This possibility was investigated by looking for a specific uptake system for GABA as the existence of such a system would strengthen the evidence for a transmitter role for this substance.

Slices (0·2 mm thick) of cortex or tectum were incubated at 18° C for 3–40 min after a 20 min pre-incubation perod, with ^{3}H-GABA. The tissue and incubation medium were then separated by centrifugation and the radioactivity in each was determined by scintillation counting. From an initial concentration of ^{3}H-GABA in the medium of $3{\cdot}75 \times 10^{-8}$M there was a rapid accumulation of radioactivity by both tissues so that, after 40 min, tissue/medium ratios of $172 \pm 8{\cdot}7$ (mean $\pm$ S.E.) for tectum and $82 \pm 5{\cdot}6$ for cortex were reached . This uptake was Na^+ and temperature dependent and did not occur when using ^{3}H-lysine, ^{3}H-leucine or ^{3}H-isoleucine at this concentration.

To provide further evidence for a transmitter role of GABA in these tissues the effect of stimulation on the efflux of GABA *in vivo* was investigated.

The exposed tectum or cortex of anaesthetized frogs was left in contact with 0·5 ml frog Ringer Locke (F.R.L.) containing ^{3}H-GABA (2·0 μCi, 2·0 Ci/mmol) for 1 hr. The cortex or tectum was then perfused with F.R.L. at 300 μl/min and the radioactivity collected in the superfusate during each 5 min period was determined by scintillation counting. The tectum or cortex was stimulated either electrically via an insulated needle electrode or by superfusion with F.R.L. containing 40 mM K^+ during one or more superfusion periods occurring at least 40 min after the start of superfusion. Neither electrical stimulation (100 Hz, 0·2 ms, 2 mA) nor K^+ produced a significant increase in the efflux of radioactivity from either tissue. Using the dansyl technique, no significant increase in the efflux of any endogenous amino acids, during superfusion with 40 mM K^+, was demonstrated.

The GABA uptake mechanism, which may explain the failure to evoke GABA release, and the high level of endogenous GABA in the frog cortex and optic tectum, suggests that GABA may have a transmitter function in these amphibian tissues.

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REFERENCE

ROBERTS, P. J., KEEN P., MITCHELL, J. F. (1973). The distribution and axonal transport of free amino acids and related compounds in the dorsal sensory neurone of the rat, as determined by the dansyl reaction. *J. Neurochem*, **21**, 199–209.

Multiplicity of transport systems for L-glutamate and L-aspartate in the retina

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In the retina it has been suggested that glutamate or aspartate may be the transmitter at synapses between receptors and second-order neurones. Light adaptation may reduce the continuous release of excitatory transmitter substance, resulting in the disfacilitatory hyperpolarization characteristic of these synapses (Dowling & Ripps, 1973). The retina possesses high affinity uptake processes for glutamate and aspartate (Neal, Peacock & White, 1973) and these may provide a mechanism for inactivating the amino acid following its release from receptor terminals.

In the present study the kinetics of the uptake of both glutamate and aspartate have been investigated. Estimates of the initial velocity of amino acid upake were obtained by incubating isolated rat retinae with radioactive L-glutamate or L-aspartate for 5 min. Preliminary studies indicated that, over the concentration range used (10^{-6}M to 10^{-3}M) the uptake of both amino acids was linear for at least 8 min. The results were analysed using various linearizing transformations of the Michaelis Menten equation: linear and non-linear fits to this equation being made using a CDC 6600 computer (Neal *et al.*, 1973).

Analysis of the uptake of glutamate and aspartate indicated that the uptake could be resolved into two components. The apparent Km values calculated from the least squares fit for the high affinity uptake of aspartate and of glutamate were about 20–25 μM, while the low affinity Km values were about 0·7 and 0·6 mM respectively. Preliminary experiments indicated that glutamate and aspartate may competitively inhibit the uptake of each other, suggesting that these amino acids may be accumulated in the retina by the same transport system.

An attempt was made to distinguish between the high and low affinity uptake processes for glutamate by studying the characteristics of the uptake process at 10^{-3}M (low affinity) and 10^{-8}M (high affinity). It was found that both systems were inhibited by *p*-hydroxymercuribenzoate (10^{-5}M), and both were virtually abolished at 0° C and by the absence of sodium in the incubation medium. The specificity of both the high and low affinity systems was very similar: thus both uptake systems were inhibited by L-cysteate and L-aspartate, and were unaffected by glycine, L-serine and L-glutamine.

Similar high affinity and multiple uptake systems for amino acids have been described in other areas of the central nervous system but also in non-neural tissues such as kidney (Mohyuddin & Scriver, 1970), bacteria (Gross & Ring, 1971), and yeast (Koytk & Rihova, 1972). Thus, while high affinity uptake processes may be utilized in the central nervous system to inactivate transmitter substances, the mere presence of a high affinity uptake process for a substance cannot be taken as evidence that the substance is a neurotransmitter.

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REFERENCES

DOWLING, J. E. & RIPPS, H. (1973). Effect of magnesium on horizontal cell activity in the skate retina. *Nature, Lond.*, **242**, 101–103.

GROSS, W. & RING, K. (1971). Active transport of glutamate in streptomyces hydrogenenans. *Biochim. Biophys. Acta*, **233**, 652–665.

KOTYK, A. & RIHOVA, L. (1972). Transport of α-aminoisobutyric acid in saccaromyces cerevisiae. *Biochim. Biophys. Acta*, **288**, 380–389.

MOHYUDDIN, F. & SCRIVER, C. R. (1970). Amino acid transport in mammalian kidney: multiple systems for imino acids and glycine in rat kidney. *Am. J. Physiol.*, **219**, 1–8.

NEAL, M. J., PEACOCK, D. G. & WHITE, R. D. (1973). Kinetic analysis of amino acid uptake by the rat retina in vitro. *Br. J. Pharmac.*, **47**, 656–657P.

The dual action of tricyclic antidepressant drugs on responses of single cortical neurones to acetylcholine

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The peripheral atropine-like action of tricyclic antidepressant drugs is well documented (Atkinson & Ladinsky, 1972), but it is not known how these drugs influence the effects of acetylcholine (ACh) at the level of the single brain cell. We used the microelectrophoretic technique in order to investigate this problem.

Spontaneously active neurones were studied in the somatosensory cortex of the halothane-anaesthetized cat. All the drugs were applied by microelectrophoresis. Repeated responses to ACh were compared following a brief application of imipramine or desipramine.

We have found that the antidepressants can both potentiate and antagonize responses to ACh. As increasingly high electrophoretic currents were used to apply the antidepressant, the following effects upon subsequent responses to ACh were observed: (1) potentiation of immediate onset; (2) delayed potentiation; (3) antagonism of immediate onset, followed by potentiation. Responses to carbachol were affected in the same way.