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The effects of gamma-hydroxybutyric acid on brain respiration *in vitro*

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Gamma-hydroxybutyric acid (GHB) has recently been shown to have specific actions on cerebral glucose metabolism, including the ability to stimulate the respiratory rate of rat cerebral cortical slices incubated *in vitro* with glucose as substrate (Taberner, Rick & Kerkut, 1972). This effect is in direct contrast to the effects of other central depressant drugs which, at equivalent concentrations, depress the respiration of brain tissue *in vitro* (McIlwain, 1966). Ahmed & Scholefield (1961) however, have reported that several short chain fatty acids can produce a temporary stimulation of the respiration of brain tissue *in vitro*, but that it is followed by an irreversible inhibition within 2 h. The present experiments were performed further to characterize this singular action of GHB on brain respiration.

Rat cerebral cortical slices were incubated in Krebs-Ringer phosphate medium (pH 7·4) at 37° under air with added substrate (10 mM). With glucose as substrate, the addition of GHB to the medium at concentrations of 0·5–4·0 mM produced a 25% increase in the respiratory rate which was maintained for at least 3 h, although GHB itself did not support respiration. As has been shown earlier, the effect could not be obtained with cerebral cortical homogenates, or with sliced homogenates of rat liver (Taberner *et al.*, 1972). Also the effect was not observed with glucose-6-phosphate, fructose, succinate, oxaloacetate, pyruvate or glutamate as the exogenous substrate. With β-hydroxybutyrate as substrate there was a very marked inhibition of respiration with GHB above 5 mM in the medium. At high concentrations of GHB (above 50 mM), the increase in respiration with glucose as substrate was no longer obtained and the respiration tended to be depressed.

Other central depressant drugs tested, including pentobarbitone and imidazoleacetic acid, invariably depressed the respiratory rate at concentrations above 0·1 mM. Oxidized glutathione which, like GHB, increases the activity of the pentose phosphate pathway in the brain (Hotta, 1962 ; Taberner *et al.*, 1972) also stimulated the rate of respiration of the tissue when glucose was the substrate. However, the effect was not as consistent as that observed with GHB, and it was not obtained with reduced glutathione.

It is confirmed that GHB, unlike other central depressant drugs, increases the rate of respiration of cerebral cortical slices *in vitro*. Since this effect could only be obtained with slices of cerebral cortex respiring with glucose as the exogenous substrate, it is possible that GHB acts by facilitating the entry of glucose into the intact cells.

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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.