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Evoked release of amino acids from the intact spinal cord

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The amino acids glycine and γ-aminobutyric acid (GABA) have both been proposed as inhibitory synaptic transmitters in the spinal cord (Werman & Aprison, 1968; Johnston, 1968) but so far there have been only two short reports concerning the release of glycine from the stimulated intact cord (Aprison, 1970; Jordan & Webster, 1971), and none concerning GABA.

In this study, the isolated and sagitally hemisected frog or toad spinal cord preparation was used (Mitchell & Phillis, 1962) and experiments were performed at 14–15° C using amphibian Ringer-Locke medium. Local electrical stimulation was applied to the ventral or dorsal roots, or to the rostral end of the cord. The cord was loaded with labelled amino acid by incubation for 40 min in a small bath (0.5 ml), containing ^{14}C-glycine ($1{\cdot}5\times10^{-5}$M), ^{3}H-GABA ($5{\cdot}0\times10^{-7}$M), ^{3}H-leucine ($1{\cdot}3\times10^{-5}$M) ^{14}C-serine ($9{\cdot}7\times10^{-5}$M), or ^{14}C-threonine ($1{\cdot}3\times10^{-4}$M). In some experiments ^{14}C-mannitol ($4{\cdot}6\times10^{-5}$M), or ^{14}C-urea ($9{\cdot}1\times10^{-5}$M) were used. After incubation the bath was emptied, washed and filled with fresh Ringer-Locke. This was removed every 2 min, an aliquot used for the assay of the radioactivity released from the tissue, and the bath refilled with Ringer-Locke.

The degree of metabolism of ^{14}C-glycine and ^{3}H-GABA was measured in pooled samples taken before, during, and after stimulation and at least 95% and 88% respectively of the radioactivity detected, was found to be accounted for by the unchanged labelled amino acids.

Electrical stimulation of the ventral and dorsal roots (150 Hz, 3 ms, 2 ma) produced no detectable changes in the spontaneous efflux of any of the compounds tested but stimulation of the rostral end of the spinal cord produced large increases in the release of ^{14}C-glycine (mean, 9·9 times pre-stimulation efflux, 5 experiments), and ^{3}H-GABA (mean, 5·5 times pre-stimulation efflux, 5 experiments). There was no significant increase in the efflux of any other labelled compounds tested.

In the presence of a calcium-free medium the evoked increase in ^{14}C-glycine, and ^{3}H-GABA efflux was reduced to 2·7 and 3·7 times pre-stimulation value respectively (mean of 4 experiments). The addition of magnesium (10 mM) resulted in a further reduction in evoked efflux of these amino acids (1·6 and 1·1 times pre-stimulation value, mean of 3 experiments).

In five experiments the cord was stimulated at a variety of frequencies (5–100 Hz), and the evoked efflux of ^{14}C-glycine was found to be proportional to the number of stimuli delivered.

These experiments on intact spinal cord tissue avoid many of the difficulties of interpretation associated with tissue slice experiments, and they demonstrate an apparently specific, and calcium dependent release of glycine and GABA when descending spinal nerve tracts are stimulated. This supports the suggestion that both glycine and GABA are synaptic transmitters in the spinal cord.

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Blockade of central GABA receptors and the convulsive actions of bicuculline, picrotoxin and leptazol

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It has been shown that the depressant effects of microiontophoretically applied γ-aminobutyric acid (GABA) on feline cortical neurones can be antagonized by bicuculline or picrotoxin applied in a similar way to the same neurones (Curtis, Duggan, Felix & Johnston, 1970; Hill, Simmonds & Straughan, 1971, 1972). When given intravenously, picrotoxin and bicuculline can be shown to reduce neurally evoked inhibitions (Brooks & Asanuma, 1965; Curtis & Felix, 1971) and these observations have been used as evidence for GABA being a cortical inhibitory transmitter. Consequently, the convulsant properties of systemically administered bicuculline and picrotoxin might be largely explained by blockade of central GABA receptors with failure of neural inhibition and consequent uncontrolled discharge of excitatory pathways.

To investigate this possibility, cats were anaesthetized with nitrous oxide and halothane, paralysed with gallamine to prevent movement artifacts and artificially respired, anaesthesia being maintained throughout the experiments. Arterial acid-base balance was routinely measured and found to be within the same limits as in spontaneously breathing animals. An area of mid-suprasylvian cortex was exposed and a silver E.Co.G. electrode placed on the surface. A seven-barrelled glass micropipette containing drug solutions for iontophoresis was inserted into the same area of cortex and extracellular action potentials were recorded through one barrel. Cells were driven by continuous application of glutamate and repeated responses to GABA were obtained before, during and after a slow intravenous infusion of convulsant. In addition to infusions of bicuculline ((20 μg/min)/kg) and picrotoxin (0·15 mg), leptazol (6 mg) was also infused as a control for the other two substances, since it has been reported not to antagonize evoked inhibitions of GABA (Krnjević, Randic & Straughan, 1966) yet acts as a convulsant at a similar level in the neuro-