was obtained from Amersham–Searle. Iodoacetic acid, *N*-ethylmaleimide and 2,4-dinitrophenol were purchased from Eastman Organic Chemicals; ouabain, serotonin creatinin sulfate and *l*-noradrenaline from Calbiochem; *p*-aminohippuric acid (PAH) from Sigma Chemical Company. Probenecid was kindly provided by the Merck Institute for Therapeutic Research, West Point, Pennsylvania.

RESULTS

Uptake of 5-HIAA by the choroid plexus. 5-HIAA, on incubation with choroid plexus of the lateral ventricles, was readily taken up. After 1 hr, the compound attained tissue/medium (T/M) concentration ratio of 8:1. The uptake as a function of time is shown in Fig. 1. A maximum uptake is obtained after 1 hr of incubation.

Influence of concentration on extent of uptake. The uptake of 5-HIAA by choroid plexus was not directly proportional to the concentration in the external medium over the whole range of concentration used. Rather, as the concentration was raised, T/M ratio declined indicating that 5-HIAA is taken up by a process that can be saturated (Fig. 2). At a concentration as high as 10^{-2}M the concentration of 5-HIAA is lower in

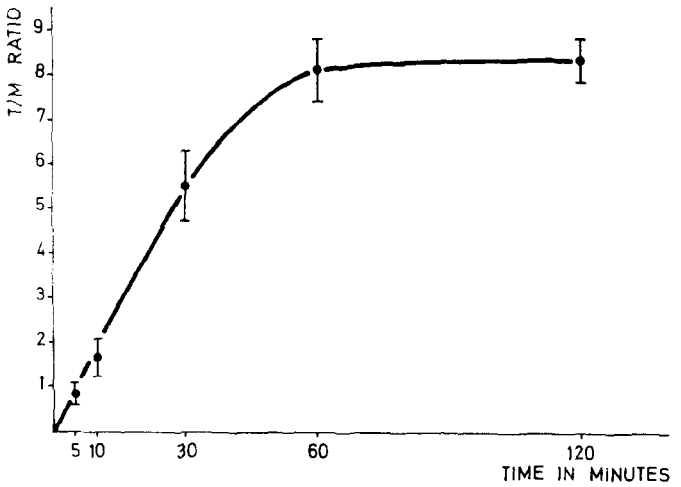

FIG. 1. Uptake of 5-HIAA by choroid plexus of the lateral ventricles as a function of time. The initial concentration of 5-HIAA in the medium was 0·01 mM. Results are given as the mean ± S.E. for five experiments.

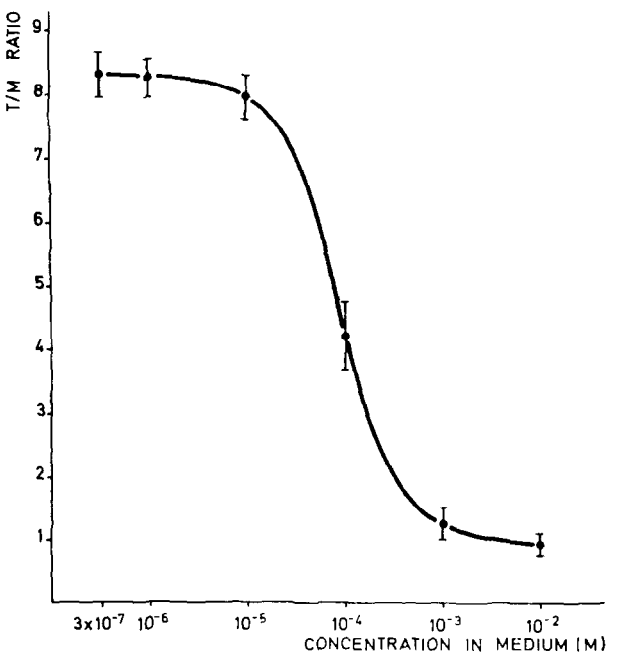

FIG. 2. Relation between concentration of 5-HIAA in the medium and 1 hr uptake of the compound by lateral choroid plexus. Results are given as the mean ± S.E. for four experiments.

the tissue than in the medium, demonstrating that there is no active transport of 5-HIAA at this concentration.

Influence of drugs and metabolic inhibitors on extent of uptake. The uptake of 5-HIAA was depressed by a number of metabolic inhibitors and drugs. Dinitrophenol, iodoacetate and *N*-ethylmaleimide were the strongest inhibitors; at 10^{-4}M they lowered the T/M ratio by 73–82 per cent. A lower inhibition was obtained with ouabain. Probenecid and PAH were also powerful inhibitors of the uptake of 5-HIAA, while serotonin and norepinephrine were inactive (see Table 1).

TABLE 1. EFFECT OF VARIOUS DRUGS ON THE 1-hr UPTAKE OF 5-HIAA BY THE CHOROID PLEXUS OF THE LATERAL VENTRICLE*

Drug	Concentration of drug (M)	Depression of T/M ratio (%)
2,4-Dinitrophenol	1×10^{-4}	76 ± 11
Iodoacetate	1×10^{-4}	73 ± 8
N-Ethylmaleimide	1×10^{-4}	82 ± 7
Ouabain	1×10^{-4}	32 ± 4
Probenecid	1×10^{-5}	46 ± 8
	1×10^{-4}	65 ± 5
	1×10^{-3}	85 ± 7
PAH	1×10^{-4}	54 ± 4
Serotonin	1×10^{-4}	7 ± 3
Norepinephrine	1×10^{-4}	4 ± 2

* The initial concentration of 5-HIAA in the medium was 0·01 mM. Results are expressed as the mean ± S.E. for four to six experiments.

Binding of 5-HIAA to homogenates of choroid plexus. The binding of 5-HIAA to choroid plexus tissue was investigated in the following way. Three or four (30–40 mg) choroid plexuses were homogenized in 5 ml of Krebs–Ringer bicarbonate containing labelled 5-HIAA (10^{-5}M). The homogenate was centrifuged at 100,000 *g* for 6 hr at 4°, and the concentration of 5-HIAA was measured in the particulate material and supernatant fluid. 5-HIAA showed some binding to tissue components. After the centrifugation there was a particulate/supernatant concentration ratio of 3·2 ± 0·4 (average four experiments ± S.E.). To distinguish between accumulation resulting from binding and accumulation resulting from active transport, *N*-ethylmaleimide, a substance that inhibits the accumulation of 5-HIAA in intact choroid plexus, was used. *N*-ethylmaleimide at a concentration (10^{-4}M) that inhibits the tissue uptake of 5-HIAA by 82 per cent, as shown in Table 1, had no appreciable effect on the extent of binding of 5-HIAA to tissue components. This result suggests that the accumulation of 5-HIAA in intact choroid plexus resulted from transport against a concentration gradient rather than from tissue binding.

Transport of 5-HIAA in brain cortex slices. To determine if there is an active transport of 5-HIAA out of the neurons that could also be blocked by probenecid, we incubated brain slices with [^{3}H]tryptophan. Tryptophan is taken up by the slices, and a small percentage is converted into [^{3}H]serotonin and [^{3}H]5-HIAA.[5,6] We used the [^{3}H]5-HIAA so formed to find out if probenecid could block the efflux of 5-HIAA.

The results of these experiments are summarized in Table 2. Probenecid did not block the uptake of tryptophan by the slices, and it did not modify either the amount of 5-HIAA present in the slices or in the medium. These experiments prove that the transport of 5-HIAA in rabbit brain cortex slices is not modified by probenecid.

TABLE 2. EFFECT OF PROBENECID ON UPTAKE OF TRYPTOPHAN AND ON FORMATION AND EFFLUX OF 5-HIAA IN RABBIT BRAIN SLICES*

	Tryptophan in the slices (counts/min)	Tryptophan in the slices (μg)	5-HIAA in the slices (counts/min)	5-HIAA in the medium (counts/min)
Control	80·200 ± 1·270	3·6 ± 0·1	981 ± 75	1·270 ± 194
Probenecid 60 m	84·700 ± 1·600	3·7 ± 0·1	970 ± 84	1·190 ± 103
Probenecid 30 m	81·500 ± 1·840	3·5 ± 0·1	930 ± 62	1·294 ± 119

* Brain cortex slices were incubated in Krebs–Ringer bicarbonate at 37° for 1 hr in presence of [^{3}H]tryptophan (10^{-4}M). In a set of experiments probenecid was added at the same time as [^{3}H] tryptophan and in another experiment probenecid was added 30 min afterwards. In all experiments the total time of incubation was 60 min, and the concentration of probenecid, when used, 10^{-4}M. The values are expressed per 100 mg tissue (wet weight) and they are average ± S.E. of four experiments.

DISCUSSION

Although the blockade of 5-HIAA efflux from the brain has been used frequently as a tool in experimental neuropharmacology, there were no data on the localization and properties of 5-HIAA transport in brain. From our experiments we may conclude that 5-HIAA is taken up by the choroid plexus by a process showing all the characteristics of active transport, e.g. transfer against a concentration gradient, saturability, substrate competition and inhibition by drugs that interfere with cell metabolism.

A transport system for organic bases has been described in the choroid plexus[7] which is similar to the transport system for organic bases excretion in the kidney.[8] It has been shown also that norepinephrine and serotonin are taken up by the choroid plexus by the same mechanism.[9] A different transport system for organic anions has been described in the kidney[10] and in the choroid plexus.[11–13] It has been shown that organic anions such as phenol red, iodopyracet and PAH are transported from CSF to blood, and that one compound inhibits the transport of the other.[11,13] We have seen that the 5-HIAA uptake by the choroid plexus is blocked by probenecid and PAH, two organic anions, but not by the basic compounds norepinephrine or serotonin. These results indicate that in the choroid plexus there is an active transport for 5-HIAA and other organic anions, and since one anion inhibits the transport of the other it may be assumed that there is a competition among these compounds for a common transport system.

The experiments on brain slices show that the efflux of 5-HIAA from the neurons into the bathing medium is not blocked by probenecid. In addition, from experiments *in vitro* with the choroid plexus, it is clear that the direction of transport is external medium to epithelial cell to blood capillary, since only the external border of the choroid epithelium is exposed to the medium. This suggests that the *in vivo* accumulation of 5-HIAA in brain after probenecid administration is due to impairment of the

removal of the 5-HIAA from the CSF at the choroid plexus (and probably also at similar vascular structures), and not to a blockade of 5-HIAA efflux from the neurons.

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