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Inhibitory effect of 1,2,3,4-tetrahydro-9-aminoacridine on the depolarization-induced release of GABA from cerebral cortex

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1,2,3,4-Tetrahydro-9-aminoacridine (THA) has an inhibitory effect on the activity of acetylcholinesterase which has led to its use in the treatment of Alzheimer's disease. Other actions of THA include the inhibition of voltage-dependent ion channels. In this paper we describe the effect of THA on the depolarization-induced release of [^{14}C]-γ-aminobutyric acid (GABA) from tissue slices of rat cerebral cortex. THA produced a dose-dependent inhibition of the 30 mM K^+-evoked release of [^{14}C]-GABA with an IC_{50} of 56 μM. The maximal response was an 84% inhibition of the evoked response. THA (up to a concentration of 1 mM) had no effect on the basal release of GABA. A similar inhibitory effect on the K^+-evoked release of [^{14}C]-GABA was seen with 4-aminopyridine (4-AP) but no inhibition was obtained with tetraethylammonium up to a concentration of 20 mM. The maximal inhibitory effect of 4-AP (39%) occurred at 1 mM (IC_{50} of 112 μM) and this response was much smaller in magnitude than that obtained with THA.

Introduction Amongst the cholinomimetic agents investigated for the treatment of Alzheimer's disease (AD), inhibitors of acetylcholinesterase (AChE) have produced the most promising results. In particular, the recent data (Summers *et al.*, 1986) on the use of 1,2,3,4-tetrahydro-9-aminoacridine (THA) have stimulated great interest in this approach. Although THA has a potent effect on AChE, other actions of THA have been explored which might explain the greater efficacy of the compound in AD compared to other cholinomimetic compounds. A similar action to the K^+-channel blocking drug 4-aminopyridine has been shown where THA inhibits voltage-dependent K^+-channels in hippocampal neurones with an IC_{50} of 30 μM for the A current (Rogawski, 1987) and the slow outward K^+-current in *Lymnaca* (Drukarch *et al.*, 1987). In order to obtain a greater insight into the possible effects on cortical function, we have investigated its action on the depolarization-induced release of γ-aminobutyric acid (GABA) from cerebral cortex.

Methods Tissue slices of rat cerebral cortex were cut (0.30 mm thickness) and immediately immersed in Krebs bicarbonate medium of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 0.5, glucose 11.1, amino-oxyacetic acid 0.1, pH 7.4, gassed with 95% O_2: 5% CO_2. Tissue slices were first incubated in Krebs bicarbonate medium (2 cm^3) containing [^{14}C]-GABA (4-amino-n [U-^{14}C]-butyric acid; 1.1 μM; 224 mCi $mmol^{-1}$, Amersham International) at 37°C for 10 min as previously described (de Belleroche *et al.*, 1982; de Belleroche & Gardiner, 1983). The slices were then transferred to a perspex block containing 8 tissue chambers and superfused with isotope-free Krebs bicarbonate medium maintained at 37°C, at a rate of 1 cm^3 min^{-1} for a further 20 min to reach a steady baseline rate of release. Tissue slices were then transferred to Krebs bicarbonate medium (1 cm^3) under control or test conditions, incubated for 10 min at 37°C and transferred to fresh medium containing 30 mM K^+ in the presence or absence of THA as indicated for a further 5 min incubation period.

The predominant ^{14}C-labelled constituent released in response to K^+-depolarization in the presence of amino oxyacetic acid has previously been shown chromatographically to be [^{14}C]-GABA (de Belleroche & Gardiner, 1983). At the end of the incubation period the tissue slices were removed and aliquots from the two incubation periods were taken for analysis of ^{14}C using liquid scintillation counting. Aquasol 2 (New England Nuclear Chemicals) was used as the scintillant. The ^{14}C content of the tissue slices was also determined following solubilization in Soluene 350 (Packard Instrument Company Inc.). [^{14}C]-GABA release is expressed as a percentage of the total [^{14}C]-GABA present in the tissue at the beginning of each individual incubation period (min^{-1}).

Results The effect of a range of concentrations of THA was tested on the basal and 30 mM K^+-induced release of [^{14}C]-GABA from tissue slices of rat cerebral cortex. THA caused a dose-dependent inhibition

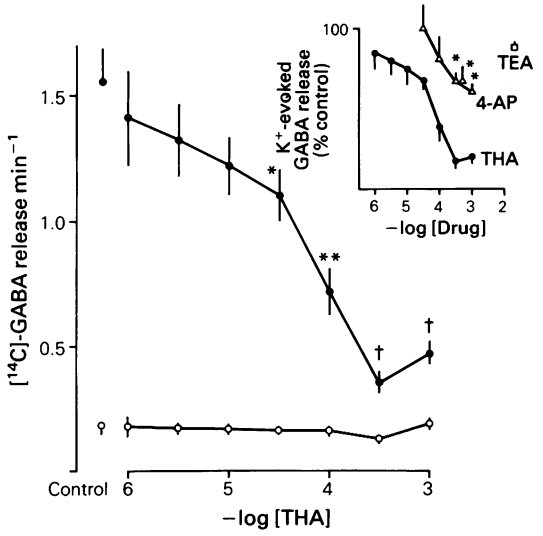

Figure 1 Effect of 1,2,3,4-tetrahydro-9-aminoacridine (THA) on the basal (○) and K^+-evoked (●) release of [^{14}C]-GABA. Tissue slices of rat cerebral cortex were pre-incubated in drug-free Krebs bicarbonate medium, then transferred to medium containing no THA or THA at various concentrations between 10^{-6} and 10^{-3} M (basal release) followed by a second incubation at the same drug concentrations but in the presence of 30 mM K^+ (K^+ evoked release). [^{14}C]-GABA released to the medium is expressed as a percentage of the tissue stores released per minute. Values are means for 5 experiments at each concentration with vertical lines indicating s.e.mean. Inset: the K^+-evoked release in the presence of drugs (release in the presence of 30 mM K^+ minus basal release in the absence of K^+) is expressed as a percentage of the drug-free controls. Values are means (vertical lines indicate s.e.mean) for 4–8 experiments for 4-aminopyridine (4-AP), 7 experiments for tetraethylammonium (TEA) and 5 experiments for THA at each concentration. (THA data calculated from the main figure.) *, **, † Indicate that the drug significantly reduced the K^+-evoked release of [^{14}C]-GABA with $P < 0.02$, $P < 0.005$ and $P < 0.001$, respectively (Student's t test).

of the K^+-evoked release of [^{14}C]-GABA but was without effect on the basal release (4.7 mM K^+) up to a concentration of 1 mM THA (Figure 1). The maximal effect of THA was an 84% inhibition of the K^+-evoked release of [^{14}C]-GABA with an IC_{50} value of 56 μM. Significant inhibition was seen at concentrations of 30 μM and above.

The action of THA was compared to that of two other K^+ channel blockers, 4-aminopyridine (4-AP) and tetraethylammonium (TEA) and the Na^+ channel blocker, tetrodotoxin (TTX). 4-AP caused a significant inhibition of the K^+-evoked release of [^{14}C]-GABA producing a maximal inhibition (39%) at 1 mM 4-AP (see inset, Figure 1) with an IC_{50} of 112 μM. TEA caused no significant inhibition up to a concentration of 20 mM TEA. A significant ($P < 0.05$) inhibition of the K^+-evoked release of [^{14}C]-GABA of small magnitude (28%) was also produced by TTX (3 μM), decreasing the release from $1.63 \pm 0.14\,min^{-1}$ (6) to $1.17 \pm 0.07\,min^{-1}$ (4). The depressed release ($P < 0.001$) of [^{14}C]-GABA in the presence of THA (300 μM), $0.48 \pm 0.04\,min^{-1}$ (6) was also significantly decreased further ($P < 0.05$) by TTX (3 μM) to $0.35 \pm 0.03\,min^{-1}$ (6), indicating a different site of action for these two drugs. (Values given are mean ± s.e.mean for the number of experiments in parentheses.

Discussion These results show that THA is able to inhibit the depolarization-induced release of [^{14}C]-GABA in a dose-dependent manner. A similar inhibition was also produced by 4-AP but not by TEA. This indicates that the action of THA is not mediated by the high conductance Ca^{2+}-activated K^+ channel ($I_{K/Ca}$) responsible for the repolarization phase of the action potential and spike after hyperpolarization, or the delayed rectifier (I_{KV}) which are both sensitive to TEA and not 4-AP (up to 2 mM). However, the transient K^+ current (I_A) responsible for prolonging interspike interval, which is widely distributed in vertebrate neurones, is activated by depolarization and is known to be inhibited by 4-AP. This current is also thought to be responsible for some of the effects of 4-AP on transmitter release (Vizi *et al.*, 1977). Although THA has also been shown to suppress action potential firing by the blockade of the fast voltage-dependent Na^+ current and depression of the TEA-sensitive slow outward K^+ current (Rogawski, 1987), TTX only produced a small inhibition of the depolarization-induced release of [^{14}C]-GABA in the preparation and TEA was ineffective.

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