matters arising

High affinity uptake of neurotransmitter amino acids

LEVI and RAITERI[1] have concluded that their results on the uptake and release of ^{3}H-GABA by rat brain synaptosomes indicate that homoexchange is a major mechanism by which radioactive GABA enters synaptosomes when low substrate concentrations are used. They therefore question the effectiveness of high affinity uptake mechanisms in terminating the postsynaptic actions of this and other neurotransmitter amino acids after their release from presynaptic nerve terminals in the central nervous system (CNS)[2,3].

I believe that their conclusion is misleading. It is based largely on two findings. First, that unlabelled GABA or glycine in low concentrations stimulate the efflux of ^{3}H-GABA or ^{3}H-glycine from synaptosomes previously labelled with these amino acids. These experiments were conducted, however, with synaptosomes that had been incubated for 10 min in 0.5 μM labelled amino acid; from our own experience with such preparations we have found that the accumulation of tritiated amino acid is by this time approaching a steady state, in which the rate of influx is approximately equal to the rate of efflux, that is, a homoexchange situation may exist. Addition of exogenous unlabelled amino acid in these conditions would therefore be expected to lead to the increased efflux of labelled amino acid observed by Levi and Raiteri[1].

These results do not contradict the hypothesis that the initial rate of influx of labelled GABA or glycine into synaptosomes or brain slices incubated *in vitro* represents a net accumulation of amino acid in the intracellular space. Previous observations[4] by Levi and Raiteri and our own results[5] have shown the initial rate of influx of labelled GABA into synaptosomes or brain slices is unaffected either by large increases in the size of the intracellular GABA pool induced by previous incubation in a medium containing a high concentration of the amino acid, or by treatment of animals with the GABA–glutamate transaminase inhibitor amino-oxyacetic acid. These findings are hard to reconcile with the notion that the accumulation of labelled GABA occurs largely by homoexchange, as if this were so the initial rate of influx of labelled GABA should be increased in such conditions.

Second, Levi and Raiteri measured the net removal of GABA from the incubation medium when synaptosomes were incubated with ^{3}H-GABA at concentrations from 1 to 10 μM. They show that the net uptake of amino acid is less than that expected from measurements of the removal of radioactivity from the medium, although approximately 40% of the total radioactivity removed could be accounted for by a net accumulation of GABA. These results, however, are in complete disagreement with those of similar experiments from the same laboratory[4] which showed that net uptake of GABA accounted for more than 95% of the uptake of ^{3}H-GABA in synaptosome preparations from various regions of rat CNS incubated with 20 μM ^{3}H-GABA. Aprison and McBride also observed a net accumulation of glycine in rat spinal cord synaptosomes when these are incubated with low concentrations of glycine (37.5–150 μM).

The results of Levi and Raiteri also disagree with our previous findings[2] which showed that a net uptake of GABA accounted for more than 80% of the total uptake of ^{3}H-GABA in rat brain slices incubated with 200 μM GABA. In these conditions the uptake of exogenous GABA led to more than a threefold increase in the total GABA content of the tissue.

Nevertheless, after a 30 min incubation with this relatively high concentration of exogenous GABA there was also a significant exchange between the endogenous GABA content of the tissue and ^{3}H-GABA in the external medium[2]. Such exchange is not unexpected and does not contradict the conclusion that high affinity transport mechanisms for amino acids are capable of a net removal of amino acid from the external medium, and can thus represent important mechanisms for terminating the actions of these pharmacologically active substances in the CNS.

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LEVI AND RAITERI REPLY—When synaptosomes are incubated in a definite volume of medium containing a very low ^{3}H-GABA concentration, the steady-state accumulation that is approached after a few minutes does not correspond to a saturation of the synaptosomal uptake capacity, but is a relative value which depends on the concentration of GABA in the medium and on the ratio between the amount of tissue and the volume of medium. If the same amount of synaptosomes is either incubated in a larger volume of medium or, even more so, if it is superfused with this medium, uptake continues to increase for a much longer period. Moreover, similar uptakes of 10 and 20 μM ^{3}H-GABA were obtained in synaptosomes that had been preincubated in the presence or in the absence of 0.5 μM unlabelled GABA, and similar results were obtained in superfusion exchange experiments when synaptosomes were preincubated in 0.05 μM ^{3}H-GABA instead of 0.5 μM. For these reasons the synaptosomes used in our experiments[1] must be considered as prelabelled, and not as preloaded to an "absolute" steady state.

If one accepts this concept, and assumes, as is generally done, that the labelled GABA mixes with the endogenous pool, then our results do show that homoexchange is an important mechanism by which labelled GABA is accumulated by synaptosomes, particularly when low GABA concentrations are used. As homoexchange has never been considered to contribute significantly to the initial rate of uptake of radioactive GABA by nerve terminals, in our opinion it is not possible, at present, to say whether the net uptake of this amino acid (which we did not deny) has or has not a high affinity component (K_m between 4×10^{-7} M (ref. 2) and 2×10^{-5} M (ref. 3)). We suggest that, even if a high affinity component did not exist, a low affinity uptake system (K_m of the order