Glutamic acid: High-affinity binding to cerebellar membranes

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Although the cerebellum is low in all known neurotransmitters, glutamate is present in abundance (Johnson & Aprison, 1971), and recent neurochemical evidence (Hudson, Valcana, Bean & Timiras, 1976; Young, Oster-Granite, Herndon & Snyder, 1974) suggests that this amino acid may be the natural transmitter of the interneuronal granule cells which provide the main excitatory input to the Purkinje cells.

We have investigated the binding of highly labelled L-[^{3}H]-glutamate (34 Ci/mmol) to rat cerebellar membranes. Specific binding was determined by subtraction of the non-specific binding component, which persisted in the presence of a 10,000 fold excess of unlabelled glutamate. The time course of specific binding was relatively slow, with equilibrium being attained after approximately 10 min, whilst non-specific binding was essentially instantaneous. Specific binding, unlike the non-specific component, was saturable and exhibited at least two components. The high-affinity system only has been investigated and was found to possess an apparent $K_D = 1.27\ \mu M$ and a binding capacity of 31.8 nmol/mg protein. Specific binding was pH and temperature sensitive and was optimal under physiological conditions. Freezing of the membranes led to a rapid and progressive loss of all specific binding properties. The specific binding was found to be associated primarily with neuronal membranes, since preparations from lung, plasma, striated muscle and kidney exhibited minimal specific binding. It is suggested that high-affinity binding of glutamate to cerebellar membranes may represent combination with its physiological receptor.

References

HUDSON, D.B., VALCANA, G., BEAN, G. & TIMIRAS, P.S. (1976). Glutamic acid: A strong candidate as a neurotransmitter of the cerebellar granule cell. *Neurochem. Res.*, **1**, 73–81.

JOHNSON, J.L. & APRISON, M.H. (1971). The distribution of glutamate and total free amino acids in thirteen specific regions of the cat central nervous system. *Brain Research*, **26**, 141–148.

YOUNG, ANN B., OSTER-GRANITE, MARY LOU, HERNDON, R.M. & SNYDER, S.H. (1974). Glutamic acid: selective depletion by viral induced granule cell loss in hamster cerebellum. *Brain Research*, **73**, 1–13.

Effects of central depressant drugs on the isolated hemisected spinal cord of the immature rat

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The immature rat spinal cord (Konishi & Otsuka, 1974) provides a convenient preparation *in vitro* for the investigation of drug receptors of the mammalian central nervous system. In 31 preparations responses of motoneurones and in some cases primary afferent terminals were elicited with both applied L-glutamate, L-homocysteate, substance P or γ-aminobutyric acid (GABA) and the effect of a series of depressant drugs on these responses was observed. Procaine hydrochloride (1 mM) was included in the perfusion medium at all times to block indirect activity (Evans & Watkins, 1975).

The psychotropic drugs chlorpromazine (50 μM), haloperidol (50 μM) and diazepam (50 μM) potentiated responses of motoneurones to L-homocysteate and to a lesser extent L-glutamate. Diazepam depressed GABA-induced primary afferent depolarization and haloperidol markedly enhanced GABA-induced primary afferent depolarizations.

The less specific depressants meprobamate (1 mM), mephenesin (1 mM), pheneturide (0.5 mM) and pentobarbitone (0.2 mM) produced depression of all motoneurone responses.

It seems possible that the potentiation of responses observed with chlorpromazine and haloperidol may relate to their reported inhibitory actions on amino acid uptake systems (Iversen & Johnston, 1971; Balcar & Johnston, 1972).

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