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.

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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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The effect of 40 mM potassium and electrical stimulation on the efflux of [^{3}H]-GABA from rat dorsal medulla *in vivo* and *in vitro*

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There have been two independent reports that 40 mM potassium causes an increase in the efflux of γ-aminobutyric acid (GABA) from the rat cuneate nucleus *in vivo* (Roberts, 1974; Assumpcao, Bernardi, Dacke & Davidson, 1977). We have also investigated the efflux of [^{3}H]-GABA from that part of the dorsal medulla containing the cuneate nucleus and our experiments do not support the conclusions of these previous workers.

Rats were anaesthetized with urethane (1.25 g/kg), the dorsal surface of the medulla was exposed and an incubation cup formed by placing a small length of tubing (3 mm internal diameter) on the pial surface and sealing it in place with silicone grease. Twenty µl of a Krebs solution containing 6.6×10^{-6} M [^{3}H]-GABA and 1.2×10^{-4} M [^{14}C]-sucrose as a spacemarker (2.8×10^6 dpm each) was placed in the cup for 30–60 min. Normal Krebs solution was then superfused over the surface at a rate of 1 ml per 10 min for 30–120 min after which a change was made to an isotonic solution containing 40 mM potassium. The radioactivity in each 10 min collection was counted after adding 5 ml Instagel (Packard) and a few drops of formic acid and quenching was estimated from the external standard channels ratio. Although multiphasic ^{3}H efflux curves were observed with small deflections immediately following the change-over to high potassium this only occurred in 5 out of 15 experiments and was always accompanied by a corresponding increase in ^{14}C efflux.

The efflux of [^{3}H]-GABA from 0.4 mm slices of rat dorsal medulla was studied *in vitro* as described for rat cerebral cortex slices by Srinivasan, Neal & Mitchell (1969). The radioactivity in each 2 min collection (1–1.5 ml) was estimated as before and the efflux was followed for 40 min before changing to an isotonic solution containing 40 mM potassium. In all 8 experiments no deflections were observed. In contrast, 40 mM potassium caused a large increase in efflux of ^{3}H from rat cortical slices similar to that described by Srinivasan *et al.* (1969). In 6 experiments with slices from rabbit dorsal medulla 40 mM potassium again failed to alter ^{3}H washout. The efflux of ^{3}H from medulla slices could be greatly increased by electrical stimulation (rectangular, 5 msec, 20 mA pulses; 60/sec for 30 s in every 2 min). This stimulation did not alter the efflux of ^{14}C and the effect on ^{3}H could be prevented by previous exposure to high potassium.

In view of these results it is suggested that any small changes in the efflux of ^{3}H from the rat cuneate nucleus *in vivo* corresponding to the superfusion of high potassium solutions may be artifactual, for instance due to shrinkage of the extracellular space (Bourke & Tower, 1966; Roberts, 1976). However the specific electrically evoked release of [^{3}H]-GABA from slices of rat and rabbit dorsal medulla lends support to the hypothesis that GABA may be a transmitter at this site.

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