Kinetics and Autoradiography of High Affinity Uptake of Serotonin by Primary Astrocyte Cultures¹

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Abstract

Primary astrocyte cultures prepared from the cerebral cortices of neonatal rats showed significant accumulation of serotonin (5-hydroxytryptamine; [³H]-5-HT). At concentrations in the range of 0.01 to 0.7 μ M [³H]-5-HT, this uptake was 50 to 85% Na⁺ dependent and gave a K_m of 0.40 ± 0.11 μ M [³H]-5-HT and a V_{max} of 6.42 ± 0.85 ($\pm$ SEM) pmol of [³H]-5-HT/mg of protein/4 min for the Na⁺-dependent component. In the absence of Na⁺ the uptake was nonsaturable. Omission of the monoamine oxidase inhibitor pargyline markedly reduced the Na⁺-dependent component of [³H]-5-HT uptake but had a negligible effect on the Na⁺-independent component. This suggests significant oxidative deamination of serotonin after it has been taken up by the high affinity system, followed by release of its metabolite. We estimated that this system enabled the cells to concentrate [³H]-5-HT up to 44-fold at an external [³H]-5-HT concentration of 10^{-7} M. Inhibition of [³H]-5-HT uptake by a number of clinically effective antidepressants was also consistent with a specific high affinity uptake mechanism for 5-HT, the order of effectiveness of inhibition being chlorimipramine > fluoxetine > imipramine = amitriptyline > desmethylinipramine > iprindole > mianserin. Uptake of [³H]-5-HT was dependent on the presence of Cl⁻ as well as Na⁺ in the medium, and the effect of omission of both ions was nonadditive. Varying the concentration of K⁺ in the media from 1 to 50 mM had a limited effect on [³H]-5-HT uptake. Autoradiography of cultures after uptake of [³H]-5-HT with Na⁺ present showed that virtually all of the cells had a grain density that was above background and that this uptake was greatly reduced when Na⁺ was omitted from the medium. These data show that, based on kinetic parameters, ionic dependence, and the pharmacology of inhibition, uptake of [³H]-5-HT by primary astrocyte cultures at submicromolar concentrations of 5-HT is indistinguishable from the high affinity uptake reported for brain tissue. High affinity uptake of serotonin has been thought to be an exclusively neuronal property, but the present findings support the concept that astroglia can also show this property.

Termination of the extracellular action of a number of neurotransmitters in the CNS is thought to be due to compartmental sequestration by specific reuptake into the nerve endings from which they were released. It is now becoming clear, however, that uptake of neurotransmitters after release need not only be by reuptake into the nerve endings, but can also involve uptake into glial cells. Uptake of amino acid transmitters by glial cells has been observed in a number of different experimental systems (Fonnum et al., 1980). Uptake of monoamine transmitters, including serotonin (5-hydroxytryptamine; 5-HT), by several glial preparations has also been reported (Henn and Hamberger, 1971; Pfister and Goworek, 1977; Suddith et al., 1978; Hoffman and Vernadakis, 1979; Pelton et al., 1981; Ritchie et al., 1981; Tardy et al., 1982; Kimelberg and Pelton, 1983; Whitaker et al., 1983). The properties of serotonin uptake into glial cells, however, generally showed some differences from high affinity uptake into brain (see "Discussion"). In this study we have examined both the kinetics of [³H]-5-HT uptake and its inhibition by clinically effective antidepressants in a well characterized rat primary astrocyte culture which is predominantly astrocytic ($\geq 95\%$ astrocytes) (Kimelberg, 1983). We show that these cultures have a high affinity uptake system for serotonin which shows the same kinetics of uptake and pharmacology of inhibition as does the high affinity uptake system reported for mammalian brain. Using autoradiography, we also show localization of uptake to almost all of the cells in these cultures. A preliminary report of this work has been presented (Kimelberg and Katz, 1984).

Materials and Methods

Primary astrocyte cultures from neonatal rat cerebral cortices were grown in 12-well trays (Costar) as described recently (Frangakis and Kimelberg, 1984). To measure uptake of [³H]-5-HT, growth medium was removed and each well was washed three times with 1 ml of a HCO₃⁻-buffered medium (in millimoles per liter: Na⁺, 145; K⁺, 4.5; Mg²⁺, 0.4; Ca²⁺, 1.3; Cl⁻, 127; SO₄²⁻, 0.4; HCO₃⁻, 25; glucose, 10; pH 7.4) at 37°C. When Na⁺ was omitted it was replaced with choline. The cells were then allowed to incubate with 1 ml of the medium/well at 37°C in a 95% air/5% CO₂ atmosphere for 20 min in a Wedco incubator. Inhibitors, when added, were present during this preincubation period. After the preincubation period, identical warmed medium, but now containing [³H]-5-HT, was added. After completion of the uptake period (usually 4 min but sometimes longer), the cells were rapidly washed seven times by rapid addition and immediate aspiration of 1 ml of ice-cold 0.32 M sucrose, buffered with 10 mM Tris-HCl, pH 7.4. A zero time uptake, measured by adding [³H]-5-HT and immediately washing the cells, was always subtracted to correct for the small equivalent volume (~ 0.2 microliter) of adherent media left after washing. In all cases, unless specified otherwise, 10^{-4} M pargyline was added to prevent oxidative deamination of 5-HT, and 10^{-5} M ascorbate was present to prevent auto-oxidation of 5-HT. The cells were then solubilized in 0.5 ml of 1 N NaOH/well at 37°C for 30 min. This was then diluted to 0.5 N NaOH and aliquots were taken for scintillation counting and protein determinations (Kimelberg and Pelton, 1983).

Autoradiography. For autoradiography, primary astrocyte cultures were grown on glass coverslips in 60-mm dishes (Corning). Cells were allowed to incubate with 0.3 μ M [³H]-5-HT in HCO₃⁻-buffered medium for 30 min in the

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Figure 1. Effect of omission of medium Na^+ and pargyline (PARG.) on $[^3\text{H}]\text{-5-HT}$ uptake. Uptake of $[^3\text{H}]\text{-5-HT}$ at a final concentration of 10^{-7} M was measured at 37°C as described under "Materials and Methods" at varying times in the presence (+) and absence (-) of Na^+ and 10^{-4} M pargyline as indicated. When Na^+ in the medium was omitted, it was replaced with choline. Final medium $[\text{K}^+]$ was 4.5 mM. Cells were 28 days old. Values are means $\pm$ SEM; n (number of wells per data point) = 3.

presence of pargyline and ascorbate (see above). To stop uptake the cells were rapidly washed seven times with 2 ml of ice-cold phosphate-buffered saline (PBS; 140 mM NaCl, 3.5 mM NaH_2PO_4 , 12 mM Na_2HPO_4 , pH 7.25). The cells were then fixed in 4% glutaraldehyde in PBS for 20 min at room temperature. After washing with PBS to remove the fixative, the coverslips were air dried, mounted on microscope slides with Fluoromount, and left overnight at 4°C to dry. The slides were then dipped in the dark in Kodak NTB2 nuclear track emulsion at 41°C , air dried for 1 hr, and allowed to develop at 4°C for 9 days. The slides were then developed in Kodak Dektol (1:2) for 2 min, stopped in 1% acetic acid for 30 sec, fixed in Kodak fixer for 4 min, and finally rinsed in three water baths for 5 min each. They were viewed using a Nikon Labophot.

Materials. 5-Hydroxy-[G- ^3H]tryptamine creatinine sulfate was from Amersham (specific activity, 9 to 23 Ci/mmol). Pargyline hydrochloride was from Sigma Chemical Co. The different antagonists used were generous gifts of different pharmaceutical companies, as follows: fluoxetine, Eli Lilly and Co.; chlorimipramine and imipramine hydrochloride, CIBA Pharmaceuticals; desmethylimipramine, USV Pharmaceuticals; amitriptyline hydrochloride, Merck, Sharp and Dohme; mianserin hydrochloride, Organon International B.V.; ipindole hydrochloride, Wyeth Laboratories.

Results

A time course for the uptake of $[^3\text{H}]\text{-5-HT}$ at 10^{-7} M and the effects of Na^+ and pargyline are shown in Figure 1. In the presence of 10^{-4} M pargyline the uptake is clearly greater than in its absence and at 30 min an intracellular concentration of 4.4×10^{-6} M can be calculated based on an intracellular volume of about $4 \mu\text{l}$ (Kimelberg et al., 1979; Kimelberg and Pelton, 1983) and an uptake of 17.5 pmol of $[^3\text{H}]\text{-5-HT}$ /mg of protein. It can be seen that the uptake is still increasing with time and has not yet reached a steady state by 30 min. The uptake of $[^3\text{H}]\text{-5-HT}$ was also markedly dependent on the presence of Na^+ in the medium. However, the magnitude of the Na^+ dependence was reduced when pargyline was absent. For Na^+ -

independent uptake there was essentially no effect due to omission of pargyline.

The marked dependence of $[^3\text{H}]\text{-5-HT}$ uptake on medium Na^+ clearly indicates that this component of uptake is of the high affinity type (Snyder, 1970; Iversen, 1974; Fonnum et al. (1980). This was further supported by kinetic studies, the results of which are shown in Figure 2. Initial rates of uptake of $[^3\text{H}]\text{-5-HT}$ over 4 min were measured as a function of varying $[^3\text{H}]\text{-5-HT}$ concentrations in the presence and absence of Na^+ in the medium. The Na^+ -sensitive component (see Fig. 2 legend) was clearly saturable, whereas the Na^+ -insensitive component increased linearly with increasing $[^3\text{H}]\text{-5-HT}$ concentration and showed no saturation within the concentration range studied, as determined by fitting the data to a computer program (see Fig. 2 legend). The K_m for the Na^+ -sensitive component was $0.40 \pm 0.11 \mu\text{M}$ and the $V_{\max}$ was 6.42 ± 0.85 pmol/mg of protein/4 min ($\pm$ SEM) as obtained from the computer program.

High affinity uptake of monoamines is also dependent on Cl^- (Fonnum et al., 1980). In Figure 3 we show that omission of Cl^- from the medium reduced $[^3\text{H}]\text{-5-HT}$ uptake by the same amount as when Na^+ was omitted. Also, the omission of both ions did not show any additivity. We also looked for any dependence of $[^3\text{H}]\text{-5-HT}$ uptake on varying external $[\text{K}^+]_o$. The effect of varying $[\text{K}^+]_o$ from 1 to 50 mM is shown in Figure 4, where it is compared with the dependence of $[^3\text{H}]\text{-5-HT}$ uptake on varying $[\text{Na}^+]_o$. There was a sharp, 37% increase in $[^3\text{H}]\text{-5-HT}$ uptake from 1 to 6 mM K^+ , but this was followed by a decline, with an essentially constant rate of $[^3\text{H}]\text{-5-HT}$ uptake being found from 10 to 50 mM K^+ . In contrast, there was a marked increase in the rate of uptake of $[^3\text{H}]\text{-5-HT}$ from about 25 to 45 mM Na^+ to a relatively constant level, with maximum levels being reached at 100 to 150 mM Na^+ .

High affinity uptake of $[^3\text{H}]\text{-5-HT}$ in the CNS is specifically sensitive

Figure 2. Dependence of $[^3\text{H}]\text{-5-HT}$ uptake on the concentration of $[^3\text{H}]\text{-5-HT}$. Varying amounts of $[^3\text{H}]\text{-5-HT}$ were added to give the final concentrations shown, and uptake was measured in the presence and absence of medium Na^+ . Na^+ was replaced with choline. Uptake of $[^3\text{H}]\text{-5-HT}$ was measured for 4 min. Cells were 25 days old. Each point is the mean $\pm$ SEM of values from three separate wells. The Na^+ -dependent curve was obtained by subtracting the uptake in the absence of Na^+ from total uptake. The curves were generated using a computer program for fitting data to the Michaelis-Menton equation based on the method of Bliss and James (1966) as previously described (Kimmelberg and Pelton, 1983). The Na^+ -dependent component was best fitted by a curve which gave a K_m of $0.40 \pm 0.11 \mu\text{M}$ and a $V_{\max}$ of $6.42 \pm 0.85 \text{ pmol/mg of protein/4 min}$.

Figure 3. Effects of omission of Na^+ or Cl^- or both on $[^3\text{H}]\text{-5-HT}$ (10^{-7} M) uptake measured for 4 min. Na^+ was replaced with choline and Cl^- was replaced with isethionate. The presence of Na^+ and Cl^- is indicated in the code on the figure as (+), and omission is indicated as (-) for the different symbols. Cells were 35 days old. Each point represents the mean $\pm$ SEM; $n = 4$.

Figure 4. Effects of varying the medium concentrations of Na^+ or K^+ on $[^3\text{H}]\text{-5-HT}$ uptake. Uptake of $[^3\text{H}]\text{-5-HT}$ was measured over a 4-min period. When $[\text{Na}^+]_o$ was varied, it was replaced with choline. When K^+ was varied, $[\text{Na}^+]_o$ was reciprocally altered to maintain constant osmolarity. Cells were 34 days old. Values are mean $\pm$ SEM; $n = 4$.

to a number of antagonists, including a variety of clinically effective antidepressants (Iversen, 1974; Fonnum et al., 1980; Green and Costain, 1981). The effects of varying concentrations of a number of these antagonists on the uptake of $[^3\text{H}]\text{-5-HT}$ are shown in Figure 5. The range of the percentage of Na^+ independent uptake found for the different cultures is also indicated and, as can be seen, all of the inhibitors seemed to inhibit approximately to this level. IC_{50} values for 50% inhibition of the Na^+ -sensitive component alone were obtained from all of these dose-response curves by inspection and are summarized in Table I in the first column. In the second column K_d values are shown, which were obtained by fitting the data to a ligand-binding curve program assuming competitive inhibition and using the K_m value of $0.40 \mu\text{M}$ found in this study for $[^3\text{H}]\text{-5-HT}$ and subtracting the Na^+ -independent component.

Figure 6 shows autoradiography of cells grown on coverslips incubated with $0.3 \mu\text{M}$ $[^3\text{H}]\text{-5-HT}$ for 30 min and fixed with 4% glutaraldehyde as described under "Materials and Methods." Figure 6A is a brightfield photomicrograph showing uptake in an area of a culture containing predominantly flat cells. Figure 6B is the corresponding phase contrast photomicrograph of the same field. As can be seen, all of the cells show a high grain density which was well above the background density seen in the cell-free areas between cells. One cell with an extremely high grain density is also present in the middle of the field. Examination of regions from all areas of the coverslip did not reveal any cells which did not show grain density above background. Figure 6C represents an area with an intermediate grain density, and Figure 6D shows the corresponding phase contrast micrograph. In this figure, both process-bearing cells and flat cells were present, both showing a grain density clearly greater than background. Figure 6E shows an area which represents the least intense uptake seen; Figure 6F is the corresponding phase

contrast micrograph showing that the area contains a confluent monolayer of cells with the characteristic flat morphology of primary astrocyte cultures (Kimelberg, 1983). When Na^+ was omitted from the medium during the initial incubation with $[^3\text{H}]\text{-5-HT}$, all of the cells showed a grain density almost identical to background levels as shown in figure 6G. Figure 6H is the corresponding phase micrograph showing the areas of the field which contained cells and the area which was cell free. This field was chosen so that a direct comparison can be made of the grain density in the two areas. Results essentially similar to those shown in Figure 6 were obtained for one other culture. Since our cultures stain 95% or better for glial fibrillary acidic protein (GFAP) (Kimelberg, 1983; H. K. Kimelberg and M. V. Frangakis, unpublished data), which included all of the morphological types in the autoradiographs which took up ^3H label, these results strongly suggest that GFAP(+) cells are taking up $[^3\text{H}]\text{-5-HT}$.

Discussion

The characteristics of the uptake of $[^3\text{H}]\text{-5-HT}$ by primary astrocyte cultures found in this study closely resembles that described for high affinity uptake of $[^3\text{H}]\text{-5-HT}$ by brain slices and subcellular fractions. These similarities include an apparent K_m of 0.11 to $0.30 \mu\text{M}$, reasonably close to our value of $0.40 \mu\text{M}$, and uptake which is Na^+ dependent (Shaskan and Snyder, 1970; Iversen, 1974; Fonnum et al., 1980). Quantitatively, our uptake is within an order of magnitude of that described for *in vitro* brain preparations. If we assume that 1 gm of brain represents 100 mg of protein (Hertz, 1982), then Shaskan and Snyder (1970) found V_{max} values in rat brain striatal or hypothalamic slices which convert to 11.2 to 14.0 pmol of $[^3\text{H}]\text{-5-HT}/\text{min}/\text{mg}$ of protein taken up. Our value is 1.6 pmol of $[^3\text{H}]\text{-5-HT}/\text{min}/\text{mg}$ of protein/min. This lower value may reflect an actual lower

Figure 5. Effects of different clinically effective antidepressants on $[^3\text{H}]\text{-5-HT}$ uptake. All of the antagonists shown were present during the 20-min preincubation period (see Materials and Methods). Uptake of 10^{-7} M $[^3\text{H}]\text{-5-HT}$ was measured for 4 min. The age of the cells from different cultures ranged from 22 to 33 days old. Percent activity refers to the control value in the presence of Na^+ for each different culture used. The $(-)\text{Na}^+$ range refers to the range of uptake found in the absence of medium Na^+ for each culture as a percentage of the control value in the presence of Na^+ . Values are means $\pm$ SEM; $n = 4$. Cl-IMI, chlorimipramine; FLUOX., fluoxetine; IMI, imipramine; AMT, amitriptyline; DMI, desmethylimipramine; IPRIND., iprindole; MIANS., mianserin.

TABLE 1

IC_{50} values for inhibition of Na^+ -sensitive $[^3\text{H}]\text{-5-HT}$ uptake

Data were obtained by inspection (shown as IC_{50}) of curves in Figure 5 or by fitting the data points shown in Figure 5 to a ligand-binding curve written by P. Munson, A. de Lean, and D. Rodland and modified for an Apple II microcomputer by M. H. Teicher (shown as K_d values). A K_m value for 5-HT of $0.40 \mu\text{M}$ was used throughout, competitive inhibition was assumed, and the Na^+ -independent activity was subtracted.

Compound	IC_{50} (μM)	K_d (μM)
Cl-IMI	0.009	0.008 ± 0.006^a
Fluoxetine	0.023	0.038 ± 0.026
IMI	0.14	0.110 ± 0.013
AMT	0.14	0.108 ± 0.055
DMI	0.62	0.482 ± 0.059
Iprindole	2.8	1.21 ± 0.82
Mianserin	4.9	8.62 ± 2.42

^a Values are means $\pm$ SE.

transport capacity in astrocytes *in situ* compared to neurons, or that uptake is lower when the cells are grown in culture. Although the rates of uptake are lower, the concentrative capacity of the cells compared to brain slices seems unimpaired. Our estimated intracellular concentration of 4.4×10^{-6} M after a 30-min incubation with 10^{-7} M $[^3\text{H}]\text{-5-HT}$ (Fig. 1) gives a tissue-to-medium ratio value of 44-fold. This value exceeds the tissue-to-medium ratio of 21 at 10^{-7} M $[^3\text{H}]\text{-5-HT}$ estimated by Shaskan and Snyder (1970) for tissue slices from rat corpus striatum, the region in which the greatest $[^3\text{H}]\text{-5-HT}$ uptake was found. Also, uptake of $[^3\text{H}]\text{-5-HT}$ had not yet reached a

steady state by 30 min, so that even higher intracellular concentrations of $[^3\text{H}]\text{-5-HT}$ would seem to be attainable. Generally, monoamine oxidase (MAO) inhibitors are added to inhibit metabolism of 5-HT, and our finding that pargyline is needed to achieve maximal uptake of $[^3\text{H}]\text{-5-HT}$ is consistent with this. It is of interest that it is the Na^+ -dependent uptake that is mainly affected by the omission of pargyline. The effect of pargyline suggests that the metabolite of 5-HT produced by MAO action (5-hydroxyindolacetic acid) is not retained as well as 5-HT itself. Thus, the effect of omission of pargyline in decreasing $[^3\text{H}]\text{-5-HT}$ uptake and steady-state content may be a consequence of a constant uptake but more rapid efflux.

Na^+ dependence has been shown for uptake of 5-HT in brain slices and synaptosomes (Bogdanski et al., 1970; Shaskan and Snyder, 1970), and Cl^- dependence for 5-HT uptake in platelets (Lindgjaerde, 1971). In contrast, increased $[\text{K}^+]_o$ had a slight inhibitory effect (Bogdanski et al., 1970). The data in Figure 3 clearly show that the uptake by astrocytes is strongly Na^+ and Cl^- dependent. Also, since the effects of simultaneous omission of Na^+ and Cl^- are not additive, it appears that the same uptake mechanism is affected by both these ions. The dependence of $[^3\text{H}]\text{-5-HT}$ uptake on varying Na^+ concentrations shows a sharp increase between 25 and 45 mM Na^+ with 50% stimulation at approximately 30 to 35 mM Na^+ . This is close to the value of 40 mM for an apparent K_m for Na^+ stimulation of 5-HT uptake in synaptosomes reported by Bogdanski et al. (1970). There was much less K^+ dependence of $[^3\text{H}]\text{-5-HT}$ uptake in astrocytes, although a small peak was found at 6 mM $[\text{K}^+]_o$. When $[\text{K}^+]_o$ was increased, $[\text{Na}^+]_o$ was concomitantly decreased. However, since a maximum effect was found at 100 to 150 mM Na^+ and K^+ was only increased to 50 mM, the lowest

Figure 6. Autoradiography of cells after incubation with [3 H]-5-HT. Uptake of $0.3 \mu\text{M}$ [3 H]-5-HT was allowed to proceed for 30 min in the presence of pargyline and ascorbate, and the cells were then fixed in 4% glutaraldehyde as described under "Materials and Methods." A to H are different fields of the same culture representative of the varying grain density found, as described in the text. A to F show cells in which uptake was measured in Na^+ -containing medium, and G and H are of cells in Na^+ -free medium. Cells were 5 weeks old and were left for 9 days before development of the emulsion. All photomicrographs on the left (A, C, E, G) are brightfield and those on the right (B, D, F, H) are phase contrast.

$[Na^+]_o$ used when K^+ was varied was 100 mM and therefore should not have been a factor. Such concentrations of $[K^+]_o$ will, however, markedly depolarize the cells (Kimelberg et al., 1979). A 40% inhibition of 5-HT uptake was reported by Bogdanski et al. (1970) in rabbit brainstem synaptosomes when K^+ was raised from 6 to 100 mM K^+ at 50 mM Na^+ . However, this occurred with a higher $[K^+]_o$ than we used and in the presence of reduced $[Na^+]_o$.

It has been shown *in vitro* that 5-HT can also be taken up by catecholamine uptake systems, and we have shown that such uptake systems are present in our primary astrocyte cultures (Pelton et al., 1981; Kimelberg and Pelton, 1983). We have not done any cross-inhibition studies, but the rank order of inhibition by a variety of antidepressants clearly conforms to the order shown for the 5-HT system, which is different from that seen for the catecholamine system. Thus, Shaskan and Snyder (1970) found that the order of effectiveness for inhibition of $[^3H]$ -5-HT uptake in hypothalamic slices was chlorimipramine > imipramine > desmethylimipramine with IC_{50} values of 3.0 to 4.5×10^{-8} , 4.0 to 6.0×10^{-7} , and 2.5 to 2.8×10^{-6} M, respectively. In our studies we found an identical rank order with IC_{50} values of 9×10^{-9} , 1.4×10^{-7} , and 6.2×10^{-7} M, respectively. These IC_{50} values are also very close to those reported for 5-HT uptake in brain synaptosomes (see Green and Costain, 1981). In a previous study (Kimelberg and Pelton, 1983) we had found an IC_{50} value for inhibition of $[^3H]$ -norepinephrine uptake by desmethylimipramine in primary astrocyte cultures of 2×10^{-9} M. In comparison, an IC_{50} of 6.2×10^{-7} M for inhibition of $[^3H]$ -5-HT uptake by desmethylimipramine was found in the present study, thus indicating considerable specificity of desmethylimipramine for the catecholamine system in the astrocyte cultures. In addition, we now find that the highly selective 5-HT uptake antagonist, fluoxetine (Wong et al., 1975), is very effective in inhibiting $[^3H]$ -5-HT uptake in primary astrocyte cultures. The IC_{50} value of 2.3×10^{-8} M for fluoxetine found in the present study is close to the value of 6×10^{-8} M quoted for inhibition of 5-HT uptake in rat brain synaptosomes (Wong et al., 1975). The much higher IC_{50} value of 4.9×10^{-6} M found for mianserin in the present study is also close to the value of 8×10^{-6} M found for rat brain synaptosomes (Green and Costain, 1981).

Other studies have also reported uptake of 5-HT by various glia preparations with varying degrees of similarity and dissimilarity to the results reported in this paper for primary astrocyte cultures. Henn and Hamberger (1971) first reported a 6-fold concentration of serotonin at an external concentration of 10^{-7} M by glial fractions isolated from rabbit brain by gradient centrifugation. Uptake of $[^3H]$ -5-HT by the all-glial cell body filum terminale preparation from frog, which was Na^+ and temperature dependent and had a K_m for $[^3H]$ -5-HT of $0.7 \mu M$, has been reported (Ritchie et al., 1981). Uptake of $[^3H]$ -5-HT by the C_6 glial tumor cell line in the presence of the MAO inhibitor nialamide has also been reported (Suddith et al., 1978). One component of this uptake was markedly Na^+ dependent. It had a somewhat high K_m of 1 to $2 \mu M$ and was inhibited by chlorimipramine and desmethylimipramine at relatively high concentrations, with IC_{50} values of about 10^{-5} M. The effect of fluoxetine was not examined. Similar results for uptake of $[^3H]$ -5-HT by C_6 glial cells were recently reported by Whitaker et al. (1983). A relatively high K_m of $2.2 \mu M$ was also found, and the IC_{50} values for chlorimipramine and desmethylimipramine were 28 and $>1000 \mu M$, respectively. In this study it was not stated whether an MAO inhibitor was added. In studies using primary cultures, Tardy et al. (1982) reported uptake of $[^3H]$ -5-HT by primary astrocyte cultures from mouse brain with a K_m of $0.17 \mu M$ and a V_{max} of 0.6 pmol/mg of protein/min. Uptake was inhibited by chlorimipramine and fluoxetine, but again only at very high concentrations of 10^{-4} M. Also, it was not mentioned whether an MAO inhibitor was added in this study. Hansson (1983) has published autoradiographic studies of uptake of 10^{-6} M $[^3H]$ -5-HT by rat primary astrocyte cultures in the presence of pargyline. The data were reported qualitatively, and "weak" accumulation of $[^3H]$ -5-HT was described relative to "intense" accumulation of 3H -glutamate.

Our results with autoradiography confirm and extend the studies of Hansson (1983).

There have been a number of experiments on the uptake of $[^3H]$ -5-HT in brain regions as studied by autoradiography which have not indicated a strong glial component. In studies at the light microscope level, uptake of $[^3H]$ -5-HT was interpreted as uptake into nerve endings since uptake was found between nerve cell bodies and in regions known to have a high concentration of serotonergic nerve endings (Fuxe et al., 1968). More direct evidence from electron microscopic autoradiography has localized around 80% of the grain clusters as being over nerve endings and axons, and only about 5% over glia and blood vessels (Aghajanian and Bloom, 1967). However, a recent study using electron microscopic autoradiography (Ruda and Gobel, 1980) did localize grains to astrocyte processes in a section from cat medulla after topical application of $[^3H]$ -5-HT and pretreatment of the animal with an MAO inhibitor. Surgical lesions of the midbrain raphe nuclei or chemical lesion by injection of 5,6- or 5,7-dihydroxytryptamine, which appear to selectively destroy serotonergic neurons (Björklund et al., 1973; Breese and Cooper, 1975), also selectively inhibited high affinity uptake of 5-HT by brain (Kuhar et al., 1972; Björklund et al., 1973). The majority of these studies certainly suggest a more intense uptake and/or storage in nerve endings but cannot definitely rule out a contribution of high affinity glial uptake associated mainly with subsequent metabolic degradation by MAO. As mentioned above, one *in vivo* study in the cat did detect uptake into CNS astrocytes (Ruda and Gobel, 1980), suggesting that our findings in culture do represent an *in vivo* situation. It is also not clear whether the lesion studies are completely specific. Thus, destruction of serotonergic neurons may have indirect effects on astroglia surrounding such nerve endings. Also, we have recently observed that chronic treatment with 5,7-dihydroxytryptamine inhibits $[^3H]$ -5-HT uptake in primary astrocyte cultures (D. M. Katz and H. K. Kimelberg, unpublished observations).

In conclusion, our studies clearly show high affinity uptake of $[^3H]$ -5-HT by rat brain astrocytes in primary culture. In its kinetics, ion dependence, pharmacology of inhibition, and, also, its concentrative capacity it is indistinguishable from that found in various brain preparations, which is usually attributed to uptake by serotonergic nerve endings. If our results on normal astrocytes in culture can be extended to astrocytes *in situ*, then uptake by such cells is likely to be a significant route for the termination of action of 5-HT and a site of action for pharmacological compounds, such as the antidepressants. Such uptake into astroglia would presumably occur in addition to reuptake into nerve endings. The relative contributions of neurons and astroglia to uptake could be assessed if selective inhibitors of astroglial and neuronal uptake can be found. Screening for such compounds could conveniently be done in astroglial and neuronal culture systems.

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