its concentration in the perfusate a readily saturable mechanism such as carrier transport or exchange diffusion would not seem to be involved. This is unlike the mechanism of localized active transport demonstrated for the removal of its 5-hydroxyindole acid metabolite, 5-hydroxyindol-3-ylacetic acid (5-HIAA), from CSF (Ashcroft *et al.*, 1968).

Tryptophan in human plasma has been shown to be strongly protein bound (McMenamy, Lund & Oncley, 1957). Therefore a possible explanation for the observed clearance of tryptophan from the CSF perfusate could be its removal by simple diffusion into the bloodstream where only a small proportion of the total concentration of tryptophan in the plasma will be diffusible.

Another explanation might be provided if tryptophan was continuously removed by metabolism in brain tissue. This possibility is currently under investigation. Preliminary studies, however, indicate no significant cerebral metabolism along the 5-hydroxyindole pathway, as evidenced by the failure of the acid metabolite 5-HIAA to rise in the CSF perfusate during the infusion of tryptophan into the perfusion system.

REFERENCES

ASHCROFT, G. W., DOW, R. C. & MOIR, A. T. B. (1968). The active transport of 5-hydroxyindol-3-ylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid from a recirculatory perfusion system of the cerebral ventricles of the unanaesthetized dog. *J. Physiol., Lond.*, **199**, 397–425.

LEVIN, E., NOGUEIRA, G. J. & GARCIA ARGIZ, C. A. (1966). Ventriculo-cisternal perfusion of amino-acids in cat brain—I. *J. Neurochem.*, **13**, 979–988.

MCMENAMY, W. H., LUND, C. C. & ONCLEY, J. L. (1957). Unbound amino-acid concentrations in human blood plasma. *J. clin. Invest.*, **36** (2), 1672–1679.

Uptake of [^{14}C]-glycine by rat spinal cord

M. J. NEAL, *Department of Pharmacology, University of Cambridge*

There is now much evidence to support the suggestion that glycine may be an inhibitory transmitter in the mammalian spinal cord. The distribution of this amino-acid in the spinal cord of the cat has been related to the presence of inhibitory interneurones (Davidoff, Shank, Graham, Aprison & Werman, 1967). Glycine hyperpolarizes spinal motorneurones (Werman, Davidoff & Aprison, 1967) and the changes in membrane permeability produced by glycine seem to be similar to those produced by a spinal inhibitory synaptic transmitter (Curtis, Hösli, Johnston & Johnston, 1968). Strychnine, which reduces spinal post-synaptic inhibition, blocks the effects of glycine on spinal motorneurones (Curtis, Hösli & Johnston, 1967).

The mechanism by which the actions of spinal inhibitory transmitters and iontophoretically applied glycine are terminated is not known. Because *p*-hydroxymercuribenzoate potentiates the inhibitory action of glycine on spinal interneurones, it has been suggested that glycine is inactivated enzymically (Curtis, Hösli & Johnston, 1968). However, an alternative method of inactivation may involve an uptake mechanism in the neural tissue of the cord similar to that described by Iversen & Neal (1968) for GABA in the cerebral cortex. Although glycine is taken up by slices of brain (Smith, 1967) there has apparently been no previous report of an uptake mechanism for glycine in the spinal cord.

Slices of rat spinal cord (0·1 × 0·1 × approx. 0·2 mm) were suspended in Krebs-bicarbonate solution and distributed volumetrically. Slices of cord equivalent to

10 mg wet weight were incubated for various times with [^{14}C]-glycine (6×10^{-7}M) in 10 ml. of oxygenated medium. The tissue was collected by rapid filtration, washed with 5 ml. of ice-cold medium and the total radioactivity was measured by liquid scintillation spectroscopy (Iversen & Neal, 1968).

There was a rapid uptake of [^{14}C]-glycine as estimated by the accumulation of radioactivity in the tissue. After incubating the slices of spinal cord for 60 min at 37° C there was a tissue : medium ratio of 30 : 1. The uptake of [^{14}C]-glycine showed saturation kinetics over a range of external glycine concentrations from 10^{-6}M to 2×10^{-4}M with an apparent *Km* for glycine $=3{\cdot}12\times10^{-5}$M and $V_{max}=$ 0·48 μ-mole/g of cord per min. The uptake of [^{14}C]-glycine was temperature-dependent, being greatly reduced at 0° and optimal at 37° C. Replacement of sodium chloride in the incubation medium by choline chloride reduced the uptake of [^{14}C]-glycine to less than 1% of the control values. Incubation of the tissue in medium containing 2,4-dinitrophenol (10^{-3}M) or with ouabain (10^{-5}M) also caused a large reduction in the uptake of [^{14}C]-glycine. Strychnine (10^{-3}M) did not affect [^{14}C]-glycine uptake but *p*-hydroxymercuribenzoate (10^{-5}M) significantly reduced the uptake of [^{14}C]-glycine into slices of spinal cord.

The results show the existence in the rat spinal cord of an efficient uptake mechanism for glycine which shows many of the characteristics of an active transport mechanism. This uptake process could act as a mechanism for terminating the inhibitory action of glycine on spinal neurones. The potentiation of the effects of glycine on motor-neurones produced by *p*-hydroxymercuribenzoate may not be due to inhibition of metabolic degradation of glycine as suggested by Curtis *et al.* (1968) but may be caused by the effect of this compound in reducing the uptake of glycine from the extracellular space.

REFERENCES

CURTIS, D. R., HÖSLI, L. & JOHNSTON, G. A. R. (1967). Inhibition of spinal neurones by glycine. *Nature, Lond.*, **215**, 1502–1503.

CURTIS, D. R., HÖSLI, L. & JOHNSTON, G. A. R. (1968). A pharmacological study of the depression of spinal neurones by glycine and related amino-acids. *Expl. Brain Res.*, **6**, 1–18.

CURTIS, D. R., HÖSLI, L., JOHNSTON, G. A. R. & JOHNSTON, I. H. (1968). The hyperpolarization of spinal motorneurones by glycine and related amino-acids. *Expl. Brain Res.*, **5**, 235–258.

DAVIDOFF, R. A., SHANK, R. P., GRAHAM, L. T., APRISON, M. H. & WERMAN, R. (1967). Association of glycine with spinal interneurones. *Nature, Lond.*, **214**, 680–681.

IVERSEN, L. L. & NEAL, M. J. (1968). The uptake of [^{3}H]-GABA by slices of rat cerebral cortex. *J. Neurochem.*, **15**, 1141–1149.

SMITH, S. E. (1967). Kinetics of neutral amino-acid transport in rat brain *in vitro*. *J. Neurochem.*, **14**, 291–300.

WERMAN, R., DAVIDOFF, R. A. & APRISON, M. H. (1967). Inhibition of motorneurones by iontophoresis of glycine. *Nature, Lond.*, **214**, 681–683.

Subcellular distribution of endogenous and [^{3}H]-GABA in rat cerebral cortex

L. L. IVERSEN* and M. J. NEAL, *Department of Pharmacology, University of Cambridge*

We have recently described the properties of an uptake mechanism for [^{3}H]-GABA in slices of rat cerebral cortex (Iversen & Neal, 1968). The present studies were undertaken to obtain more information on the cellular location of [^{3}H]-GABA uptake in relation to the storage sites for endogenous GABA in the rat cortex, by comparing the subcellular distribution of the exogenous and endogenous amino-acid in cortical homogenates.