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COMMENTARY

OCCURRENCE AND FUNCTIONAL SIGNIFICANCE OF SEROTONIN AND CATECHOLAMINE UPTAKE BY ASTROCYTES

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Serotonin (5-HT), and the catecholamines norepinephrine (NE) and dopamine (DA), are important nervous system transmitters. Traditionally, these and other transmitters have been viewed as excitatory or inhibitory "switching" mechanisms at synapses in the nervous system. There is now a growing interest in the involvement of the catecholamines and serotonin in more "modulatory" effects on brain function [1], and also in the development of the nervous system [2]. An important factor in allowing these transmitters to effectively fulfill any of these roles is, of course, the ability to control both the initiation and termination of their action. In the mammalian nervous system, initiation of action is thought to be due to release of transmitters from presynaptic nerve endings by the exocytosis of vesicles in which these transmitters are stored. Termination of the action of transmitters, after they have combined with postsynaptic receptors and achieved their specific effects, is thought to be mainly by re-uptake mechanisms located in the same nerve terminals from which they were released. This scheme is now an established textbook tenet (e.g. Refs. 3–5) and is well-accepted in the literature, e.g. "both in the PNS and CNS, the high affinity membrane systems for catecholamines appear to be exclusively neuronal" and "there is strong evidence for the existence of a high-affinity transport of 5-HT into peripheral and central serotonergic neurons" [6]. However, many synapses in the CNS are surrounded by the processes of astroglial cells (astrocytes) [7–10]. These cells, together with oligodendroglia, constitute the macroglia which represent the major portion of the non-neuronal cells in the CNS. This perisynaptic location of astrocytes has long led neuroscientists to seriously consider that these cells may have a role in transmitter uptake. Thus, Lugaro as early as 1907 [9] wrote "Elsewhere, I have presented the argument that the action that is carried out at the level of the neuronal articulation between the nervous termination and the dendrites and cellular bodies of successive neurons is of a chemical nature. Every nervous termination suffers a chemical modification and this chemical modification in turn gives stimulus to another neurone. If this is true, the interneuronal articulation (i.e. synapse) would be the center of the chemical exchange, and this would comprise therefore in all the most proximal, vacant interstitial spaces, a region for infiltration of the protoplasmic prolongations or feathery extensions of the neuroglia, perhaps with the purpose of collecting and instantly processing the smallest amount of waste product." If we interpret "waste product" as released transmitters, we come up with a pretty good description of glial uptake of released transmitters at the synapse.

In this commentary I will review recent evidence indicating that there may indeed be significant uptake of catecholamines and serotonin into astrocytes, in addition to the already established evidence for neuronal re-uptake. I will also present possible reasons why such uptake has not been seen so far *in situ*, and speculate on the possible roles for astrocytic uptake based on the limited data available.

Uptake systems for catecholamines and serotonin in brain

The major evidence to date from studies *in situ* indicates that concentrative uptake of added, exogenous monoamine transmitters is localized to neurons not glia, and specifically to the neurons synthesizing and releasing such transmitters. Such uptake is thought to occur by high affinity uptake systems. These uptake systems are inhibited specifically by several classes of compounds, many of which have important psychological and other nervous system effects and are clinically successful drugs [5, 11]. The evidence for such neuronal uptake will be considered in more detail later but, in brief, it is based on observations that high affinity uptake is very active in synaptosome preparations and is inhibited by specific surgical or chemical lesioning of neuronal tracts. Furthermore, histochemical and autoradiographic studies have shown that the pattern of localization of added transmitters corresponds to the pattern for endogenous levels of transmitters in neurons [12, 13]. Autoradiography at the electron microscopic level has also localized uptake of exogenous, labeled transmitters to nerve endings [14–18].

What then is the evidence for any significant extraneuronal uptake of 5-HT and catecholamines and why should this question even be raised? The reason comes mainly from work showing that glial preparations isolated from brain tissue [19] and a variety of glial cultures [20–30] show high affinity uptake of 5-HT and catecholamines. One possible reason for the general failure to observe concentrative uptake into extraneuronal sites in the CNS *in situ* is that it

is followed by metabolism. This appears to be the case in the corpus striatum in which significant glial metabolism of DA has been reported [31, 32]. Thus, inhibitors of metabolizing enzymes have to be used to observe any significant retention of labeled transmitters, probably because their metabolites are more permeable [33]. In addition, even under these conditions the amount of label retained is likely to be considerably smaller than in nerve terminals since astrocytes do not appear to contain a concentrative vesicle type uptake system as occurs in neurons.

In addition to the high affinity uptake systems for catecholamines and serotonin mentioned above, there are also low affinity uptake systems. These are termed uptake$_2$ while the high affinity uptake systems are termed uptake$_1$ [34]. The uptake$_1$ system has been thought of as being the primary means of inactivation of transmitters after they are released from synaptic nerve terminals and represents re-uptake into nerve terminals. This system shows a relatively high affinity for the substrate with K_m values of 0.2 to 0.4 μM. Also, uptake is highly dependent on the presence of Na^+ in the medium which is generally thought to be due to co-transport of the transmitter with Na^+, allowing concentration of the transmitter intracellularly because of utilization of the free energy available in the inwardly directed Na^+ electrochemical gradient. The number of sodium ions transported per molecule can vary and the free energy available increases dramatically when >1 Na^+ is transported, as discussed in detail for γ-aminobutyric acid (GABA) [35]. Specific inhibitors of the high affinity uptake system for the different amines are available, such as fluoxetine for 5-HT [36] and benzotropine [37] for DA. Also, the rank order of a class of inhibitors, such as the tricyclic antidepressants, is unique to either the NE, DA or 5-HT high affinity uptake system. These inhibitors are usually highly potent and inhibit effectively in the range of 0.01 to 1 μM [11].

Uptake$_2$ or low affinity uptake has been thought to be the only type present in glial cells in the CNS, thus distinguishing monoamine transmitter uptake in such cells from re-uptake into neuronal nerve endings [5, 33, 34]. Such systems are also common outside the CNS [33, 34]. They are specific transport systems, since the uptake is saturable, but show relatively high K_m substrate values in the range of 2 to 200 μM. Also, these systems are not dependent on Na^+ and show no stereochemical selectivity for (+) and (−) isomers in the case of NE. They are sensitive to inhibitors different from those effective for the high affinity systems. Thus, the low affinity catecholamine system in non-CNS tissues is sensitive to inhibition by O-methylated catecholamines, haloalkylamines and steroids [33, 34]. Low affinity uptake systems are thought to be associated mainly with metabolism by catechol-*O*-methyl transferase (COMT), but surprisingly COMT itself has a high affinity for catecholamines, and effects of COMT inhibitors subsequent to uptake are seen only at low concentrations of catecholamines (3–12 μM) [33]. There have been few studies on the low affinity system in the CNS. However, COMT is well known to be present in the CNS and appears to be localized to astrocytes and oligodendrocytes [38], but this is clearly only suggestive of the existence of the low affinity systems in glial cells since this enzyme can metabolize catecholamines taken up by a high affinity system localized in glia since, as mentioned above, its K_m is in the micromolar range. The concentrations that monoamines normally reach in the region of their release are also unknown, so that the necessity for a low affinity, high capacity system is not clear. It has been suggested that catecholamines can reach very high concentrations in the synaptic vesicles in which they are stored [39] so that concerted release of a number of these vesicles could produce high concentrations in the synaptic cleft. Also high concentrations of catecholamines could be reached under pathological conditions.

Localization of catecholamine and 5-HT uptake in brain in situ

Important conclusions on cellular uptake sites for neurotransmitters have come from work applying histochemistry or autoradiography techniques to brain tissue. In the case of amino acid transmitters, uptake of radiolabeled glutamate and GABA has been clearly observed in astrocytes *in situ* by autoradiography [13, 40–44]. In contrast, the results of studies on the localization of radiolabeled monoamines in glia *in situ* by autoradiography have been largely negative. This was studied for catecholamines in rat cerebral cortex by light and electron microscopy in the presence of an inhibitor of monoamine oxidase (MAO) [16, 43], or by light microscopy using both histofluorescence and autoradiography [6, 12, 13, 15]. Uptake was interpreted as being into nerve endings because the autoradiographic pattern of uptake corresponded with the pattern of adrenergic nerve endings as visualized by specific histofluorescence for endogenous catecholamines. The presence of an MAO inhibitor, nialamide, was initially found to be essential to show such uptake [6, 15]. It should be pointed out that, in order to detect uptake into astrocyte cultures by autoradiography, it appears necessary to inhibit both the metabolizing enzymes COMT and MAO, and even under such conditions astrocytes in primary culture only appear able to concentrate catecholamines 5- to 10-fold over the concentrations present in the medium [22, 24]. Thus, *in situ* the concentrations that monoamines reach in neuronal elements may be much greater than those reached in astrocytes so that experimental conditions sufficient to localize monoamines in neurons *in situ* by autoradiography may be insufficient to localize the relatively lower levels in astrocytes.

In one study on the uptake of [^{3}H]6-OHDA in 1-day-old rat brain, ^{3}H label was found in both neurons and non-neuronal cells such as ependymal, meningeal and glial cells [17], but only the uptake of [^{3}H]6-OHDA into neurons was blocked by nomifensine, indicating that only the neurons contained a high affinity uptake system. Uptake into glia only occurred after a 4- to 6-hr delay, suggesting that it was the result of uptake of labeled, degenerated neuronal debris due to the action of 6-OHDA. However, this study was done on 1-day-old rats, and the major astrocytic proliferation is only just beginning at this time [44, 45]. Also, any astroglia present in

1-day-old rats are likely to be functionally immature. In support of the concept that glia will take up catecholamines and metabolize rather than concentrate them, recent studies [31, 32] have shown that subsequent to the gliosis induced by injection of kainic acid into adult rat striatum there is both increased [^{3}H]DA uptake and increased production of the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), due to the action of MAO, and of HVA (homovanillic acid), due to the action of MAO and COMT. When a neuronal uptake blocker, nomifensine, was added, production of the O-methylated derivative, HVA, actually increased [31, 32], and it has been shown that soluble COMT is largely localized to astrocytes and oligodendrocytes *in situ* [38]. Astrocytes *in situ* contain at least the MAO-B isozyme [46–48], and primary cultures of mouse astrocytes contain both MAO-A and MAO-B, although in these cultures the A form predominates [49].

In the case of studies on uptake of 5-HT *in situ*, there has again been largely negative evidence from autoradiographic studies of uptake into astrocytes in mammalian CNS. Uptake of [^{3}H]5-HT was interpreted as uptake into nerve endings because uptake was found between nerve cell bodies in regions known to have a high concentration of serotonergic nerve endings [12]. More direct evidence from electron microscope autoradiography localized about 80% of the grain clusters as being over nerve endings and axons, and only about 5% over glia and blood vessels after intraventricular injection of [^{3}H]5-HT in rat brain [14]. However, there is one reported exception to these negative findings of astrocytic uptake of [^{3}H]5-HT in mammalian CNS. Ruda and Gobel [18], using electron microscopy, localized grains to astrocytic processes in layers I and II of the dorsal horn of cat medulla after topical application of [^{3}H]5-HT and pretreatment of the animal with an MAO inhibitor, in addition to finding uptake of [^{3}H]5-HT into serotonergic nerve endings of different types. Also, in the filum terminale of the frog, which contains only glial cell bodies, increased grain density over such cell bodies and their processes was seen after incubation of slices of this tissue with [^{3}H]5-HT [50].

Surgical lesions of the midbrain raphe nuclei or chemical lesions of serotonergic nerves by injection of 5,6- or 5,7-dihydroxytryptamine, which appear to selectively destroy serotonergic neurons [51], also inhibits high affinity uptake of 5-HT by brain [52, 53]. Similar results have been obtained for uptake of NE after pretreatment with 6-OHDA [54], which is thought to selectively lesion noradrenergic nerve terminals due to its uptake on the catecholamine high affinity uptake system. However, it is not clear whether these lesion studies are completely specific. Thus, destruction of nerve terminals may have indirect effects on astroglia surrounding them. Also, we have observed recently that 6-OHDA is toxic to primary astrocyte cultures, and that 5,7-dihydroxytryptamine, although not clearly toxic, inhibited 5-HT uptake in such cultures (H. K. Kimelberg, D. M. Katz and R. A. Waniewski, unpublished observations).

Thus, the majority of these studies certainly suggest a more intense uptake and/or storage of catecholamines and serotonin into nerve terminals, but cannot definitely rule out a contribution of high affinity uptake into astrocytes associated mainly with subsequent metabolism.

Localization of catecholamine and 5-HT uptake in isolated brain fractions

Uptake into brain slices, total homogenates or crude, predominantly mitochondrial (10,000–50,000 *g*) membrane fractions [55] *in vitro* could be into neurons, glia, or membrane vesicles of varying size derived from neurons, glia or other cellular CNS constituents. It would not be expected, for example, that shearing forces due to homogenization would differentiate between nerve process terminals or glial processes. Thus, a mixture of vesiculated synaptic nerve terminals, axonal and dendritic processes, glial processes and other vesiculated plasma membrane fractions could be expected. Further purification of the 10,000–50,000 *g* fraction by gradient centrifugation will enable predominantly synaptosome-containing fractions to be obtained, but it is still likely that such fractions will contain other vesiculated components. Indeed, when membrane fractions obtained from homogenization of the C_6 glioma line were mixed with brain homogenates, they were found to be present in the "synaptosome" band after gradient centrifugation [56]. Although this experiment does not necessarily show the fate of glial cells *in situ* after homogenization of brain, it illustrates the problems of obtaining pure brain components by subfractionation procedures.

The earliest report of concentrative uptake of monoamines by glia appears to have been that by Henn and Hamberger [19] using glial fractions isolated by gradient centrifugation from rabbit cerebral cortices or whole brain. They found a 6- and 3-fold concentrative uptake for 5-HT and NE at a concentration of 0.1 μM after 5- and 15-min incubations respectively. At 0.5 μM and 40 min of incubation, DA showed a 5-fold cell to medium ratio. By comparison, synaptosomal fractions showed a 49-fold cell to medium ratio after 5 min of incubation with 0.1 μM 5-HT and a 10-fold ratio for 0.1 μM NE after 15 min of incubation. At 0.1 μM, a 53-fold concentrative uptake of GABA by the glial fraction was noted. However, the uptake of NE in all fractions was 35–50% inhibited by 5 μM desmethylimipyramine (DMI), a preferential inhibitor of high affinity NE uptake. More recent studies on uptake of NE in primary astrocyte cultures (see below) have also shown marked sensitivity to DMI. It should be noted that, although 80–90% of the isolated fractions was reported to be glia, the proportion of the fraction that was astrocytic is unclear [19].

Uptake of catecholamines in glial cultures

The first report of uptake of catecholamines by glial cells in culture appears to have been by Pfister and Goworek [20] who showed, by histofluorescence, localization of NE and DA at 10^{-4} M in cells identified as both glia and neurons in explant cultures from neonatal rat cerebral cortices. Hoffman and Vernadakis [21] also reported glial uptake of NE in whole brain cultures from 8-day chick embryos. Fifty percent inhibition of this uptake at the relatively high

concentration of 10^{-5} to 10^{-4} M DMI was observed. Subsequently, rat primary astrocyte cultures were shown to take up [^{3}H]NE or [^{3}H]DA [22]. At catecholamine concentrations of 0.1 μM, uptake was inhibited by omission of Na^+ from the media, pretreatment of the cultures with ouabain, or when uptake was measured at 4°. It was noted [22] that [^{3}H]DA uptake was less sensitive to these manipulations than was [^{3}H]NE. Conversely, uptake of [^{3}H]NE or [^{3}H]DA at higher concentrations (0.1 mM) was not Na^+ dependent and was not inhibited after pretreatment of the culture with ouabain. This may represent the low affinity or uptake$_2$ system, but detailed studies on this component were not done. Maximum accumulation in the cells of the catecholamines added at 0.1 μM only reached about 10 relative to the concentration in the medium, comparable to the low concentration ratios seen in bulk isolated glia by Henn and Hamberger [19], as discussed above. In primary astrocyte cultures, uptake of DA, added in the concentration range of 10^{-7} to 10^{-4} M, was associated with metabolism to DOPAC (due to MAO and aldehyde dehydrogenase action) and HVA (due to the action of COMT on DOPAC). The oxidatively deaminated and O-methylated derivative of NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), was also found after exposure of the cells to 10^{-6} or 10^{-4} M NE [22]. Later studies [25] showed that uptake of [^{3}H]NE at 10^{-7} M was very sensitive to inhibition by tricylic antidepressants, with DMI more effective than amitriptyline (AMT) with IC_{50} values of 2×10^{-9} and 4×10^{-8} M respectively. These results are comparable to the order of potency and sensitivity that was found for inhibition of NE uptake in brain [11, 34, 36, 57]. The K_m for [^{3}H]NE uptake was 0.35 ± 0.05 μM, and DA and DMI were competitive inhibitors of such uptake. Competitive inhibition by DA together with greater sensitivity to DMI suggests that the uptake system is an NE system, which is known *in situ* to transport DA as effectively or more effectively than its normal substrate, NE [58].

It should be noted that high affinity uptake in primary astrocyte cultures is greatest in the presence of both pargyline and tropolone, inhibitors of the catecholamine-metabolizing enzymes MAO and COMT respectively. As mentioned above, we observed metabolites due to the action of both enzymes after incubation of primary cultures with DA or NE [22]. As also previously mentioned there is immunocytochemical evidence for localization of soluble COMT in astrocytes, as well as oligodendrocytes, *in situ* [38], and recently for MAO-B in both primary astrocyte cultures and in astrocytes *in situ* [46]. Both MAO and COMT activities have also been measured in primary astrocyte cultures [49, 59]. Recently, it has been found that astrocytes in primate brain selectively stain with antibodies to MAO-B rather than MAO-A [46, 47]. This is different from studies on the enzyme activities of primary mouse astrocyte cultures where MAO-A activity predominates [49]. The MAO-A/MAO-B ratio varied from 8.7 after 2 weeks of growth to 5.8 after 10 weeks. These ratios were decreased by about one-half when the cells were chronically treated with DBcAMP. The results on MAO localization *in situ* [46, 47] are also puzzling because only MAO-B was found in serotonergic neurons, but serotonin is a preferred to substrate for MAO-A [4]. On the other hand, after gliosis due to injection of kainic acid into rat corpus striatum, increased production of HVA was found, but this was only significantly inhibited by the MAO-A inhibitor clorgyline and not by the MAO-B inhibitor deprenyl [31].

As mentioned previously, autoradiographic studies *in situ* failed to localize uptake of catecholamines to glial cells, whereas GABA and several amino acid transmitters have been so localized [13, 55]. There have been a few studies on autoradiographic localization of catecholamines in glial cultures. In agreement with the studies *in situ*, Hosli *et al.* [60] and Hosli and Hosli [61] found no uptake of ^{3}H-labeled catecholamines in glia in explant cultures from rat CNS, relative to a clear localization in neurons. Hansson [62] reported "weak" accumulation of both DA and NE in nialamide-treated cultures, relative to "strong" accumulation of GABA and "intense" accumulation of aspartate and glutamate. In a more detailed autoradiographic study on [^{3}H]NE and [^{3}H]DA uptake in primary rat astrocyte cultures from cerebral cortex, we have observed detectable but heterogeneous cellular localization of silver grains, which was also Na^+-dependent, sensitive to 10^{-7} M DMI and AMT, and required tropolone as well as pargyline for maximum intensity [23, 24].

Thus, the demonstration of high affinity uptake of catecholamines in astrocyte cultures is only seen when metabolism by both MAO and COMT is inhibited. Quantitative studies on uptake, however, indicate that the maximum concentrations that catecholamines reach intracellularly appear to be only 10-fold above that in the medium, so that they are observed by autoradiography in astrocyte cultures only under optimal conditions. Thus, uptake into astrocytes may not have been identified *in situ* relative to the much greater concentrations obtainable in nerve terminals since experimental conditions may have been used that show only the sites of the most intense uptake.

Uptake of 5-HT in glial cultures

Uptake of [^{3}H]5-HT by the C_6 glial tumor cell line in the presence of the MAO inhibitor nialamide appears to have been the first report of 5-HT uptake by glial cultures [26]. One component of this uptake was markedly Na^+ dependent. It had a somewhat high K_m of 1–2 μM and was inhibited by C1-IMI and DMI at relatively high concentrations, with IC_{50} values of around 10^{-5} M. The effect of the specific 5-HT inhibitor fluoxetine was not examined. Similar results for [^{3}H]5-HT uptake by C_6 cells were reported recently by Whitaker *et al.* [27]. Again, relatively high K_m values of 2.2 μM and IC_{50} values for C1-IMI and DMI of 28 and >1000 μM, respectively, were reported. Zimelidine and mepyramine had IC_{50} values of 19 and 25 μM respectively. In this study it was not stated whether an MAO inhibitor was added. Recently Tardy *et al.* [28] reported uptake of [^{3}H]5-HT by primary astrocyte cultures from mouse brain with a K_m of 0.17 μM and a V_{max} of 0.6 pmole/mg protein/min. Uptake was inhibited by C1-IMI and

Table 1. IC_{50} values for inhibition of Na^+-sensitive [^{3}H]5-HT uptake by rat primary astrocyte cultures

Compound	IC_{50} (μM)
Cl-IMI	0.009
Fluoxetine	0.023
IMI	0.14
AMT	0.14
DMI	0.62
Iprindole	2.8
Mianserin	4.9

IC_{50} values (concentrations needed to inhibit Na^+-dependent [^{3}H]5-HT uptake, approx. 80% of total, by 50%) are given for a number of clinically effective antidepressants. All the antagonists shown were present during the 20-min preincubation period. Uptake of 10^{-7} M [^{3}H]5-HT was then measured for 4 min. Homogeneous astrocyte cultures were prepared from neonatal rat cerebral cortex and were used after 22–23 days growth. (See Ref. 29 for further details.)

fluoxetine, but again only at very high concentrations of around 10^{-4} M. It was also not mentioned whether an MAO inhibitor was added in this study.

We have also recently observed significant uptake of [^{3}H]5-HT by rat primary astrocyte cultures [29, 30]. This uptake showed a high affinity for the Na^+ sensitive component of [^{3}H]5-HT uptake with a K_m of 0.40 μM, and [^{3}H]5-HT uptake was also very sensitive to specific inhibitors, such as the clinically effective antidepressants. The order of effectiveness of inhibition for the antidepressants tested was chlorimipramine > fluoxetine > imipramine = amitriptyline > desmethylimipramine > iprindole > mianserin. The IC_{50} values are shown in Table 1.

The values shown in Table 1 are very close to those found for inhibition of [^{3}H]5-HT uptake in various brain preparations [11, 34, 57, 63]. Inhibition by the specific inhibitor fluoxetine, with an IC_{50} value of 2.3×10^{-8} M, is close to the value of 6×10^{-8} M reported for inhibition of 5-HT uptake in rat brain synaptosomes [36]. Omission of the MAO inhibitor pargyline markedly reduced the Na^+-dependent component of [^{3}H]5-HT uptake, but it had a negligible effect on the Na^+-independent component, suggesting significant oxidative deamination of serotonin by MAO and subsequent release of its metabolite 5-hydroxyindoleacetic acid (5-HIAA) after it has been taken up by the high affinity system [29]. We estimated that this system enabled the cells to concentrate [^{3}H]5-HT up to 40- to 50-fold at an external [^{3}H]5-HT concentration of 10^{-7} M [29]. Quantitatively, the rate of uptake by the primary astrocyte cultures was estimated to be about 10% of that reported for rat brain striatal or hypothalmic slices [63]. These lower values may reflect an actual lower transport capacity of astrocytes *in situ* compared to neurons, or a lower uptake in the cells when grown in culture.

Hansson [62] reported "weak" autoradiographic grain localization over primary astrocyte cultures after exposure of cultures to [^{3}H]5-HT. We found that virtually all the cells had a grain density that was above background after uptake of [^{3}H]5-HT in Na^+-containing medium. This uptake was reduced to close to background levels when Na^+ was omitted from the medium [29]. We also found that, by fixing the cells with 4% paraformaldehyde and simultaneously staining immunocytochemically for glial fibrillary acidic protein (GFAP), we could localize [^{3}H]5-HT by autoradiography over GFAP(+) cells [30]. This grain localization was inhibited when 10^{-7} M fluoxetine or chlorimipramine was present, or by omission of Na^+ from the medium.

Speculation on roles for astroglial uptake of catecholamines and 5-HT

As can be appreciated from the foregoing review, at the present time the evidence for uptake of catecholamines and 5-HT by astrocytes comes overwhelmingly from studies on isolated preparations and glial cultures. The failure to localize uptake into astrocytes *in situ* may be simply due to the lack of definition of light microscopic studies, coupled with the fact that astrocytes do not appear to concentrate these monoamines to anywhere near the same extent as do neurons. In *in vitro* preparations containing only astrocytes, such lower uptake can be detected using quantitative measurements of uptake of radioactive label. In the case of autoradiographic localization, the fixed cultures can be developed for a time sufficient to obtain an adequate grain density. In tissue, conditions will be optimized for the regions showing the greatest localization, namely neuronal nerve endings, and regions of lower uptake, such as glia, may then be missed. In most *in situ* studies, inhibitors of MAO were added to eliminate metabolism [6], but in the case of the catecholamines, inhibition of COMT also appears to be important to detect uptake at low catecholamine concentrations in primary astrocyte cultures [23, 24]. This absence of COMT inhibitors may be one reason for failure to observe glial localization *in situ*. Chemical or surgical lesion studies to eliminate specific nerve terminals cannot be considered definitive because of the possibility of indirect effects of neuronal destruction on surrounding astrocytes. Also, the chemicals used may also be toxic to glial cells or affect transmitter uptake. For example, we have found that treatment of primary astrocyte cultures with 6-OHDA or 5,7-dihydroxytryptamine affects 5-HT uptake. After exposure of cultures to 10^{-4} M 5,7-dihydroxytryptamine for 3 days in the absence of serum, there was only a 10% reduction in cell protein relative to serum-free controls, but a 47% reduction in the Na^+-dependent component of [^{3}H]5-HT uptake, expressed on a per mg protein basis. In contrast, 2-day exposure to 10^{-4} M 6-OHDA seemed toxic to the cells, as indicated by loss of up to 90% of the cell protein remaining as an attached cell monolayer (H. K. Kimelberg, D. M. Katz and R. A. Waniewski, unpublished experiments).

Accepting that the work described in this commentary indicates that astrocytes can take up both catecholamines and serotonin by a high affinity system and thus have the potential of competing with neuronal terminals for uptake of the released transmitters, the question of whether and to what extent this activity occurs in the CNS and what its role might

be remains unanswered. Studies so far indicate that the uptake$_1$ system in astrocytes *in vitro* behaves pharmacologically like the uptake $_1$ system in various brain preparations, so that at present it does not seem possible to selectively inhibit astrocytic uptake and determine its effect on neuronal function *in situ*. Further pharmacological studies on this point should be done and if selective inhibition can be obtained it might enable this question to be addressed *in situ*. Mianserin and iprindole do block uptake of [^{3}H]5-HT at relatively high concentrations (see Table 1), but inhibition of 5-HT uptake in synaptosomes by 8 μM mianserin has also been reported [11].

The likely fate of the catecholamines and 5-HT taken up into astrocytes appears to be metabolism and removal—a pass-through, uptake and metabolism system, rather than uptake and storage in presynaptic vesicles for re-release, as seems to occur in nerve endings. Figure 1 is a diagram depicting the close relationships of astrocytes to a varicosity which represents the presynaptic specialization of many catecholaminergic or serotonergic axons in the mammalian CNS [35, 7, 64, 65]. Portions of postsynaptic neuronal dendrites or cell bodies with receptors (cross-hatched areas) for the transmitters, are also shown. In some cases, the junctional membrane differentiation characteristic of chemical synapses is absent [66], suggesting action at a distance or non-synaptic type effects of catecholamines or 5-HT. Thus, these receptors may or may not be closely apposed to the presynaptic varicosity, as shown. High affinity uptake, with co-transport of Na^+, is indicated on both the astrocytes and presynaptic varicosity. Uptake of the released transmitter at low concentrations occurs because of the high affinity of the transport process, and concentrative uptake is thought to be achieved in part through co-transport of one or more sodium ions utilizing the energy of the inwardly directed Na^+ electrochemical gradient. This gradient is maintained by operation of the ubiquitous ATP driven (Na^+,K^+) pump which pumps out Na^+ accumulated intracellularly, and this pump is indicated in the lower astrocytic profile. Transmembrane transport routes and the diffusional pathways for transmitters released by fusion of synaptic vesicles with the presynaptic membrane are shown as dashed lines. Transmembrane transport of Na^+ or K^+ is shown as solid arrows. As recently reported by Sweadner [67] for sympathetic neurons in culture, norepinephrine release may also occur by reversal of the high affinity uptake system under appropriate conditions. The fate of recovered transmitters in the presynaptic varicosity could be either repackaging in the vesicles, or metabolism by MAO (A or B) localized to outer mitochondrial membranes. COMT appears to be localized only to glia,

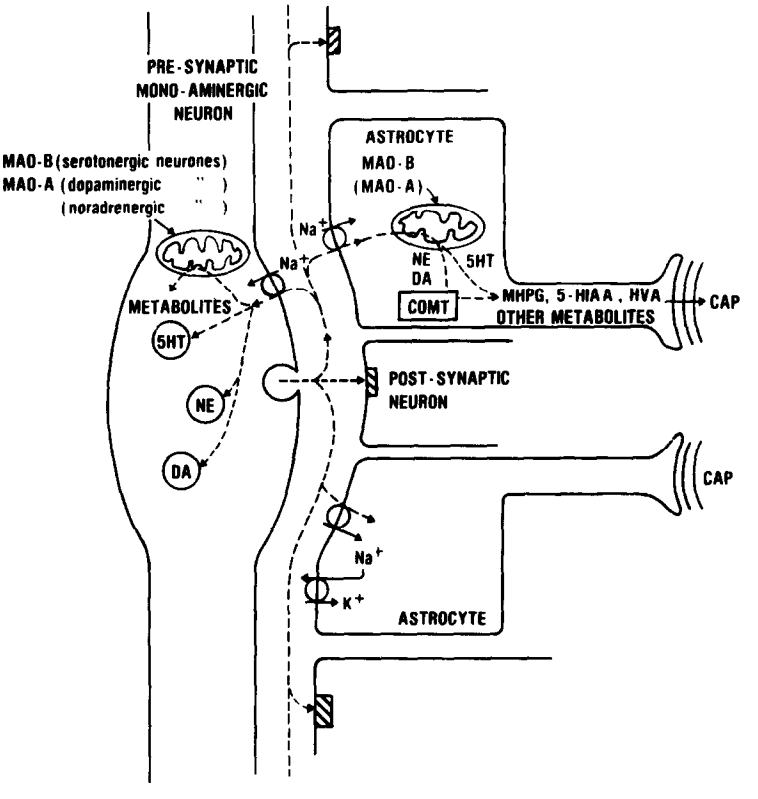

Fig. 1. Uptake of serotonin or catecholamines at a generalized monoaminergic presynaptic varicosity. See text for description. Abbreviations: Cap, brain capillary; MAO-A and MAO-B, A and B isozymes of monoamine oxidase; COMT, catechol-*O*-methyltransferase; 5-HT, serotonin; NE, neorepinephrine; DA, dopamine; MHPG, 3-methoxy-4-hydroxyphenylglycol; 5-HIAA, 5-hydroxyindoleacetic acid; and HVA, homovanillic acid.

and MAO-B is detectable by immunocytochemistry in both astrocytes and serotonergic neurons *in situ* [46, 47]. In a recent commentary in this journal [68] it was pointed out that significant metabolism may occur intraneuronally without release, but this would be due to the action of MAO alone. Also, it was noted that metabolism of DA can occur after a chemical lesion of DA nerves by 6-hydroxydopamine, and this is consistent with metabolism of DA in glia [68].

In the astrocytic processes, no other fate for the transmitter taken up other than inactivation by metabolism seems likely at the present time. In preliminary studies, we obtained no evidence for depolarization-induced release of [^{3}H]5-HT when the cells were exposed to elevated [K^+] in the medium. The presence of both MAO and COMT suggests that a wide variety of metabolites can be produced within the astrocyte. It appears from our studies on astrocyte cultures [29] and other studies [33] that such metabolites permeate membranes more readily than the parent amines, and thus can exit by diffusion through the cell membrane anywhere within the neuropil. Alternatively, there could be specific membrane transport systems located at the perivascular surface of an astrocytic process (end-foot [7]) facing capillaries (cap, see Fig. 1) and also in astrocytic processes facing CSF located beneath the pia mater and subependymal zone, which would preferentially direct such metabolites into the blood and CSF respectively, for removal from the CNS. This would represent the waste product envisioned by Lugaro ([9], and see quotation at the beginning of this commentary), but formed after, rather than as a direct result of, chemical synaptic transmission. Some of the intramembraneous assemblies preferentially localized in astrocytic membranes at the perivascular and CSF structural facing sites [69] might represent the basis of such transport processes.

The cellular localization of the MAO isozymes and the uptake and release of monoamines have recently been the objects of renewed interest in relation to Parkinsonism symptoms induced by inadvertent administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP). It appears that MPTP itself is not toxic but is converted to a toxic oxidized derivative, MPP^+, by MAO-B. MPP^+ is selectively toxic to neurons of the substantia nigra, the degeneration of which then causes the symptoms of Parkinsonism [70–72]. It has been suggested that MPP^+ is taken up by the high affinity DA uptake system in these cells, while a major site of MPTP oxidation is via MAO-B action in astrocytes [72]. It is not clear how MPTP gets into astrocytes, but this question and the production of MPP^+ could easily be studied in primary astrocyte cultures. Toxicity of MPTP to substantia nigra neurons has already been shown in explant cultures, which presumably also contain astrocytes [73]. It would be of interest to see if MPP^+ is or is not taken up into astrocytes by a high affinity system. The proposed scheme for MPTP toxicity in which neurons take up a metabolite released by MAO-B action in astrocytes constitutes an interesting reversal of the uptake and metabolic inactivation of neuronally released transmitters by astrocytes, as described in this commentary.

Recently it has been hypothesized that transmitter uptake may be due to internalization of receptors, as seen in desensitization or down-regulation [74]. Thus, a separate specific uptake system for transmitters was proposed to be unnecessary, and such a dual role for receptors would explain why some drugs have effects on both receptors and uptake systems. Since astrocytes, particularly in culture, are now known to have a large variety of receptors [75, 76], such a route of uptake, if it does prove to be significant, could also apply to astrocytes.

Does uptake by astrocytes represent a first or second line of defense for removing released transmitter monoamines from the synaptic cleft and extracellular space, thus terminating their actions? The close proximity of astrocytic processes to the synapse [7–10, 64], plus the possible existence of a high affinity uptake system in such cells, as documented in this commentary, suggests that astrocytic uptake could compete equally with re-uptake into neuronal terminals. Since the uptake system in astrocytes is also sensitive to clinically effective antidepressants, the therapeutic effects of such agents may thus be mediated to some extent by their action on astrocytes. Some of the effects seen after administration of antidepressants do seem consistent with inhibition of uptake and metabolism of monoamines by astrocytes. For instance, it has been reported that 30–50% of the O-methylated derivative of NE, MHPG, which is found in urine, is of CNS origin [39], and COMT has been shown to be localized to astrocytes and oligodendrocytes in the CNS [38]. Thus, inhibition of uptake of NE into astrocytes could explain the reported decrease of urinary or plasma MHPG levels in depressed patients after treatment with imipyramine (IMI) and AMT [77] or DMI [78]. Long-term treatment with AMT has been reported to decrease CSF levels of 5-HIAA [79], and again this could be a consequence of inhibition of 5-HT uptake and deamination in astrocytes. All these actions of the tricyclic antidepressants would contribute to increasing the levels of released norepinephrine and serotonin, in concordance with the original amine hypothesis where at least some types of depression were considered to be due to low effective levels of these transmitters [80]. However, in rats, while acute treatment with DMI decreases the levels of an O-methylated and deaminated metabolite of NE, chronic treatment with DMI increases this level [81]. Chronic treatment, however, might be expected to lead to an adaptive increase in uptake sites (up-regulation).

The involvement of astrocytic uptake as a significant means of terminating the action of neurotransmitter monoamines in the CNS seems rarely to have been considered, but it may well play an important role. Future studies in this area could shed light on this important aspect of neuron–astroglia interrelationships and its role in both normal and abnormal brain function.

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