Release of [±^{3}H]-cis-3-aminocyclohexanecarboxylic acid ([^{3}H]-ACHC) from central neurones

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The conformationally-restricted γ-aminobutyric acid (GABA) analogue, cis-3-aminocyclohexanecarboxylic acid (ACHC) is a relatively selective inhibitor of neuronal GABA transport systems (Bowery, Jones & Neal, 1976). Recently we have shown that ACHC is also a substrate for the neuronal GABA transport systems but has little affinity for glial transport sites (Neal & Bowery, 1977). In the present study we have examined the effect of depolarizing stimuli on the release of [^{3}H]-ACHC from small slices of cerebral crotex and from frog retinae. Autoradiographical studies have shown that these tissues take up [^{3}H]-GABA mainly into neurones (see Bowery *et al.*, 1976 for references) and we have found that in the frog retina, [^{3}H]-ACHC is taken up into the same small population of neurones (horizontal cells) as [^{3}H]-GABA.

Slices of rat cerebral cortex (0.25 × 2 mm) or individual frog retinae were incubated at room temperature for 30 min in Krebs' bicarbonate solution containing [^{3}H]-ACHC (0.1 μM). The tissue was then transferred to a small chamber and superfused with medium at a rate of 1.2 ml/minute. Fractions (2.4 ml) were collected and the radioactivity estimated by liquid scintillation counting (Bowery *et al.*, 1976). Since ACHC is apparently not metabolized in central nervous tissue (Neal & Bowery, 1977) it is probable that the radioactivity released in the present experiments was unchanged [^{3}H]-ACHC.

The resting spontaneous release of [^{3}H]-ACHC from frog retinae and cortical slices (fractional rate coefficient = 0.001–0.002 min^{-1}) was consistently more rapid than that of [^{3}H]-GABA in the presence of 0.1 mM amino oxyacetic acid which prevents GABA metabolism (rate coefficient = 0.0003–0.0007 min^{-1}). Exposure of cortical slices or frog retinae to K^+ (KCl added to Krebs' solution – final concentration 25 mM) for 4 min, evoked rapid increases in the efflux of [^{3}H]-ACHC. The increases (peak of evoked release/resting release) were 5.3 ± 0.59 and 4.9 ± 0.70 (mean ± s.e. mean of 6 determinations) in cortical slices and frog retinae respectively. In parallel experiments, K^+ (25 mM) evoked larger increases in [^{3}H]-GABA release from cortical slices and frog retinae, the increases in efflux rate being 15.2 ± 0.89 and 35 ± 11.7 respectively (mean ± s.e. mean of 6 determinations). The potassium evoked release of [^{3}H]-ACHC and [^{3}H]-GABA was calcium dependent.

Veratridine (10 μM for 4 min), which releases [^{3}H]-GABA from neurones but not from glia (Bowery & Neal, 1977), evoked a large increase in the release of [^{3}H]-ACHC from cortical slices (approximately 10 times resting release, mean of 2 experiments).

These experiments provide further evidence for the neuronal localization of [^{3}H]-ACHC and show that the analogue is released by depolarizing stimuli.

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Measurement of the antagonism of glycine by strychnine in the immature rat spinal cord *in vitro*

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The electrophoretic application of drugs to single neurones together with systemic application of strychnine *in vivo* has shown that strychnine is a specific antagonist of both post-synaptic inhibition and the depressant action of glycine in the mammalian spinal cord (see Curtis & Johnston, 1974).

However, in such *in vivo* studies, receptors are not in equilibrium with a known concentration of antagonist, thus quantitative assessment of antagonism is difficult. The *in vitro* spinal cord of the neonatal rat offers the possibility of quantifying such antagonism. In this preparation, neutral amino acids produce depolarizing responses of motoneurones which can be recorded extracellularly via the ventral root (Otsuka & Konishi, 1976).

Figure 1a shows the effect of strychnine on responses of motoneurones (VR) produced by glycine, β-alanine, taurine and γ-aminobutyric acid (GABA). Figure 1b shows data from another experiment in which the effect of two concentrations of strychnine is