Calcium dependent release of isotopically labelled γ-aminobutyric acid (GABA) from rat dorsal medulla *in vivo*

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Recently Hill, Mitchell & Roberts (1977) reported that they were unable to evoke consistent increases in [^{3}H]-GABA efflux from rat dorsal medulla *in vivo* on potassium (K^+) stimulation. This apparently contradicts an earlier report by Assumpção, Bernardi, Dacke & Davidson (1977) that K^+-stimulation evoked a significant increase of [1-^{14}C]-GABA efflux from this region. We have repeated these experiments using [2,3-^{3}H]-GABA and [1-^{14}C]-GABA and find consistent, statistically significant, Ca^{++}-dependent increases in efflux of both isotopic species of GABA from the dorsal medulla.

Details of the experimental techniques have been reported previously (Assumpção, Bernardi, Dacke & Davidson, 1976, 1977). We would draw particular attention to the 60 min period of closed cycle superfusion of the exposed pial surface with solutions containg 2 μCi ml^{-1} (4×10^{-5}M) of [1-^{14}C] or 10 μCi ml^{-1} (18×10^{-8}M) of [2,3-^{3}H]-GABA. The total volume of the system (450 μl) was over twenty times the volume of labelling solution used by Hill *et al.* (1977) and therefore more likely to maintain stable specific activity of the isotope during the labelling period.

Next, the pial surface was superfused with surrogate cerebrospinal fluid (CSF). Five minute fractions of superfusate were collected for counting by liquid scintillation spectrometry. In some experiments the superfusate was changed to one containing elevated (40 mM) K^+, pH and osmolarity being initially checked for constancy. Consistent increases in efflux of [^{14}C]-GABA (mean increase in 6 experiments, 33%) and [^{3}H]-GABA (mean increase in 6 experiments, 21%) were observed.

When $CaCl_2$ in the superfusing fluid was replaced with $MgCl_2$ the increase in [^{3}H]-GABA efflux was reduced to 1%. A similar Ca^{++}-dependency of [^{14}C]-GABA efflux was observed.

(L)-[G-^{3}H] leucine did not respond to K^+ stimulation indicating that K^+ evoked GABA release is not an artifact caused by extracellular space changes as suggested by Hill *et al.* (1977) but supports our hypothesis for release from an intracellular source.

Preliminary experiments indicate that direct electrical stimulation of the pial surface (200 Hz, 1.5 mA for 10 min) also increases [^{14}C]-GABA efflux.

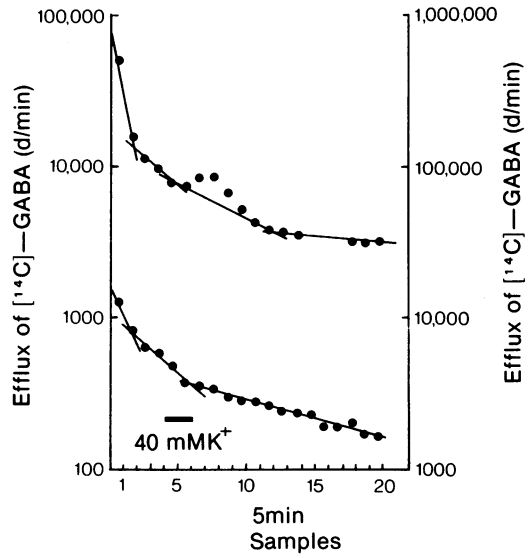

Figure 1 Effect of high potassium stimulation on the efflux of [2,3-^{3}H]-GABA from the superfused cuneate region of the rat dorsal medulla, with normal Ca^{2+} (upper graph, left ordinate) and Ca^{2+}-free superfusates (lower graph, right ordinate). Each graph shows data from a single experiment. Increase in efflux is calculated as the increase in sample radioactivity (DPM) compared with that predicted from the least square line of best fit computed from the remaining points in the phase. The lower graph shows clearly the reduction in efflux in Ca^{2+}-free conditions. Rats were pretreated with amino-oxyacetic acid (20 mg/kg, i.p.) to prevent GABA catabolism. Abscissa indicates number of serial 5 min superfusate fractions collected. Horizontal bar indicates 10 min period of stimulation with high K^+ superfusate.

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