TABLE 1. *Effect of imipramine* (10^{-4}mol/*ml*), *desipramine* (10^{-4}mol/*ml*) *and nialamide* (10^{-3}mol/*ml*) *on the accumulation of* ^{14}*C-5-HT into the GSCs of* Helix pomatia. *The figure in brackets represents the number of experiments performed.*

	Accumulation of ^{14}C-5HT into GSCs	Accumulation of ^{14}C-5-HT into brain tissue
%Inhibition of ^{14}C-5-HT accumulation caused by imipramine	24±6% (5)	51±4% (5)
% Inhibition of ^{14}C-5-HT accumulation caused by desipramine	18±3% (4)	40±5% (4)
% Increase of ^{14}C-5-HT accumulation caused by nialamide	3±1% (5)	48±5% (5)

It is concluded from these studies that the tryptophan hydroxylating enzyme (tryptophan-hydroxylase) is present in 5-HT containing cells (GSCs) alone. From the specific accumulation of ^{14}C-5-HT by cell somata containing the amine (GSCs), and from the effects of imipramine, desipramine and nialamide upon the accumulation process and the metabolism of the amine by whole nervous tissue (which consists of many 5-HT containing synapses), it is deduced that 5-HT is probably inactivated in two ways: by enzymatic oxidation and by reuptake into synaptic terminals.

REFERENCES

BRIEL, G., NEUHOFF, V. & MAIER, M. (1972). Microanalysis of amino acids and their determination in biological material using dansyl chloride. *Hoppe-Seyler's 2. Physiol. Chem.*, **353**, 540–553.

COTTRELL, G. A. (1970). Direct postsynaptic responses to stimulation of serotinin-containing neurons. *Nature, Lond.*, **225**, 1060–1062.

COTTRELL, G. A. & OSBORNE, N. N. (1970). Serotonin: Subcellular localization in an identified serotonin-containing neuron. *Nature, Lond.*, **325**, 470–472.

KOE, B. K. & WEISSMAN, A. (1966). p-Chlorophenylalanine: A specific depletor of brain serotonin. *J. Pharmacol. Exp. Therap.*, **154**, 499–516.

OSBORNE, N. N. (1972). The *in vivo* synthesis of serotonin in an identified serotonin-containing neuron of *Helix pomatia*. *Intern. J. Neuroscience*, **3**, 215–219.

OSBORNE, N. N. (1973). The analysis of amines and amino acids in micro-quantities of tissue. In: *Progress in Neurobiology*, ed. by G. A. Kerkut & J. W. Phillis (Pergamon Press) in press.

OSBORNE, N. N., BRIEL, G. & NEUHOFF, V., (1971). Localization of GABA and free amino acids in *Helix*, and the *in vitro* metabolism of ^{14}C-glucose and ^{14}C-glutamic acid. *Intern, J. Neuroscience*, **1**, 265–272.

OSBORNE, N. N. & NEUHOFF, V. (1973). Biochemical studies on identifiable neurons of molluscs. *Naturwissenschaften*, **60**, 78–87.

Amino acids in the frog central nervous system

J. F. MITCHELL and R. A. YATES*

Department of Pharmacology, University of Bristol Medical School, University Walk, Bristol BS8 1TD

In a preliminary attempt to characterize the optic nerve transmitter in the frog the concentrations of a number of amino acids and related compounds in the cerebral cortex, optic tectum and optic nerve of the frog were measured as their ^{3}H-dansyl derivatives by the modified method described by Roberts, Keen & Mitchell (1973). A unique, or high concentration of a compound in the optic nerve and/or tectum, compared with other parts of the nervous system, might suggest a transmitter role for that substance at optic nerve terminals. In these studies no compound was found which occurred uniquely in the optic nerve or tectum.

In a comparison of the levels of nine amino acids in the optic nerve, tectum and cerebral cortex, the relative concentration of GABA in the cortex, (3·1±0·4 μmol/g wet weight (mean±S.E.)) and in the tectum (2·2±0·3 μmol/g) when compared with the optic nerve (0·4 μmol±0·01 μmol/g), was the most outstanding feature. This finding, and the large body of evidence which indicates that GABA is likely to be an inhibitory transmitter in the central nervous system of mammals, suggested that GABA, while almost certainly not being the optic nerve transmitter, might nevertheless have a transmitter function in the frog cortex and tectum.

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.

R.A.Y. is an MRC Junior Research Fellow.

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

M. J. NEAL and R. D. WHITE*

Department of Pharmacology, The School of Pharmacy, University of London, London, WC1N 1AX

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).