## Efflux characteristics of isotopically labelled *y*-aminobutyric acid (GABA) and L-glutamate in the rat cuneate nucleus

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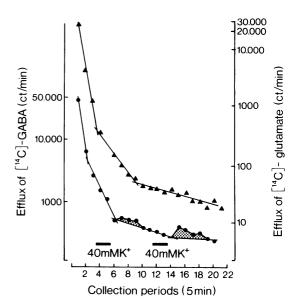
The experiments reported here are part of a series designed to investigate the efflux characteristics of isotopically labelled putative neurotransmitters in the *in vivo* superfused rat cuneate nucleus. Details of the superfusion and isotope-labelling methods in the electrophysiologically identified cuneate nucleus of the chloralose-urethane anaesthetized rat have already been reported (Assumpção, Bernardi, Dacke & Davidson, 1976).

After a 60 min period of superfusion with a solution containing 2  $\mu$ Ci/ml of 1-[14C]-GABA or L-glutamate, the pial surface of the exposed cuneate nucleus was superfused with artificial cerebrospinal fluid (Merlis, 1940) at 62.5  $\mu$ l/min and 5 min fractions collected for estimation of radioactivity by liquid scintillation spectrometry. The results, when plotted semi-logarithmically against time, with computed lines of best fit, revealed multi-phase spontaneous effluxes for both GABA (Figure 1) and glutamate.

In some experiments the superfusate was changed to one containing elevated (40 mM)  $K^+$  for one or more 10 min periods. This caused significant (P < 0.01) increases in the efflux of labelled GABA which could be measured by the departure of the observed points from those predicted by the line of best fit computed from the remaining points in that particular phase (Figure 1). Roberts (1974) measured evoked GABA release from the dorsal column nuclei as a percentage increase in isotope efflux over spontaneous release, but it is clear from Figure 1 that a percentage measurement may not be particularly meaningful unless it is also known at which phase in the efflux it is calculated.

Interestingly, although GABA and glutamate are respectively the major inhibitory and excitatory transmitter candidates in the cuneate nucleus (for review see Davidson, 1976), we have obtained no evidence for a potassium evoked increase in glutamate efflux such as reported by Roberts (1974). The reason for this discrepancy in our results is not clear at present.

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Effect of high potassium stimulation on the efflux of 1-[14C]-glutamate (upper graph, ▲) and GABA (lower graph, ●) from the superfused cuneate nucleus of rats pretreated with amino-oxyacetic acid (20 mg/kg, i.p.). The ordinate for the upper graph is on the right, for the lower graph on the left. The abscissa indicates the number of 5 min fractions collected in each experiment; horizontal bars indicate 10 min periods of stimulation with high (40 mm) K+ C.S.F. The hatched areas in the second and third phases on the lower graph indicate the K+-evoked GABA efflux as an increase in sample radioactivity compared with that predicted from the least squares line of best fit computed from the remaining points in the phase. No corresponding increase in glutamate efflux can be detected in the upper graph.

## References

ASSUMPÇÃO, J.A., BERNARDI, N., DACKE, C.G. & DAVIDSON, N. (1976). A superfusion technique for isotope efflux studies and concurrent electrophysiological investigation in the rat cuneate nucleus. J. Physiol. Lond. (in press).

DAVIDSON, N. (1976). Neurotransmitter Amino Acids. Academic Press: London.

MERLIS, J.K. (1940). The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. Am. J. Physiol., 131, 67-72.

ROBERTS, P.J. (1974). The release of amino acids with proposed neurotransmitter function from the cuneate and gracile nuclei of the rat in vivo. Brain Res., 67, 419-428.