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Tetrahydroaminoacridine Blocks Potassium Channels and Inhibits Sodium Inactivation in *Myxicola*[1]

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ABSTRACT

In voltage-clamped *Myxicola* giant axons internally and externally applied tetrahydroaminoacridine (THA) blocked K^+ channels with a dissociation constant of 100 μM and slowed their rate of activation. At a concentration of 10 μM, internal THA primarily slowed inactivation of conducting Na^+ channels. At 100 μM the decline of the Na^+ current during depolarizing pulses was biphasic, with an initial phase 2 to 3 times faster than in control axons. In the presence of THA there was a steady-state inward current accompanied by an increase in amplitude and time constant of Na^+ tail currents, as if THA blocked Na^+ channels by first entering them and then rendered THA-occluded channels resistant to fast inactivation. THA did not alter activation, prepulse-induced fast inactivation or slow inactivation. The effects of THA on voltage-dependent axonal ion channels might account for central nervous system hyperexcitability seen in some patients treated with THA. Because THA is a potent, centrally active anticholinesterase, even subtle ion channel-directed effects might contribute to its putative antidementia action in clinical states involving a central nervous system deficiency of acetylcholine by selective augmentation of acetylcholine release and/or negation of autoreceptor effects of endogeneous acetylcholine.

THA (1,2,3,4 tetrahydro-9-aminoacridine; Tacrine) is a potent, centrally active anticholinesterase that appears to relieve some of the symptoms of moderate-to-severe senile dementia of the Alzheimer type (Summers *et al.*, 1986). Plasma levels of THA taken from the same experimental subjects (Summers *et al.*, 1986; Park *et al.*, 1986) ranged from 5 to 70 ng/ml, corresponding (for 50 ng/ml) to 0.2 μM, a concentration in the correct range for THA inhibition of both cholinesterases and acetylcholinesterases (Heilbronn, 1961; Tonkopii *et al.*, 1976; Bajgar *et al.*, 1979). The availability of a clinical pharmacological correlate for a drug in such an early stage of its application is unusual. Although the more potent anticholinesterase physostigmine (Bajgar *et al.*, 1979) has been studied more extensively (see review by Jorm, 1986), it is not known whether it will be as well tolerated clinically. THA is structurally similar to 9-aminoacridine, a well known blocker of open Na^+ channels (Yeh, 1979; Yamamoto and Yeh, 1984) and the central aromatic ring of THA corresponds structurally to 4-AP, a well known K^+ channel blocker (Yeh *et al.*, 1976; Dubois, 1982; Soni and Kam, 1982). Because THA was also shown to possess an analeptic effect comparable to 4-AP in the ketamine/diazepam-anesthetized macaque (Martinez-Aguirre and Crul, 1979), it appeared possible that THA might have ion channel-directed effects that could modify conduction and/or synaptic transmission and contribute to its clinical efficacy. The experiments reported here demonstrate that THA indeed blocks K^+ channels, but has an additional, substantially more potent, effect on Na^+ channel inactivation.

Methods

Myxicola axons were dialyzed internally and voltage clamped by standard methods (Bullock and Schauf, 1978, 1979). For Na^+ current measurements the external solution was usually 20% Na^+ (millimolar): NaCl, 86; $CaCl_2$, 10; $MgCl_2$, 50; Tris, 347; and pH 7.30 ± 0.05, 5°C) to minimize net inward current and thus series resistance error, whereas the internal solution was 600 mM Cs^+ glutamate (pH 7.30; 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. For K^+ currents the internal solution was 600 mM K^+ glutamate buffered with 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid to pH 7.30 ± 0.05, with 10^{-6} M tetrodotoxin added externally. Series resistance was compensated, leak and capacity currents subtracted by analog circuitry and data acquired by a Nicolet 535 Signal Averager. Currents were recorded under standard conditions, after addition of THA and 15 min after return to control solutions. A total of 12 axons were examined, with data given as means ± S.E.

Sodium and K^+ activation were characterized using half-activation times and peak inward or maximum steady-state currents respectively. Time constants for inactivation of open (conducting) Na^+ channels were determined assuming a single exponential decline. The time course

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ABBREVIATIONS: THA, tetrahydroaminoacridine; 4-AP, 4-aminopyridine; CNS, central nervous system; ACh, acetylcholine.

of fast inactivation produced by conditioning depolarizations was measured with a variable-duration prepulse to a fixed potential, followed by a 0.5 msec return to the holding potential (−80 mV), and then by a constant test pulse to +20 mV (Schauf, 1974). Steady-state fast inactivation was measured using 50 msec prepulses of variable amplitude and a test pulse to 20 mV. To measure slow inactivation axons were held at −160 mV, depolarized to −30 mV for 100 msec to 10 sec and maximum Na^+ currents measured during subsequent test pulses to +20 mV (Schauf *et al.*, 1976). Recovery from slow inactivation was measured using a 1-sec prepulse to 20 mV to load the slow inactive state and returning to −160 mV for variable times before a test pulse to +20 mV.

Results

Effects of THA on the K^+ conductance. External and internal THA at a concentration of 0.5 mM completely blocked K^+ currents in *Myxicola* giant axons. At 100 to 200 μM THA decreased K^+ currents and slowed the rate of K^+ activation by 50 to 75% (fig. 1), but did not affect its voltage-dependence. Before THA application the average half-maximal activation time for the K^+ conductance at 0 mV was 2.11 ± 0.12 msec, whereas the voltage for half-maximal activation was 11.6 ± 1.5 mV. The corresponding values in THA were 3.24 ± 0.23 msec (an average increase of 52%) and 10.5 ± 2.2 mV (no significant difference). The dose-response curve for internal THA was well described assuming a single THA binding site with a dissociation constant (K_d) of 100 μM (solid squares and dashed line in fig. 2). In a few experiments THA was applied externally in 440 mM K^+ and was equally effective on outward and inward K^+ currents. Rate of block with external application was slower than if THA was applied internally, but the final levels (and K_d) were comparable.

Effects of THA on the Na^+ conductance. For exposure times of 10 to 15 min and a concentration of 10 μM internal

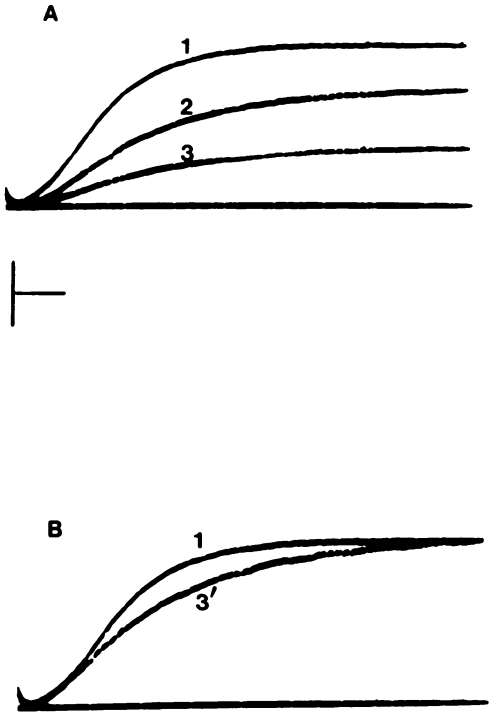

Fig. 1. Effects of internal 100 μM THA (A; trace 2) and 200 μM THA (trace 3) on K^+ currents compared to control (trace 1) at a potential of +20 mV. Slowing of K^+ activation becomes apparent when THA records are scaled to have the same final value as control (record labeled 3′ in B). Current and time scales are 0.5 mA/cm² and 1.0 msec.

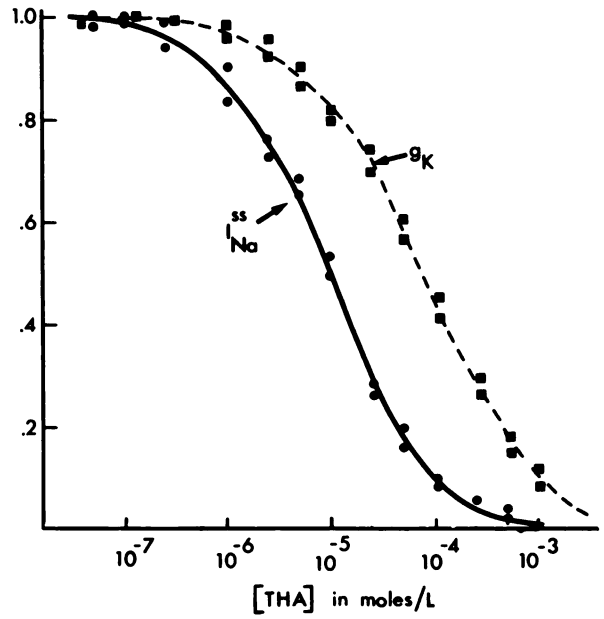

Fig. 2. Dose-response curves for THA. The ordinate represents the relative effect of THA at the concentrations indicated on the abcissa. Filled circles were obtained from the THA-dependent increase in steady-state inward current, whereas the filled squares were calculated using the inhibition of the K^+ conductance. The dashed line is the prediction for a single binding site with a dissociation constant of 100μM, whereas the solid line is the prediction for a single binding site with a dissociation constant of 10 μM. Data are from two different axons.

THA accelerated inactivation and decreased the maximum inward Na^+ currents by 35 to 50% (average 41 ± 4% in 8 axons). Inward Na^+ currents failed to inactivate completely in the presence of THA, even for long-lasting depolarizations (fig. 3). The eventual increase in steady-state inward current was dose-dependent and not strongly voltage-dependent. At 10 μM THA steady-state inward current was 20 to 30% of peak inward current, whereas for 100 μM THA steady-state inward current saturated at 74 ± 7%. The effects on inactivation seen with varying exposure times to THA is shown more clearly in figure 4. Relatively short exposures (2 min or less) primarily slowed Na^+ inactivation and did not affect the maximum inward Na^+ current (records in A). Longer applications progressively reduced maximum inward current, induced noninactivating inward currents (records B and B1) and accelerated the initial phase of Na^+ inactivation (records in C and C1).

The time constant characterizing inactivation of conducting Na^+ channels in untreated axons averaged 1.44 ± 0.22 msec at 0 mV, declining to 0.74 ± 0.07 msec at +80 mV. In axons exposed to 10 μM THA for 10 to 15 min inactivation was biphasic with average values for fast and slow components of 0.49 ± 0.08 msec and 10.4 ± 2.6 msec at 0 mV, and 0.22 ± 0.04 msec and 4.7 ± 1.3 msec at +80 mV. When Na^+ was included in the internal solution, THA was found to block outward Na^+ currents to a much greater extent than inward currents. Steady-state currents in the presence of THA were as sensitive to external tetrodotoxin as control Na^+ currents and had the same reversal potential. These complex effects on inactivation of conducting Na^+ channels are similar to those reported previously for gallamine on *Myxicola* (Schauf and Smith, 1981).

Presence of steady-state inward current was accompanied by a slowing and an increase in magnitude of the Na^+ tail currents that occur on membrane repolarization (C2 in fig 4). At negative

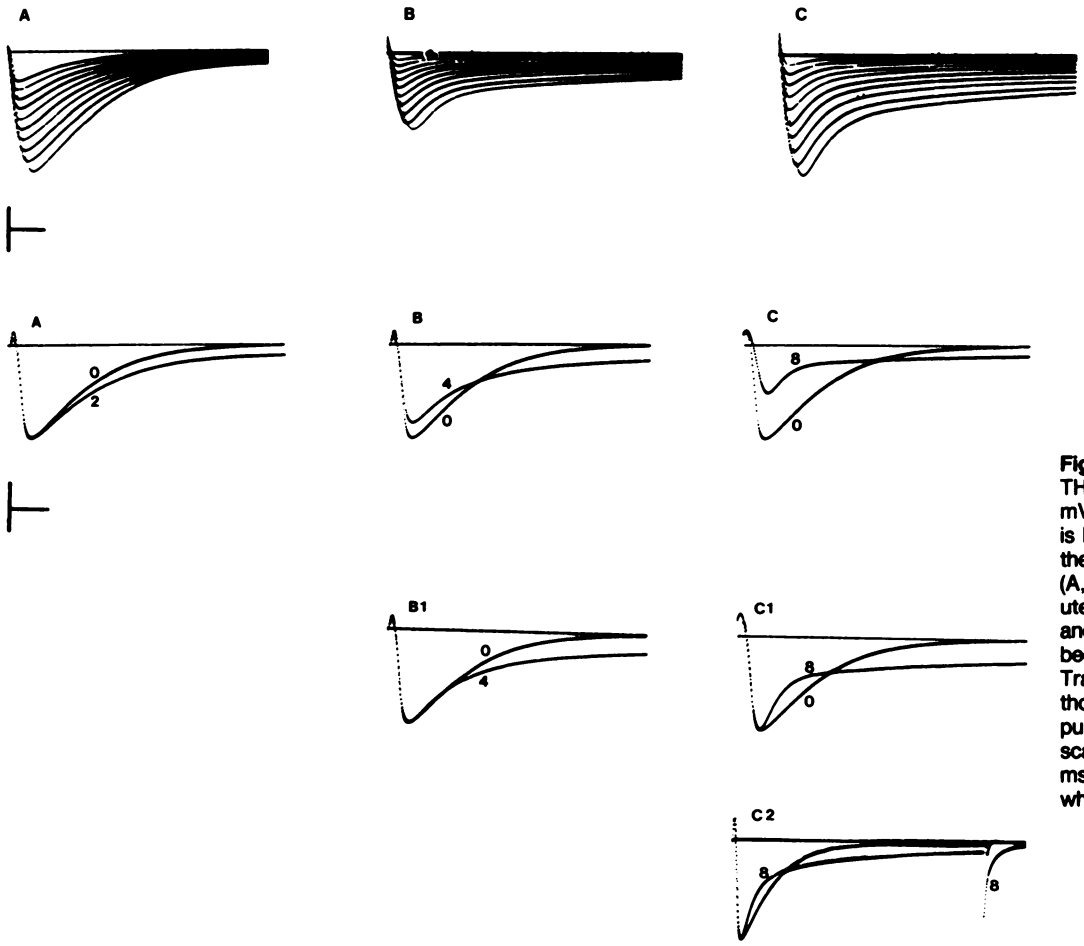

Fig. 3. Effects of 10 μM THA on Na^+ currents in *Myxicola* at potentials between −20 and +70 mV (10-mV increments). Traces in A were obtained before THA application and those in B after 10 min. In C records from B were scaled to the same magnitude as the control records. Current and time scales are 0.3 mA/cm^2 and 1.0 msec.

Fig. 4. Effect of internal 10 μM THA on Na^+ inactivation at −10 mV. The Na^+ current before THA is labeled 0 in all traces, whereas the numbers on the other traces (A, B, C) indicate the time in minutes after THA application. In B1 and C1 the results in THA have been scaled for comparison. Traces in C2 are the same as those in C1, except that a longer pulse was used. Current and time scales are 0.3 mA/cm^2 and 1.0 msec, respectively, for all but C2, where the time scale is 2.0 msec.

potentials the effect of THA on tail currents was relatively small, but for positive potentials Na^+ tail currents were quite prolonged and also showed an initial plateau, as if THA could not easily dissociate from open channels. Although THA decreased maximum inward currents for long exposure times the time course of activation was never altered, behavior expected if the rapid decline in inward current was not an effect of inactivation, but reflected blockade of open Na^+ channels by THA.

Because the effect of THA on steady-state Na^+ current saturated at high concentrations, a dose-response curve could be derived by dividing the steady-state inward current by peak current at a given dose and normalizing to the maximal ratio observed at 1 mM (filled circles and solid line in fig. 2). The dissociation constant was 10 μM, an order of magnitude lower than that derived from the effect of THA on the K^+ conductance.

External 100 μM THA had no effect on the Na^+ conductance, or did 100 μM internal THA change time- or voltage-dependence of Na^+ activation (Fig. 5). In control experiments half-maximum activation occurred at −24.5 ± 2.6 mV, whereas after 100 μM THA the value was −22.8 ± 2.1 mV. Although THA affected the inactivation of conducting Na^+ channels, it had no effect on the rate or voltage-dependence of inactivation produced by depolarizing prepulses. Steady-state inactivation was quantitated using the expression (Hodgkin and Huxley, 1952):

$$h_\infty = \frac{1}{1 + \exp\left[(V_h - V)/k_h\right]}$$

where V_h is the voltage for 50% inactivation and k_h is a shape parameter. Before THA k_h was −7.5 ± 0.6 mV and V_h was −44.2 ± 2.1 mV. In THA the corresponding values were −7.2 ± 0.4 mV and −45.7 ± 2.4 mV. The time constants for prepulse inactivation at −30 mV and +20 mV were 6.7 ± 1.1 msec and 1.1 ± 0.29 msec, respectively, in untreated axons and 7.2 ± 1.5 msec and 0.9 ± 0.25 msec after THA application. Recovery time constants were similarly unaffected.

In THA-treated axons the maximum Na^+ conductance and residual noninactivating Na^+ currents were reduced to the same extent by long-lasting (seconds) depolarizing prepulses or repetitive stimulation in the same way as in control experiments. THA therefore did not appear to alter the slow Na^+ inactivation process. Effects of THA on gating currents were examined in only a few experiments and are not illustrated. Activation charge movement was insensitive to THA, but THA decreased charge movement at the end of a depolarizing pulse, consistent with enhanced charge immobilization.

Discussion

In *Myxicola* axons internal and external THA blocked K^+ channels with a dissociation constant of 100 μM and slowed K^+ activation. Other well characterized K^+ blockers include tetraethylammonium chloride$^+$, 4-AP (Dubois, 1982; Yeh *et al.*, 1976) and dendrotoxin (Halliwell *et al.*, 1986; Penner *et al.*, 1986; Schauf, 1987; Weller *et al.*, 1985). In contrast to 4-AP (Yeh *et al.*, 1976), THA had no use-dependence. Micromolar internal concentrations of THA initially slowed inactivation of

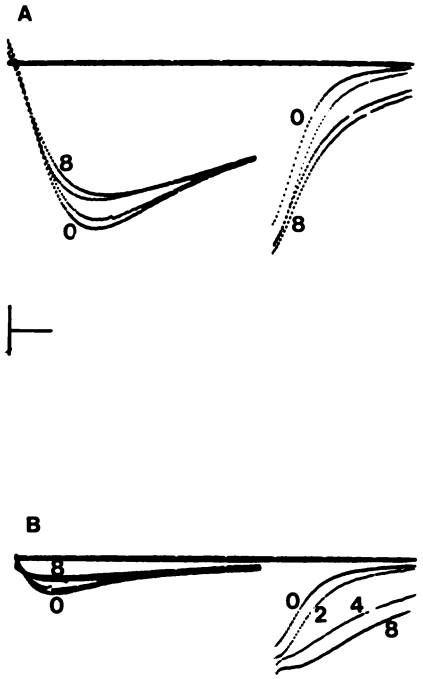

Fig. 5. Effects of 100 μM THA on Na^+ tail currents in *Myxicola*. The traces in A were obtained after repolarization to −80 mV after a depolarization to 0 mV, whereas those in B followed a depolarization to +80 mV. The numbers by each trace indicate the time in minutes after exposure to THA. Current and time scales are 0.2 mA/cm² and 0.5 msec.

conducting Na^+ channels, with the decline of Na^+ currents during depolarizing pulses becoming biphasic during prolonged exposure.

In the presence of THA there was a significant steady-state inward current accompanied by an increase in the amplitude and time constant of Na^+ tail currents. Such effects might arise if THA interacted with two sites in the Na^+ channel. For example, the rapid initial phase of inactivation accompanied by slowed tail currents could correspond to THA entry and block of open Na^+ channels, whereas THA-induced resistance to fast inactivation is the result of drug binding at another site associated with the inactivation gate. THA did not alter Na^+ activation or slow inactivation. These effects correspond most nearly to gallamine (Schauf and Smith, 1981), although THA-induced inward Na^+ tail currents were not as slow or "hooked" as those in gallamine, suggesting easier dissociation of THA from its binding site.

Ion channel-directed effects of THA may contribute to its toxicology. Clinical doses of THA range up to 3 mg/kg (Summers *et al.*, 1981; Kaye *et al.*, 1982; Summers *et al.*, 1986). These doses may produce the CNS side effects of emesis and diaphoresis, but seizures have not been reported. Doses in the range of 35 mg/kg in rats and mice produce generalized seizures (Summers *et al.*, 1980). In the rodent this would exceed the K_d of THA for the Na^+ channel effects in *Myxicola*. Combined effects of THA on Na^+ and K^+ currents could prolong presynaptic action potentials, potentiate synaptic transmission and thus contribute to the seizures observed after toxic doses of THA in rodents.

In the treatment of Alzheimer's disease with THA plasma levels are an order of magnitude lower than those observed here for ion channel-directed effects. Any clinical comparison also involves unknown species specificities, differences in temperature and ionic composition, sensitivity of end-organs and variations in presynaptic activity. Nevertheless, because THA is a highly potent anticholinesterase, one might speculate that even a modest prolongation of action potentials resulting from altered Na^+ inactivation could potentiate the postsynaptic cholinergic actions of THA, thus making THA a promising drug for the treatment of diseases characterized by CNS deficiencies of ACh. Although the therapeutic possibilities for physostigmine cannot be overlooked, doses of physostigmine greatly in excess of those needed for the maximal inhibition of cholinesterases appeared to have no significant effects on action potentials (Cantoni and Loewi, 1944; Crescitelli *et al.*, 1946; Toman *et al.*, 1947), though it should be noted that these early studies could have missed modest effects on Na^+ inactivation. Pharmacodynamic considerations also favor THA in comparison to physostigmine as the latter has a short duration of action (Summers *et al.*, 1980).

In an independent series of experiments the effects of the muscarinic ACh receptor agonist RS86 (Palacios *et al.*, 1986) were examined. In contrast to THA, no effects of RS86 on Na^+ or K^+ conductances in *Myxicola* were evident at concentrations up to 1 mg/ml. Controlled trials of individualized doses of this agent were negative in eight Alzheimer's subjects (Bruno *et al.*, 1986).

Presynaptic muscarinic receptors (autoreceptors) may act to inhibit the release of ACh in the CNS (Polak and Meeuws, 1966; Polak, 1971; Hadhazy and Szerb, 1977). Thus, in Alzheimer's disease both anticholinesterases and direct agonists might be self-defeating in that they inhibit the release of endogenous ACh. However, in the case of THA, the ion channel effects described could negate the autoreceptor effects and, in this way, increase the release and effectiveness of the remaining endogeneous ACh.

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