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# TRANSPORT OF SEROTONIN AND NOREPINEPHRINE BY THE RABBIT CHOROID PLEXUS *IN VITRO**

YOSHIHIRO TOCHINO† and LEWIS S. SCHANKER

Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Md., U.S.A.

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**Abstract**—When pieces of rabbit choroid plexus were incubated aerobically at 37° with solutions of serotonin-2-$^{14}$C or DL-norepinephrine-7-$^{14}$C, the tissue took up the amines by a process showing all the characteristics of active transport. Uptake against a concentration gradient occurred by a saturable process that was inhibited by anaerobic conditions and by low concentrations of ouabain, reserpine, and certain metabolic inhibitors. Serotonin and norepinephrine were bound to homogenates of choroid plexus, but the characteristics of the binding were such that binding would not account for the bulk of amine accumulation seen in the intact tissue. The uptake of hexamethonium, a substance previously shown to be actively transported by choroid plexus, was competitively inhibited by serotonin and norepinephrine, suggesting that the three compounds share a common transport process.

PREVIOUS work from this laboratory has shown that the choroid plexus *in vitro* takes up the quaternary ammonium compounds hexamethonium, decamethonium, and $N^1$-methylnicotinamide by a process of active transport.[1] Since decamethonium and $N^1$-methylnicotinamide were found to inhibit competitively the uptake of hexamethonium, and since the characteristics of uptake of the three cations were similar, it was concluded that the compounds are transported by the same process.

The present investigation shows that the choroid plexus can also take up by active transport the primary amines serotonin and norepinephrine. Evidence is presented that the two amines and the quaternary ammonium compounds share a common uptake process.

## METHODS

Male albino rabbits weighing 1·8–2·1 kg were killed by an intravenous injection of air, and the brain was quickly exposed. The choroid plexuses of both lateral ventricles and of the fourth ventricle were excised and placed in a chilled petri dish lined with filter paper that had been slightly moistened with Krebs-Ringer phosphate solution. Each plexus weighed approximately 10 mg. In most experiments, the plexus from each lateral ventricle was cut into two or three segments of equal length, and the plexus from the fourth ventricle cut into two portions.

Each piece of tissue (3–5 mg) was placed in 4 ml of Krebs-Ringer phosphate solution (pH 7·4) containing 1 g glucose/liter and various concentrations of $^{14}$C-labeled serotonin, DL-norepinephrine, or hexamethonium. The resulting mixtures, contained in 20-ml beakers, were shaken in a Dubnoff metabolic shaker (90 oscillations/min) at 37° in an atmosphere of oxygen or nitrogen. When the latter gas was used, oxygen

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† Visiting scientist. Present address: Shionogi Research Laboratory, Fukushima-ku, Osaka, Japan.

was removed from the medium by bubbling nitrogen through it for 15 min prior to adding the tissue. After an incubation period, the tissue was removed from the beaker, blotted on slightly moistened filter paper, and weighed.

For the measurement of radioactivity, the tissue was digested in 0·5 ml of 2·5 N KOH solution by occasional shaking over a 3-hr period at room temperature. One tenth ml of tissue digest, or 0·025 ml of the final incubation medium together with 0·1 ml of 2·5 N KOH solution, was added to 18 ml of a scintillation fluid,[2] and the radioactivity measured with a Packard Tri-Carb liquid scintillation spectrometer, model 314X. In all measurements of radioactivity, the total number of counts exceeded 5,000, and was at least 5 times the background.

Results were expressed as a (wet weight) tissue-to-medium concentration ratio of compound. 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.

*Materials*

Serotonin-2-$^{14}$C hydrogen oxalate (specific activity 0·94 mc/mmole), DL-norepinephrine-7-$^{14}$C acetate (specific activity 28 mc/mmole), and hexamethonium-methyl-$^{14}$C chloride (specific activity 3·5 mc/mmole) were obtained from the New England Nuclear Corp. Serotonin creatinine sulfate was obtained from the California Corp. for Biochemical Research. DL-Norepinephrine hydrochloride and L-epinephrine bitartrate were obtained from the Winthrop Laboratories, hexamethonium chloride and ouabain from Nutritional Biochemicals Corp., iodoacetic acid and 2,4-dinitrophenol from Eastman Organic Chemicals, and N-ethylmaleimide from the Gallard-Schlesinger Chemical Mfg. Co. Reserpine phosphate (lyophilized) was kindly provided by Ciba Pharmaceutical Products, Inc.

In this paper, the term norepinephrine always refers to the DL form, and the term epinephrine to the L form of the compound.

*Chromatographic identification of amines taken up by the choroid plexus*

To determine whether serotonin and norepinephrine are taken up by the choroid plexus without undergoing metabolic alteration, tissue (9–11 mg) that had been incubated with one of the $^{14}$C-labelled compounds for 3 hr was homogenized in 2 ml of 80% methanol. The homogenate was centrifuged, the resulting supernatant fluid applied to strips of Whatman no. 3MM filter paper, and the chromatograms were developed ascendingly with two of the following solvent systems: *A*, acetic acid: *n*-butanol: water (1:5:4); *B*, *n*-propanol: ammonium sulfate: 0·1 M sodium phosphate buffer, pH 6·8 (2:60:100); *C*, *n*-butanol saturated with 1 N HCl solution. For controls, the pure $^{14}$C-labeled compounds were added to a methanol extract of tissue that had been incubated in the absence of the amines, and the mixtures chromatographed. Radioactive spots were detected with a Vanguard model 880 automatic chromatogram scanner.

Norepinephrine and serotonin did not appear to undergo metabolic change in these experiments, since the radioactivity extracted from the tissue (80–90% recovery) was chromatographically identical with that of the controls. For example, in solvent *A*, the radioactivity from labeled norepinephrine gave a single spot, $R_f$ value 0·20; and in solvent *C*, there was a single spot ($R_f$ 0·11) and a trace streak ($R_f$ 0·36 to 0·51)

both in the experimental and the control preparations. In the case of serotonin, solvent *A* revealed a major spot (85% of the radioactivity, $R_f$ 0·68) and a small spot (15%, $R_f$ 0·27); and solvent *B* showed a large spot (60%, $R_f$ 0·49), a smaller spot (35%, $R_f$ 0·28), and a trace spot ($R_f$ 0·65) both in the experimental and the control preparations. The formation of multiple chromatographic spots has been reported previously for a wide variety of pure organic bases including a number of catecholamines.[3–5] The spots appear to result from multiple associations between the organic base and various acidic moieties present in both the developing solvent and the sample applied to the paper.[3–5] To distinguish between multiple spot formation and the presence of true metabolites, Beckett *et al.*[4] have suggested that the reference compound and the experimental sample be treated and chromatographed under identical conditions; in the present study, the control preparations served this purpose.

## RESULTS

### *Uptake of serotonin and norepinephrine by the choroid plexus*

Serotonin and norepinephrine (0·1 mM), on incubation with choroid plexus of the lateral ventricle, were readily taken up by the tissue. After 1 hr, the compounds attained tissue/medium (T/M) concentration ratios of 14–16 (Table 1). Accumulation of the amines was also seen with choroid plexus of the fourth ventricle; however, the T/M ratios were somewhat lower, 9–10.

TABLE 1. ONE-HOUR UPTAKE OF SEROTONIN AND NOREPINEPHRINE BY THE CHOROID PLEXUSES

The initial concentration of the compounds in the medium was 0·1 mM. Results are expressed as the mean of 9–12 experiments ± S.E.

| Compound | T/M ratio | |
|---|---|---|
| | Lateral plexus | Fourth plexus |
| Serotonin | 14·4 ± 2·4 | 9·0 ± 2·1 |
| Norepinephrine | 16·1 ± 1.6 | 10·1 ± 2·4 |

The uptake of serotonin and norepinephrine as a function of time is shown in Fig. 1. It can be seen that both compounds entered the lateral plexus at approximately the same rate, with a half-time for equilibration of about 25 min. The steady-state T/M ratios, reached after 2 hr, were 15 for serotonin and 19 for norepinephrine.

### *Influence of concentration on extent of uptake*

The uptake of serotonin by choroid plexus was not directly proportional to the concentration in the external medium. Rather, as the concentration was raised, the amount of compound taken up per gram tissue tended to approach a maximal value (Fig. 2). For example, at a concentration of 0·01 mM, the 1-hr uptake was 0·25 μmole/g; and at concentrations 10-, 30- and 200-fold greater, the uptake was increased by 5-, 10- and 27-fold respectively. These results indicated that serotonin is taken up by a process that can be saturated.

A similar relation between concentration of compound and extent of uptake was

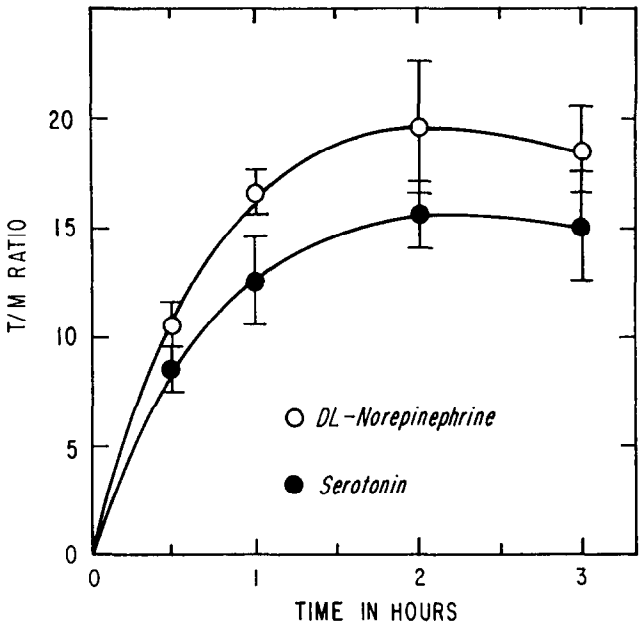


FIG. 1. Uptake of norepinephrine and serotonin by choroid plexus of the lateral ventricles as a function of time. The initial concentration of compound in the medium was 0·1 mM. Results are given as the mean ± S.E. for 5 experiments.

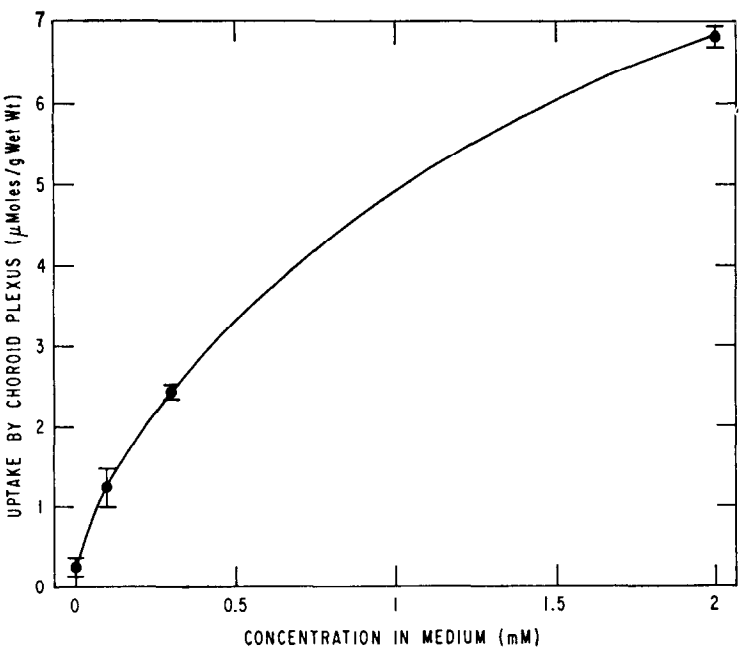


FIG. 2. Relation between concentration of serotonin and 1-hr uptake of the compound by lateral choroid plexus. Results are given as the mean ± S.E. for 5 experiments.

noted for norepinephrine (Fig. 3). For instance, at a concentration of 0·1 mM, the 1-hr uptake amounted to 1·4 μmole/g; and on raising the concentration 10- and 50-fold, the uptake was increased by 4- and 11-fold respectively.

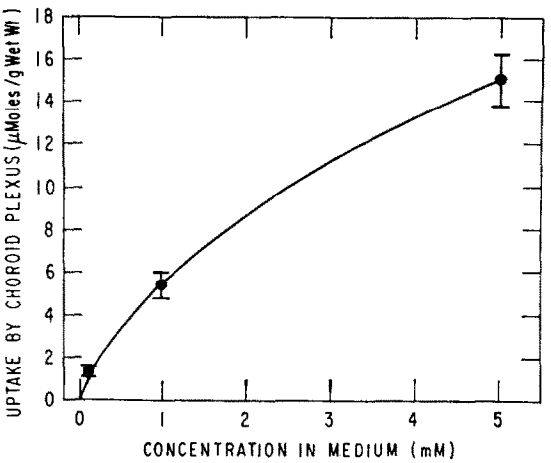


FIG. 3. Relation between concentration of norepinephrine and 1-hr uptake of the compound by lateral choroid plexus. Results are given as the mean ± S.E. for 5 experiments.

*Influence of anaerobic conditions, drugs, and metabolic inhibitors on extent of uptake*

The uptake of serotonin by the lateral choroid plexus was depressed by a number of metabolic inhibitors and drugs (Table 2). Iodoacetate and N-ethylmaleimide were

TABLE 2. EFFECT OF METABOLIC INHIBITORS AND OTHER SUBSTANCES ON THE ONE-HOUR UPTAKE OF AMINES BY THE CHOROID PLEXUS OF THE LATERAL VENTRICLE*

| Amine | Inhibitor | Concentration of inhibitor (M) | Depression of T/M Ratio (%) |
|---|---|---|---|
| Serotonin | Nitrogen atmosphere | | 17 ± 9 |
| | Iodoacetate | $1 \times 10^{-3}$ | 89 ± 0·1 |
| | | $1 \times 10^{-4}$ | 75 ± 2 |
| | | $1 \times 10^{-5}$ | 25 ± 5 |
| | 2,4-Dinitrophenol | $1 \times 10^{-3}$ | 38 ± 13 |
| | | $1 \times 10^{-4}$ | 14 ± 7 |
| | N-Ethylmaleimide | $1 \times 10^{-4}$ | 75 ± 5 |
| | | $1 \times 10^{-5}$ | 27 ± 3 |
| | Reserpine | $3 \times 10^{-5}$ | 19 ± 6 |
| | Ouabain | $1 \times 10^{-4}$ | 23 ± 3 |
| | | $1 \times 10^{-5}$ | 15 ± 4 |
| Norepinephrine | Nitrogen atmosphere | | 72 ± 6 |
| | Iodoacetate | $1 \times 10^{-4}$ | 61 ± 5 |
| | 2,4-Dinitrophenol | $1 \times 10^{-3}$ | 55 ± 9 |
| | | $1 \times 10^{-4}$ | 30 ± 6 |
| | N-Ethylmaleimide | $1 \times 10^{-4}$ | 79 ± 4 |
| | Ouabain | $1 \times 10^{-4}$ | 30 ± 3 |
| | | $1 \times 10^{-5}$ | 10 ± 3 |

*The initial concentration of serotonin and norepinephrine in the medium was 0·1 mM. Results are expressed as the mean ± S.E. in 6–12 experiments.

the strongest inhibitors; when used at a concentration of $10^{-4}$M, they lowered the T/M ratio by 75%. Lower degrees of inhibition were obtained with ouabain, reserpine, and 2,4-dinitrophenol. Substituting nitrogen for the usual oxygen atmosphere also depressed uptake, but the degree of depression was slight—17%.

The uptake of norepinephrine was inhibited by iodoacetate, N-ethylmaleimide, 2,4-dinitrophenol, and ouabain, and the degrees of inhibition were similar to those seen in the previous experiments with serotonin (Table 2). In contrast, a nitrogen atmosphere had a much greater effect on the uptake of norepinephrine (72% depression) than on that of serotonin.

In experiments with the fourth ventricular plexus, ouabain was tested as an inhibitor of amine uptake. The results were much like those obtained with the lateral plexuses: serotonin uptake was inhibited by 29% and 13% in the presence of $10^{-4}$ and $10^{-5}$ M ouabain respectively; and norepinephrine uptake was inhibited by 43% and 8% by these concentrations of the drug.

*Interaction of hexamethonium and various amines with the uptake process*

The quaternary ammonium cations hexamethonium, decamethonium, and $N^1$-methylnicotinamide have been shown to be taken up by the choroid plexuses by a process of active transport.[1] The compounds compete with one another for uptake, suggesting that they share the same transport mechanism. In looking for evidence that serotonin and norepinephrine—both predominantly cations at pH 7·4—might also be taken up by this process, the two amines (and also L-epinephrine) were tested as possible inhibitors of the transport of hexamethonium. In addition, hexamethonium, L-epinephrine, and serotonin were tested as inhibitors of norepinephrine uptake.

The results (Table 3) show that epinephrine, norepinephrine, and serotonin (1 mM) markedly inhibited the uptake of 0·1 mM hexamethonium by both the lateral and fourth plexuses. Similarly, hexamethonium, epinephrine, and serotonin (1 mM) strongly inhibited the uptake of 0·1 mM norepinephrine.

TABLE 3. EFFECT OF VARIOUS AMINE COMPOUNDS ON THE ONE-HOUR UPTAKE OF HEXAMETHONIUM AND NOREPINEPHRINE BY THE CHOROID PLEXUSES*

| Compound | Test substance | Concentration of test substance (M) | Per cent depression of T/M ratio | |
|---|---|---|---|---|
| | | | Lateral plexus | Fourth plexus |
| Hexamethonium | Epinephrine | $10^{-3}$ | 76 ± 6 | 65 ± 5 |
| | | $10^{-4}$ | 37 ± 9 | |
| | Norepinephrine | $10^{-3}$ | 49 ± 5 | 44 ± 7 |
| | Serotonin | $10^{-3}$ | 93 ± 2 | 90 ± 2 |
| Norepinephrine | Hexamethonium | $10^{-3}$ | 52 ± 9 | |
| | Epinephrine | $10^{-3}$ | 69 ± 6 | |
| | Serotonin | $10^{-3}$ | 88 ± 2 | |

*The initial concentration of hexamethonium and norepinephrine in the medium was 0·1 mM. Results are expressed as the mean ± S.E. in 5–10 experiments.

To investigate the nature of the inhibition, the uptake of hexamethonium from solutions of various concentrations of the compound was measured in the presence of 1 mM serotonin or norepinephrine. The results, plotted graphically according to the method of Lineweaver and Burke,[6] were compared with the uptake of hexamethonium in the absence of the inhibitors (Figs. 4 and 5) .The common intercept of the curves on the vertical axis indicated that both serotonin and norepinephrine were competitive inhibitors of the uptake of hexamethonium.

*Binding of amines to homogenates of choroid plexus*

The binding of serotonin and norepinephrine to components of choroid plexus tissue was investigated in the following way. Thirty mg of tissue was homogenized (Tenbroeck glass homogenizer) in 10 ml of Krebs-Ringer phosphate glucose solution containing the amine (0·1 mM). The homogenate was centrifuged at 100,000 *g* for 18 hr at 0°–5°, and the concentration of amine measured in the particulate material and supernatant fluid. In some experiments, the homogenate was incubated under oxygen at 37° for 1 or 2 hr prior to the centrifugation.

As shown in Table 4, both amines showed binding to tissue components. Serotonin had a particulate/supernatant concentration ratio of about 4·4, and norepinephrine a ratio of about 5·2. There was little or no difference between incubated and non-incubated samples.

The question arose to what extent the binding of the amines accounted for their accumulation in the intact choroid plexus. Accordingly, a qualitative test was employed to distinguish between accumulation resulting from binding and accumulation resulting from transport against a concentration gradient. Iodoacetic acid, a substance known to interfere with the accumulation of the amines in intact choroid plexus, was tested for possible interference with the binding of the amines to a homogenate of the tissue. As shown in Table 4, iodoacetate, in a concentration that inhibits the tissue uptake of serotonin and norepinephrine by 61–75%, had no appreciable effect on the extent of binding of the compounds to tissue components. The results suggested that most of the accumulation of the amines in the intact choroid plexus resulted from transport against a concentration gradient rather than from tissue binding.

TABLE 4. BINDING OF SEROTONIN AND NOREPINEPHRINE TO HOMOGENATES OF CHOROID PLEXUS*

| Compound | Particulate/supernatant concentration ratio | | | |
|---|---|---|---|---|
| | No incubation | Incubation, 1 hr | Incubation, 2 hr | Iodoacetate (no incubation) |
| Serotonin | 4·5 ± 0·2 | 4·3 ± 0·2 | 4·4 ± 0·3 | 4·3 ± 0·2 |
| Norepinephrine | 5·0 ± 0·2 | 5·2 ± 0·3 | 5·4 ± 0·3 | 5·1 ± 0·2 |

*Homogenates of choroid plexus containing either serotonin or norepinephrine (0·1 mM), and in some cases 0·1 mM iodoacetate, were incubated under oxygen for 0, 1, or 2 hr at 37° and then centrifuged at 100,000 *g* for 18 hr at 0°–5°. The concentration of amine in the particulate material (wet weight) and the supernatant fluid was measured, and binding was expressed as the ratio of the concentrations. Results are given as the mean ± S.E. for 4 experiments.

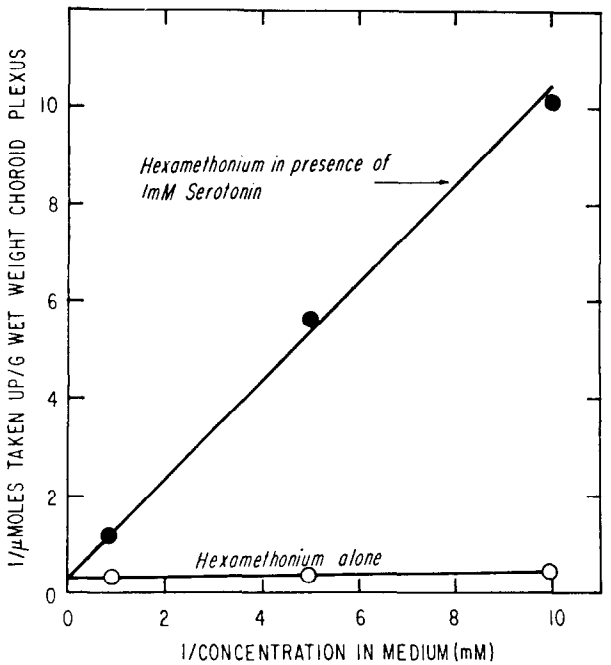


FIG. 4. Uptake of hexamethonium by choroid plexus of the lateral ventricles in the presence and absence of 1 mM serotonin. To avoid the variability among animals, the lateral plexuses of a single animal were cut into a total of 6 pieces, and all were incubated simultaneously for 1 hr to obtain the values plotted in the graph. The graph is typical of those obtained in 3 separate experiments.

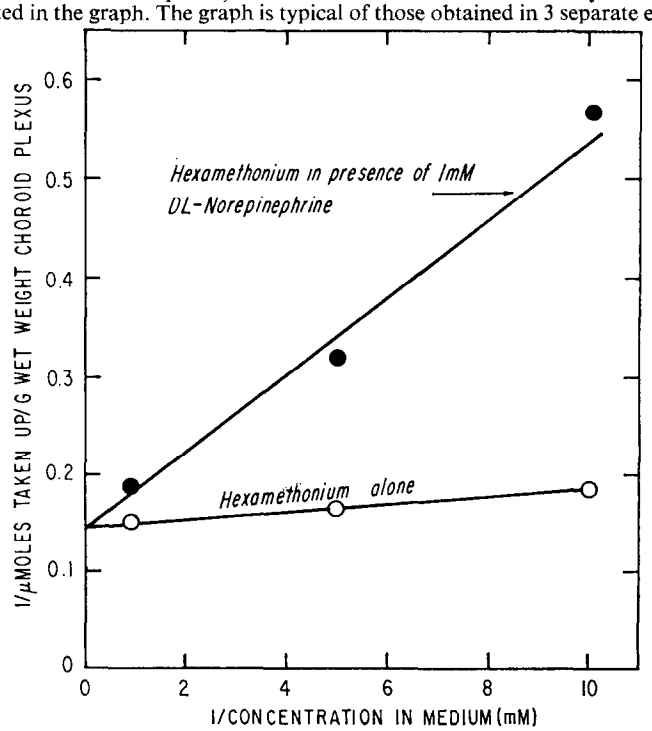


FIG. 5. Uptake of hexamethonium by choroid plexus of the lateral ventricles in the presence and absence of 1 mM norepinephrine. Typical of 3 experiments (see legend of Fig. 4 for details).

## DISCUSSION

The active transport process by which the choroid plexus takes up hexamethonium and certain other quaternary amines appears to be shared by the primary amines serotonin and norepinephrine. The latter two compounds act as competitive inhibitors of the transport of hexamethonium and are themselves taken up by the tissue by a process showing the characteristics of active transport, e.g. transfer against a concentration gradient, saturability, substrate competition, and inhibition by substances that interfere with cell metabolism. The secondary amine, epinephrine, may also share the same uptake process, for it inhibits the transport of both hexamethonium and norepinephrine.

The only notable difference in the characteristics of transport of serotonin and norepinephrine is the degree to which the uptakes depend on the presence of molecular oxygen. For instance, in an atmosphere of nitrogen the uptake of norepinephrine is depressed by 72%, and that of serotonin by only 17%. This difference may mean that uptake occurs in two or more stages; the compounds share a common pathway at one stage of their uptake, and move through independent pathways at another stage.

The transport of a variety of structurally dissimilar organic cations by a common mechanism is reminiscent of organic base excretion in the kidney. In that organ, the tranport process for bases appears to be shared by many quaternary ammonium ions as well as by the ionized forms of a number of primary, secondary, and tertiary amines.[7–9]

The choroid plexus further parallels the kidney in having additional transport processes for organic anions such as phenol red and chlorphenol red, and for monosaccharides such as glucose and galactose.[1, 10–12]

Since very little is known about the relative rates of transfer of serotonin and norepinephrine between brain, plasma, and cerebrospinal fluid (CSF), it is difficult to assess the physiological significance of a choroid plexus "pump" for the amines. Nevertheless, sufficient qualitative information is available to suggest that *in vivo* the pump acts to remove the amines from the CSF. In the first place, there is evidence from experiments *in vivo* that hexamethonium is readily transported by the same pump from CSF to blood.[1, 13] In addition, it is well known that serotonin and norepinephrine do not pass readily in the opposite direction, that is from blood to CSF. Moreover, 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 choroidal epithelium is exposed to the medium. And finally, it has recently been reported that after intraventricular injection in rats, norepinephrine-$^3$H rapidly leaves the CSF; half the dose moves into the peripheral circulation in less than 5 min, and at least 50% of this is unchanged norepinephrine-$^3$H; the other half of the dose quickly enters the brain where it becomes localized.[14]

The operation of the pump in the direction CSF-to-blood would in part explain the extremely low concentrations of endogenous serotonin and norepinephrine in normal CSF.[15] Another factor helping to keep these concentrations low would be the ability of brain tissue to remove the amines from CSF by various uptake, storage, and metabolic processes. In addition, there are the blood-brain and blood-CSF barriers, which tend to keep polar compounds such as serotonin and norepinephrine out of the central nervous system.

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