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COMMENTARY

UPTAKE AND METABOLISM OF γ-AMINOBUTYRIC ACID BY NEURONES AND GLIAL CELLS

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The role of γ-aminobutyric acid (GABA) as an inhibitory substance in invertebrate and vertebrate nervous system is now well established [1]. Roberts [2] has recently reviewed in this journal various aspects of the biochemical pharmacology of this substance in mammalian CNS. In the present review we will concentrate on one aspect of the biochemical disposition of GABA in CNS, namely its uptake by specialized transport mechanisms in neurones and glial cells. The existence of such transport mechanisms is well documented for other neurotransmitters, particularly the biogenic amines [3], and transmitter uptake by nervous tissues is thought to represent a general mechanism whereby the postsynaptic actions of neurotransmitters are terminated following their neural release [4]. In common with the other transmitter-specific neurones, GABA-inhibitory neurones in mammalian CNS appear to possess high-affinity uptake sites specific for GABA on their external surfaces and this may be related to that function. However, high-affinity uptake sites for GABA, with a different substrate and inhibitor specificity also exist on the surface of a variety of glial cells, where their function is as yet not clear.

UPTAKE OF GABA BY INHIBITORY NEURONES

Many studies have shown that GABA is rapidly accumulated from the external medium by small slices of brain tissue, or by homogenates containing synaptosomes [5–12]. The uptake of ^{3}H-GABA by such preparations is mediated by a high-affinity transport mechanism with an apparent K_m in various regions of rat brain of approximately 20 μM [10, 13, 14]. Levi and Raiteri [15] have shown that the detectability of the high-affinity component of GABA uptake in brain slice preparations is critically dependent upon the dimensions of the slices used. This component predominates in small prisms (dimensions 0·1 × 0·1 × 3 mm) prepared from rat cerebral cortex by a mechanical chopper [13], but in brain slices of 0·4 mm thickness the high-affinity uptake is masked by a low-affinity component, presumably reflecting GABA uptake by a less specific "neutral amino acid" transport process [16]. The reason for this difference in the behaviour of tissue slices of increasing thickness is not known; it may explain, however, why the high affinity component of GABA uptake was not described in earlier studies of amino acid uptake by conventional brain slices. The accumulation of GABA by the high affinity mechanism in small brain slices or in synaptosome preparations appears to reflect a net uptake of amino acid, rather than a homo-exchange process. Thus the initial rate of ^{3}H-GABA uptake in such preparations is not influenced by preloading with non-radioactive GABA, or by treatment of animals with amino-oxyacetic acid (AOAA) which increases the endogenous GABA pool size [17, 18]. In common with other high-affinity neurotransmitter uptake mechanisms, the uptake of GABA is highly dependent on the presence of sodium ions in the external medium [11, 13, 19]. The rate of uptake of GABA by brain slices or synaptosome preparations from different brain regions correlates closely with the endogenous GABA content in such regions [14, 18, 20]. The uptake mechanism in brain slices is apparently associated largely with nerve terminals, as shown by the finding that homogenates of rat cerebral cortex retain up to 75 per cent of the ^{3}H-GABA uptake sites present in small tissue slices of the same tissue [14]. In homogenates of tissue labelled by prior exposure of small slices to ^{3}H-GABA, or after incubation of the homogenate, the labelled amino acid is mainly recovered in fractions with a density characteristic of synaptosomes, after sucrose density gradient centrifugation [14, 21]. The particles containing ^{3}H-GABA sedimented to the same position on such gradients as fractions containing particle-bound endogenous GABA and the biosynthetic enzyme glutamic acid decarboxylase, suggesting that ^{3}H-GABA is taken up by nerve terminals which normally contain and synthesize the amino acid. The selectivity of this process for those terminals which contain GABA as opposed to other transmitters is strongly suggested by the observation that synaptosomes labelled with radioactive GABA could be partially separated from other synaptosome populations labelled with catecholamines or with other amino acids, such as glycine [22, 24]. These conclusions were greatly strengthened by the results obtained by electron-microscopic autoradiography of small brain slices and homogenates labelled by exposure to ^{3}H-GABA [25, 26]. It was found that a high proportion of the labelled amino acid (about 75 per cent) could be retained in such tissue preparations after fixation with glutaraldehyde, which appears to effect a true covalent fixation of GABA and other amino compounds to tissue macromolecules. These initial autoradiographic studies showed that both in brain slices and in homogenates there was a high degree of localization of the labelled amino acid over nerve terminals, or synaptosomes, which accounted for more than 70 per cent of the autoradiographic

activity. Furthermore, the labelling of nerve terminals occurred in an "all or none" manner, so that some nerve terminals or synaptosomes were heavily labelled, while others were devoid of label. The proportion of terminals which became labelled after exposure to ^{3}H-GABA varied from 15 per cent of the total terminal population in cerebellar homogenates to more than 50 per cent in slices or homogenates of *substantia nigra*. In homogenates of rat spinal cord, it was possible to show that the population of terminals labelled to exposure to ^{3}H-GABA was distinct from another population which became selectively labelled after exposure to [^{3}H]glycine [26], adding further weight to the notion that the ability to accumulate exogenous ^{3}H-GABA is a special property of those nerve terminals that normally contain and release this amino acid transmitter.

The use of homogenates or small slices of brain tissue, however, does not provide much useful morphological information on the anatomical identity or distribution of the GABA inhibitory neurones. The preservation of fine structure is poor in small brain slices after incubation *in vitro*. One way of overcoming this problem is to make use of purified synaptosome complexes of identified origin, such as the cerebellar glomeruli particles, in which autoradiographic studies have shown that the high affinity uptake sites for ^{3}H-GABA are exclusively located over the inhibitory Golgi axon terminals [27]. Alternatively, somewhat better preservation of tissue morphology can be obtained by using thin slices of brain sectioned from fresh chilled tissue by using the Oxford Instrument "Vibratome". The distribution of ^{3}H-GABA in coronal sections of rat spinal cord has been examined in this way at the light microscope level [28]. The morphological distribution of GABA uptake sites, with the highest density in the *substantia gelatinosa* region of dorsal grey, and in Clarke's nucleus, corresponds well with the known distribution of endogenous GABA in the spinal cord, and with the proposed function of GABA as the transmitter mediating the process of presynaptic inhibition at primary afferent terminals in this region of CNS [29].

Another approach, which allows excellent preservation of fine structure, is to administer the labelled amino acid *in vivo* and to fix the brain *in situ* by perfusion of fixative solution through the vasculature. This may be achieved by administration of labelled GABA into the CSF, or by direct microinjection into a brain region in an anaesthetized animal. After the injection of ^{3}H-GABA into CSF, in animals pretreated with AOAA to inhibit its metabolism, the amino acid penetrated rather poorly into the brain, so that intense labelling was seen only over structures that were near the ventricular system. Autoradiographic examination of such tissue, however, revealed several important features [30]. In addition to labelling over nerve terminals in various regions of the brain, intense labelling was also seen over certain neuronal perikarya. In the superficial layers of the cerebellar cortex, for example, the cell bodies of stellate inhibitory interneurones were prominently labelled. Since GABA uptake sites would be expected to exist not only at nerve terminals but all over the surface of GABA-inhibitory neurones, and since the stellate neurones are thought to use GABA as their transmitter, their result supports the hypothesis that neuronal uptake sites for GABA are restricted to those inhibitory neurones that use this transmitter. On the other hand, neurones known to use transmitters other than GABA, for example the noradrenaline-containing cells of the *locus coeruleus*, or cerebellar granule cells, were devoid of labelling after injection of ^{3}H-GABA. In experiments on the localization of ^{3}H-GABA in various tissues other than CNS (see below) we have also observed that sympathetic nerve terminals in the pineal, cholinergic nerve terminals in the sympathetic ganglia and neurosecretory terminals and axons in the posterior pituitary gland lack the ability to accumulate exogenous GABA. Similar selectivity of uptake of labelled GABA into cell bodies and axons and terminals of inhibitory cerebellar interneurones has been observed in dissociated cell cultures of rat cerebellum by Lasher [31]. He found that not only the stellate cells became labelled after incubation with ^{3}H-GABA, but larger cells which seem to correspond to Purkinje neurones also accumulated the amino acid. The Purkinje cells are perhaps the best characterized GABA-inhibitory neurones in mammalian CNS, and so would be expected to take up the labelled amino acid. In studies *in vivo*, however, we have rarely observed autoradiographic activity over Purkinje cells in the cerebellum, and this has also been the experience of others [32, 33]. It may be that the tightly wrapped glial sheath which surrounds such cells in the intact cerebellum constitutes an effective barrier limiting access of ^{3}H-GABA. This would explain why Purkinje cells show an ability to accumulate exogenous GABA when the glial sheath is disrupted, as in dispersed cell cultures, or in fragments of cerebellar tissue transplanted into the anterior chamber of the eye [34] or maintained in tissue culture [35].

Further evidence for the specificity of ^{3}H-GABA uptake by GABA-inhibitory neurones in CNS was provided by Hattori *et al.* [36]. In a combined autoradiographic and biochemical study, they found that ^{3}H-GABA was taken up predominantly into nerve terminals in small slices of rat *substantia nigra*. In agreement with our own findings, they observed labelling of 48 per cent of the terminals in this tissue. After lesions of the globus pallidus or hemitransections anterior to the *substantia nigra* there was a marked reduction in glutamic acid decarboxylase activity in *substantia nigra*, and a parallel reduction in the labelling of nerve terminals by ^{3}H-GABA. Storm-Mathisen [37] has also reported a reduced uptake of ^{3}H-GABA in rat *substantia nigra* after similar lesions.

Although the results obtained after injections of ^{3}H-GABA *in vivo* were encouraging, a further unexpected problem became apparent at that time. this was the observation that after such injections, accumulations of ^{3}H-GABA were seen not only over neuronal cell bodies and nerve terminals, but also over a variety of glial cells and ependymal cells lining the ventricular cavities. In fact this non-neuronal labelling was often so prominent that it obscured any detailed analysis of the distribution of the labelled neuronal elements; similar observations were made by Hokfelt and Ljungdahl [38, 40]. Glial cells had not appeared to represent a prominent com-

ponent of ^{3}H-GABA uptake in our previous studies of incubated brain slices or homogenates, either because glial cells are damaged in such *in vitro* preparations, or because the uptake of ^{3}H-GABA by glial cells is relatively slow by comparison with neuronal uptake (see below). We have subsequently examined the glial uptake of GABA in various model systems in order to find out more about the properties and possible functional implications of this process.

UPTAKE OF GABA BY GLIAL CELLS

Since both GABA-inhibitory neurones and glial cells accumulate exogenous GABA in CNS tissue, we have studied the properties of the glial uptake component in simpler systems, in which GABA-inhibitory neurones are not present. Bowery and Brown [41] described a high affinity uptake for ^{3}H-GABA in rat superior cervical sympathetic ganglia, and autoradiographic examination of sympathetic ganglia after incubation with labelled GABA showed that the satellite glial cells and Schwann cells were responsible for this uptake [42]. Schon and Kelly [43, 44] observed a similar uptake of ^{3}H-GABA into the satellite cells of rat dorsal root ganglia, which represent a useful model tissue since such ganglia contain no sympathetic terminals—the main cell types present being the large sensory neurones and their satellite glial cell sheaths. In sensory ganglia incubated *in vitro* in media containing low-concentrations of ^{3}H-GABA, there is a slow accumulation of unchanged ^{3}H-GABA. The accumulation of ^{3}H-GABA was greatly increased if AOAA was present in the incubation medium; under such conditions the uptake continued linearly for 4 hr and tissue:medium ratios of up to 100:1 could be attained, similar to those observed in small slices of rat cerebral cortex in previous studies. However, the accumulation of GABA by brain slices was much more rapid, and approached a steady state after 1 hr of incubation. The accumulation of ^{3}H-GABA by brain slices is also unaffected by the presence of AOAA in the incubation medium, although at high concentrations this compound can inhibit ^{3}H-GABA accumulation [45]. GABA uptake by the satellite cells in sensory ganglia was similar to that in brain slices or homogenates in being markedly dependent on the presence of sodium ions in the external medium, and exhibiting high affinity for the substrate, with a K_m of 10 μM [43]. Like the neuronal uptake, the system in sensory ganglia was not inhibited by related amino acids such as glutamate, glycine and glutamine, and was potently inhibited by the GABA analogues 3-hydroxy-GABA, 2-fluoro-GABA and trans-4-aminocrotonic acid. The uptake process in sensory ganglia, however, showed important differences in inhibitor sensitivity from that in brain slices or homogenates (Table 1). The most notable differences were seen with L-2,4-diaminobutyric acid (DABA), which is a potent inhibitor of GABA uptake in small brain slices or homogenates (K_i = 50 μM), but was some 20 times less potent in inhibiting GABA uptake in sensory ganglia, and β-alanine which was some 200 times more potent as an inhibitor of GABA uptake in sensory ganglia than in brain slices.

The presence of a similar GABA uptake system in glial cells has also been detected by autoradiographic and biochemical studies in other parts of the nervous system. An uptake which is sensitive to β-alanine and relatively insensitive to inhibition by L-DABA was found to occur in satellite cells in sympathetic ganglia, in pituicytes in the posterior pituitary gland and in gliocytes in the pineal gland [46, 47]. In the isolated rat retina a somewhat anomalous situation exists, since the glial Muller cells represent the predominant site of uptake [48–50], but the uptake process is sensitive to inhibition by L-DABA and relatively insensitive to β-alanine. Species comparisons of GABA uptake into retinal tissues have revealed striking differences. In rabbit, goldfish, frog, pigeon and chicken retinae uptake occurs mainly into neuronal cells, especially into certain amacrine cells [50, 52]. In the rat, however, the Muller cells are largely responsible, but these appear to show the uptake properties characteristic of neuronal sites elsewhere in the nervous system [53].

From these observations it is apparent that glial cells in many regions of the nervous system possess high affinity uptake sites for GABA. As mentioned above, glial accumulations of ^{3}H-GABA have also been observed in various regions of the brain after administration of labelled GABA *in vivo*. Other

Table 1. Comparison of kinetic parameters and inhibitor potencies in rat sensory ganglia and small slices of cerebral cortex*

	^{3}H-GABA uptake		^{3}H-DABA uptake	^{3}H-β-alanine uptake	
	Ganglia	Cortex	Cortex	Ganglia	Cortex
K_m (μM)	10	22	31	33	55
V_{max} (moles/g^{-1} min^{-1})	2·1	115	33	2·3	12·5
IC_{50} for GABA (μM)	—	—	117	18	33
IC_{50} for L-DABA (μM)	700	50	—	470	270
IC_{50} for β-alanine (μM)	120	21,000	>10,000	—	—
IC_{50} for 3-hydroxy GABA (μM) *ca*	100	100	100	—	—

* Results from Iversen and Johnston [14], Schon and Kelly [43, 66] and Dick and Kelly (unpublished results).

studies have reported a prominent uptake of GABA, mediated by high-affinity process in glial cells separated from brain by a bulk gradient centrifugation technique [54, 55]. Similar high affinity uptake sites (K_m 13–30 μM) have been reported in various glial tumour cells maintained in tissue culture [56] and glial cells in cultures of cerebellar tissue [31]. An uptake of labelled GABA by glial or satellite cells has also been reported in invertebrate preparations, in the *lamina ganglionaris* of *Musca* and *Drosophila* [57] and in satellite cells surrounding motor nerve terminals in lobster nerve-muscle preparations [58]. In slices of human glial tumours incubated *in vitro*, a high affinity uptake of ^{3}H-GABA could also be detected, although the rate of uptake was considerably slower than that seen in slices of normal rodent brain [59].

The possibility that the uptake of ^{3}H-GABA by glial cells *in vitro* in the rat sensory ganglia sympathetic ganglia, pineal and pituitary could be an artifact induced by damage to these cells during the incubation procedures can be excluded, since these sites also become prominently labelled after intra-arterial injection of ^{3}H-GABA in the intact animal [47].

Biochemical analysis of rat pineal, posterior pituitary and sympathetic and sensory ganglia showed that all of these tissues, which lack GABA-inhibitory neurones, contained measurable amounts of endogenous GABA, GABA:glutamate transaminase (GABA-T) and glutamic decarboxylase (GAD) activities [46, 47]. These findings strongly suggest that GABA plays some role in glial cells unrelated to its inhibitory neurotransmitter function. This undoubtedly contributes to the complexities of the compartmentation of GABA metabolism in mammalian CNS, although the precise functional significance of the glial compartment of GABA metabolism is not yet clear. It is possible that the high-affinity uptake sites for GABA in glial cells in CNS might represent an important mechanism for removing extracellular GABA after its release from inhibitory nerve terminals. GABA taken up in this way would be rapidly degraded by GABA-T in the glial cells. Recent histochemical evidence showing high GABA-T activity in glial cells in both retina and brain would support such a hypothesis [60, 61]. However, it is not clear why such a mechanism should be present both in inhibitory nerve terminals and in glial cells, and this explanation does not account for the function—if any—of high-affinity glial uptake sites in the peripheral nervous system where there are no GABA-releasing nerve terminals.

STUDIES WITH ^{3}H-DABA AND ^{3}H-β-ALANINE

Because the glial uptake mechanism for GABA is preferentially inhibited by β-alanine, and the neuronal uptake mechanism by DABA, we have examined the possibility that these substances might act as selective substrates for the two uptake mechanisms. Simon and Martin [62] showed that ^{14}C-DABA was taken up by synaptosome fractions from the rat brain, and that this uptake was sodium-dependent and could largely by inhibited by an excess of GABA. They used labelled DABA of relatively low specific activity, and were thus obliged to use relatively high concentrations of the amino acid for most of their studies. We have recently studied the uptake of high specific activity ^{3}H-DL-DABA (sp. act. 8·7 Ci/m-mole) by rat brain and other tissues (Dick and Kelly, unpublished results). The radioactive amino acid was accumulated rapidly by small slices of rat cerebral cortex, using the techniques employed in previous studies of ^{3}H-GABA uptake [13]. The accumulation of ^{3}H-DABA was temperature-dependent and required the presence of sodium in the incubation medium. The results obtained from kinetic studies and examination of inhibitors of DABA uptake suggest that DABA acts as an alternative substrate for the neuronal high-affinity uptake sites for GABA in inhibitory neurones. Thus the K_m for DABA uptake (31 μM) was similar to the K_i for inhibition of ^{3}H-GABA uptake in the same preparation by non-radioactive DABA. Similarly, the K_i for GABA as an inhibitor or ^{3}H-DABA uptake was similar to its own K_m when used as a substrate. DABA uptake was also inhibited by the same compounds previously found to be effective inhibitors of ^{3}H-GABA uptake (Table 1). Electron microscopic autoradiographic examination of small slices of cortex labelled with ^{3}H-DABA gave a result closely comparable to that previously obtained with ^{3}H-GABA with certain nerve terminals representing the major sites of uptake. In contrast, ^{3}H-DABA was poorly accumulated by isolated rat sensory ganglia, with a tissue:medium ratio not exceeding 1:1 after 1 hr incubation. Autoradiographic studies showed that there was no selective accumulation of labelled DABA over the satellite glial cells in these tissues. ^{3}H-DABA may thus represent a useful tool for the selective labelling of GABA-inhibitory neurones in the mammalian CNS for autoradiographic studies. In addition to the selective accumulation of this amino acid by inhibitory neurones and not by glial cells, its use has the added advantage that, unlike GABA, DABA is not rapidly metabolised in brain. After micro-injection of ^{3}H-DABA into a cerebellar folium in the intact rat brain we have observed a selective localization of radioactivity of the labelled compound over stellate neurone cell bodies and other inhibitory neurones, similar to that seen after administration of ^{3}H-GABA. The neuronal sites labelled with ^{3}H-DABA correspond closely with those recently shown by immunofluorescence histochemical studies to contain GAD [63, 64]. A selective accumulation of ^{3}H-DABA was also seen by electron-microscopic autoradiography over the inhibitory terminals of Golgi neurone axons in cerebellar glomeruli, while the other components of the glomeruli were devoid of label [65]. This pattern of labelling was very similar to that seen after incubation of isolated cerebellar glomeruli particles with ^{3}H-GABA, when a selective accumulation of label over the Golgi axon terminals was also seen [27]. We believe that micro-injections of ^{3}H-DABA into the intact brain followed by autoradiographic examination will allow a detailed identification and mapping of GABA-inhibitory neurones and their terminals in many regions of CNS in the future.

Conversely, studies with ^{3}H-β-alanine indicate that it acts as a selective substrate for the high affinity GABA uptake sites in glial cells. ^{3}H-β-alanine was well concentrated in isolated rat sensory ganglia by a slow uptake process similar to that for GABA,

and autoradiographic examination showed a selective accumulation by satellite glial cells identical to that observed in previous studies with ^{3}H-GABA. A similar slow uptake of ^{3}H-β-alanine was found to occur in small slices or homogenates of rat cerebral cortex. Autoradiographic studies of brain slices labelled with ^{3}H-β-alanine showed an absence of labelling over nerve terminals, with accumulations of label over structures presumed to be glial cell processes [66]. The glial elements surrounding small blood vessels, and glial cells in a satellite position to large neurones were particularly prominent sites for the high-affinity uptake of this amino acid. Labelled particles other than synaptosomes were also observed in brain homogenates incubated with ^{3}H-β-alanine, suggesting perhaps that fragments of glial cytoplasm or "gliosomes" may exist in such homogenates. Kinetic studies of ^{3}H-β-alanine uptake by brain slices showed that it was taken up by a high-affinity, sodium-dependent mechanism which has the properties described previously for the glial uptake of GABA. Because in the case of GABA this glial component of uptake in brain slices and homogenates is small by comparison with the neuronal uptake component it is unlikely to have seriously interfered with previous estimates of the neuronal uptake of GABA by such preparations. Indeed, the glial component can only be studied by using a substrate such as β-alanine which is not taken up by the neuronal mechanism.

Although β-alanine was relatively ineffective as an inhibitor of GABA uptake in the isolated rat retina, incubation with ^{3}H-β-alanine led to a selective accumulation of label over the Muller cells. The complexity of uptake mechanism in retinal tissue, however, is evidenced by the findings that in rabbit retina ^{3}H-β-alanine is taken up selectively by amacrine cells and some ganglion cells [67, 68].

SUMMARY AND CONCLUSIONS

The existence of high-affinity uptake sites for GABA in both inhibitory neurones and glial cells in the nervous system now seems well established. Recent findings, described in this review, indicate that the neuronal and glial transport mechanisms for GABA can be distinguished by their different chemical specificity, and DABA and β-alanine appear to represent preferred substrates for the neuronal and glial sites respectively. The uptake of GABA by inhibitory neurones is a phenomenon similar to that observed for several other transmitter substances, and is probably a mechanism that plays an important role in terminating the actions of GABA after its release from inhibitory nerve terminals in CNS. The selective uptake of GABA and DABA by inhibitory neurones also provides a method for identifying and mapping the distribution of GABA-inhibitory neurones by autoradiographic techniques [69].

The high-affinity uptake of GABA by glial cells, however, is more difficult to understand in terms of a possible physiological function. The existence of such sites may have important implications for the metabolic compartmentation of GABA in the CNS. For example, an uptake of GABA by glial cells, following its release from inhibitory nerve terminals, has been suggested as one part of a hypothetical cycle of carbon substances between neurones and glia [70, 71]. The precise metabolic or physiological function of such a transfer, however, is not immediately apparent. Furthermore, not all glial cells in the nervous system are located in the vicinity of GABA-releasing nerve terminals. The glial cells in peripheral ganglia or in pineal or pituitary glands, for example, would not be exposed to GABA from such a source, since none of these organs contain GABA-inhibitory neurones. Since the glial cells in such tissues seem to contain GABA normally, and to possess all of the enzymic machinery for GABA metabolism, it seems likely that GABA plays some part in glial function unrelated to its inhibitory transmitter role in other parts of the nervous system. Exogenous ^{3}H-GABA can be released from satellite glial cells in sympathetic or sensory ganglia in response to depolarising stimuli [72, 73], and inhibitory receptors for GABA exist on the surface of ganglionic neurones [74, 75]. It is, therefore, possible to speculate that GABA could function as an inhibitory modulator, released from glial sites and controlling the excitability of adjacent neurones.

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