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

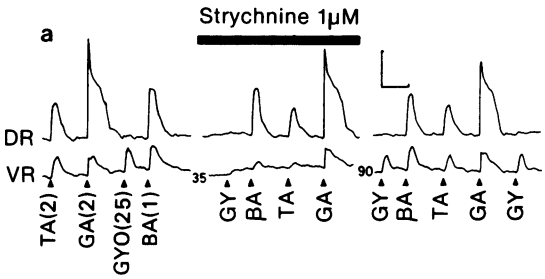

Figure 1a Effect of strychnine on depolarization of motoneurones (VR) and primary afferent terminals (DR) produced by Taurine (TA), GABA (GA), glycine (GY) and β-alanine (βA); concentration (mM) shown. Interval between traces shown in minutes. Calibration 1 mV, 10 minutes.

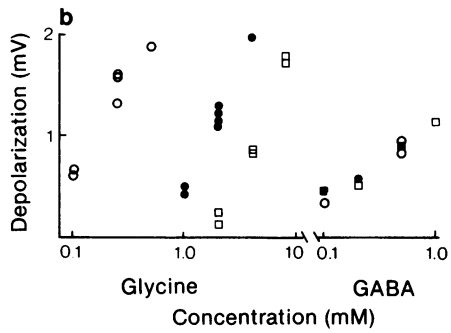

Figure 1b Effect of strychnine 1 μM (●) and 4 μM (□) on responses, measured as in (a) of motoneurones to glycine or GABA (○).

Ringer solution containing procaine 1mM was superfused at 1 ml/minute. Temperature 20°C. Amino acids applied in 2 ml doses.

shown on responses to glycine and GABA. It can be seen that in the case of glycine a parallel shift of the dose-response plot is obtained which allows estimation of the dose ratio for antagonism.

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Structure-activity studies on an excitatory glutamate receptor of leech neurones

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Glutamate receptors are present on a wide range of animal tissues (Gerschenfeld, 1973; Curtis & Johnston, 1974). There is evidence for more than one type of glutamate receptor, aspartate preferring receptors and glutamate preferring receptors (Morgan, Vrbova & Wolstencroft, 1972; Johnston, Curtis, Davies & McCulloch, 1974). In the present study an attempt is made to determine the preferred form of the excitatory glutamate receptor on leech Retzius cells.

Intracellular recordings were made from Retzius cells from *Hirudo medicinalis* and *Haemopis sanguisauga*. The potentials were amplified using conventional methods and displayed on a Hewlett-Packard pen recorder. The segmental ganglia were removed from the animal and placed in a bath of 20 ml volume. The ganglia were bathed in leech Ringer: NaCl (115); KCl (4); $CaCl_2$ (2); glucose (10); Tris HCl (10); pH 7.4. Drugs were dissolved in leech Ringer and applied over the preparation in a volume of 0.2 ml. The equipotent molar ratio (EPMR) for each analogue was calculated for at least five experiments from the ratio of the number of nmol producing comparable responses. Glutamate was taken as the standard. If the EPMR was greater than one then the compound was less potent than glutamate. The results on *Hirudo* are summarized in Table 1. Similar results were obtained for *Haemopis*.

Since ibotenic acid is approximately equipotent with glutamate and aspartate is less potent this suggests that glutamate may be interacting with the receptor in an extended conformation. Quisqualic acid and kainic acid are also capable of interacting with the receptor in an extended form. The high potencies of these compounds on leech glutamate receptors is of interest and agrees in the case of quisqualic acid with the observations of Shinozaki & Shibuya (1974) on the potency of this compound at the crayfish neuromuscular junction.

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