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Tetrahydroaminoacridine (THA) reduces voltage-dependent calcium currents in rat sensory neurons

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Tetrahydroaminoacridine (THA) is a centrally active anticholinesterase that also interacts with neuronal K^+ and Na^+ channels and cardiac Ca^{2+} channels. The effects of THA on neuronal voltage-dependent Ca^{2+} channels are not known. We tested the effects of THA (25 nM–250 μM) on the Ca^{2+} current components of acutely dissociated rat nodose ganglion and dorsal root ganglion (DRG) neurons using the whole cell patch clamp recording technique. THA reduced the low-threshold (T) and high-threshold (N/L) Ca^{2+} current components in a concentration-dependent manner ($IC_{50} \cong 125$ μM for T; $\cong 80$ μM for N/L). Minimal current reduction was seen below ~ 10 μM. Our results show that THA reduces voltage-dependent Ca^{2+} currents in rodent sensory neurons suggesting another means by which THA may affect Ca^{2+}-dependent physiologic processes.

Tetrahydroaminoacridine (THA) is a centrally active anticholinesterase that has been tested in the palliative treatment of patients with Alzheimer's disease [6, 21]. In addition to its anticholinesterase activity, THA is known to have effects on several types of neuronal voltage-dependent ion channels. THA is structurally related to 4-aminopyridine, a K^+ channel blocker, and has been shown to block K^+ currents in several studies [1, 3, 4, 7, 10, 12, 13, 15, 16, 18, 20]. THA can also block inward Na^+ currents [16] and inhibit Na^+ inactivation [18]. In studies of guinea pig ventricular myocytes [15] and rabbit sinoatrial node [13], THA reduced slow inward voltage-dependent Ca^{2+} currents. The effects of THA on neuronal voltage-dependent Ca^{2+} currents are not known.

We examined the effect of THA on the Ca^{2+} current components of acutely dissociated rat nodose and dorsal root ganglion (DRG) neurons using the whole cell patch clamp technique. These neurons have Ca^{2+} current components [2, 9, 19] similar to those initially described in chick DRG neurons [5] and subsequently in mouse DRG neurons [8]. These Ca^{2+} current components include a transient low-threshold current (T), a transient high-threshold current (N), and a slowly inactivating high-threshold current (L). Our results show that THA reduced T and N/L Ca^{2+} currents in rat nodose and DRG neurons.

Nodose ganglia were taken from 5–10-day-old rats, placed in oxygenated Ca^{2+}- and Mg^{2+}-free buffer, treated with collagenase (1 mg/ml), and incubated at 37°C in a 93% air/7% CO_2 atmosphere for 30 min. Following addition of enzyme inhibitor (5% fetal calf serum), the neurons were triturated, centrifuged, resuspended, plated, and reincubated prior to use. DRGs were taken from 15–25-day-old animals (~ 20 ganglia/animal), placed in Minimal Essential Medium (MEM), minced with micro-dissecting scissors, treated with collagenase (3 mg/ml), and incubated for 50 min before addition of trypsin (1 mg/ml), with continued incubation for 10 min. The ganglia were removed, placed in MEM, centrifuged, triturated, recentrifuged, resuspended, plated, and reincubated prior to use.

Recordings of Ca^{2+} currents were made with glass micropipettes (1.5–2.5 MΩ) filled with a solution of (in mM): CsCl 140, CsOH 30, HEPES 10, EGTA 10, ATP 5 and GTP 0.1 (pH 7.2–7.3, ~ 300 mOsm). Neurons were bathed in a solution of (in mM): choline Cl 67, TEA 100, glucose 5.6, KCl 5.3, $CaCl_2$ 5.0, $MgCl_2$ 0.8, HEPES 10 (pH 7.35, ~ 320 mOsm). Cesium and TEA were used to block K^+ currents; choline was used to block Na^+ currents. Following sealing of the micropipette to the neuron and rupture of the membrane patch, the neuron was hyperpolarized to a holding potential

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(V_h) = −90 mV to remove steady-state inactivation of Ca^{2+} currents. The program pCLAMP (Axon Instruments) was used to generate voltage step commands and to digitize (5.6 kHz) and store current traces. All experiments were conducted at room temperature.

T currents were isolated by evoking currents from a V_h = −90 mV at clamp potentials (V_c) at or positive to −55 mV. Typically, T currents were evoked by using voltage steps of 100 ms duration delivered every 5 s in 5 mV increments ranging from a V_c = −65 to −20 mV. T currents were measured at the point of peak inward current and at 100 ms. Maximal T current was the largest evoked peak T current evoked at a V_c (usually −35 mV) negative to that at which more slowly inactivating current components (N/L) were evoked. This resulted in slight underestimation of T current magnitude but allowed for examination of those currents with little high-threshold current contamination. T currents were stable and showed little change in amplitude over the course of an experiment. T current magnitude varied among neurons from barely detectable to greater than 1.5 nA.

N/L currents were evoked from a V_h = −90 mV (occasionally −80 mV) at a V_c at or positive to −20 mV and were measured at the point of peak inward current and at 100 ms. Maximal N/L current was the largest evoked high threshold current measured at peak current. Currents were evoked at 1 min intervals and declined 30–50% during a 20 min recording. Current rundown was estimated for each neuron from serial maximal N/L currents evoked at 1 min intervals approximately 5 min after patch rupture and prior to application of THA.

T and N/L currents were leak subtracted for analysis using the inverse of currents evoked from hyperpolarizing voltage commands equal to the depolarizing commands used in evoking inward currents. Current activation, current inactivation, and voltage-dependency were assessed for control and THA. Current activation was estimated using the time to the point of peak inward current. Current inactivation was estimated using the ratio of current at 100 ms/peak current. Voltage dependency was assessed by inspection of the current–voltage relation.

THA was prepared on the day of experiments by dissolving it in external solution. The THA solution was drawn up into a blunt tipped (10–15 μm) micropipette which was kept out of the bath solution when not in use. THA was tested by positioning the micropipette tip ~50 μm from the cell soma and applied by passive diffusion shortly before and during voltage step commands. The effects of THA applied by pressure ejection (1 psi) were the same as those by passive diffusion. Control micropipettes contained external solution and were substituted sequentially with micropipettes containing different concentrations of THA.

THA was tested in 25 neurons: 20 nodose and 5 DRG. There was no observed difference in the effects of THA on the evoked Ca^{2+} currents for the two neuronal types, so data were pooled for analysis. Fig. 1 shows the con-

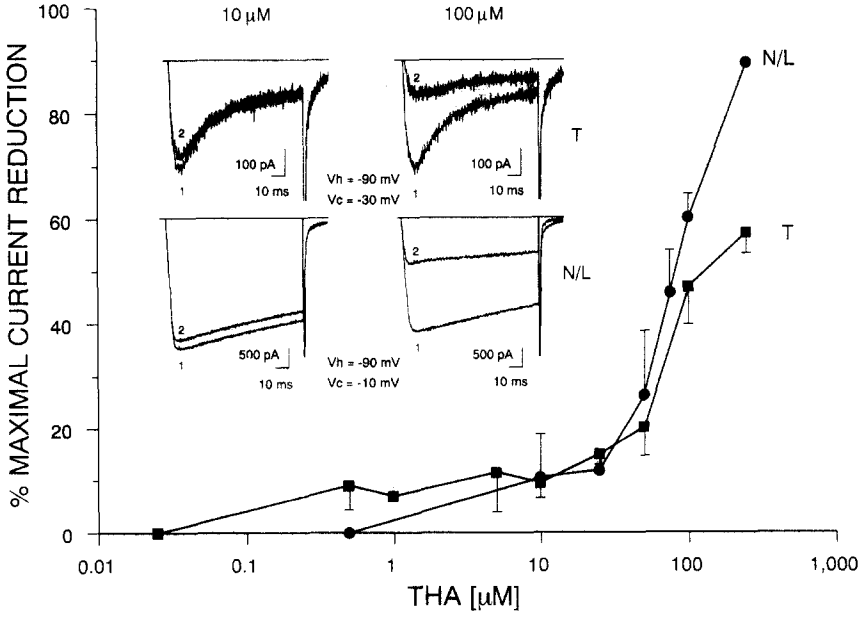

Fig. 1. Concentration-response curves for maximal T and N/L Ca^{2+} currents tested in 25 nodose and dorsal root ganglion (DRG) neurons with THA (25 nM–250 μM). Each point represents the mean percent reduction of control maximal currents obtained by testing with THA in 1–4 neurons (bars indicate S.E.M.). For each neuron studied, specific concentrations of THA were tested by single or multiple applications. Inset: leak-subtracted T and N/L currents in a nodose neuron for control (1) and THA (2) at 10 μM and 100 μM.

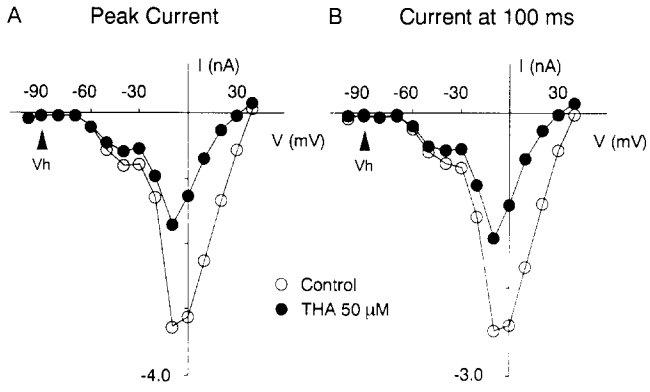

Fig. 2. Current-voltage relations for Ca^{2+} currents measured in a DRG neuron in the absence and presence of THA (50 μM). Ca^{2+} currents were evoked from a $V_h = -90$ mV at a V_c ranging from -100 to $+40$ mV. A: THA's effect on peak current amplitude. B: THA's effect on the current amplitude measured at 100 ms (note change in ordinate scale).

vous system have an increased sensitivity to THA. Beneficial effects have been reported for nimodipine, a dihycentration-response curve of THA (25 nM–250 μM) on maximal T and N/L current reduction for all neurons studied. THA decreased peak current in a concentration-dependent manner for both T ($IC_{50} \cong 125$ μM) and N/L ($IC_{50} \cong 80$ μM) currents. Minimal current reduction was seen below ~ 10 μM. At the highest concentration tested (250 μM), T current was reduced by 57%, and N/L current by 90%. Fig. 1 (inset) demonstrates the concentration-dependent reduction of T and N/L currents in a nodose neuron by THA (10 μM and 100 μM). T currents recovered fully within 1 min of THA application. N/L currents recovered fully within 1 min with THA (10 μM), but took 3 min to recover with THA (100 μM) (not shown). Current activation was not changed with THA. Similarly, the amount of current inactivation was not changed with THA (10 μM), however, current inactivation appeared to decrease with THA at higher concentrations (100 μM), an effect seen in other neurons as well. This suggests that the apparent decreased inactivation for T currents was due to either the late current consisting of a T current and a reduced N/L current component or that there were two phases of T current inactivation with the THA effect greater on the initial, larger component. For N/L currents, the initial current was reduced to a greater extent than late (100 ms) currents. This could be due to greater reduction of the more rapidly inactivating N current than the slowly inactivating L current by THA or that THA causes a small increase in activation rate. Fig. 2 shows the current-voltage relation for Ca^{2+} currents measured at peak current (A) and at 100 ms (B) when tested with THA (50 μM). THA did not change the voltage-dependency of the evoked currents at peak or 100 ms.

The main finding of this study was that THA reversibly reduced whole cell voltage-dependent T and N/L Ca^{2+} currents in a concentration-dependent manner in rat nodose and DRG neurons. These results demonstrate that in addition to THA's known anticholinesterase activity and its effects on K^+ and Na^+ currents in neuronal preparations, it can also cause marked reduction of neuronal voltage-dependent Ca^{2+} currents. Ca^{2+} current reduction occurred without effect on current activation or inactivation, or on voltage dependency at THA concentrations $\leqslant 75$ μM. Several studies have shown THA's ability to block different K^+ conductances but THA has little to no effect on Ca^{2+}-activated K^+ conductances [1, 3, 20]. Thus the block of Ca^{2+} currents seen in these experiments probably does not contribute to a secondary reduction of K^+ currents. THA's reduction of both T and N/L currents suggests that it may have diverse effects on Ca^{2+}-dependent physiologic processes. N currents may be primary regulators of the inhibition of release of neurotransmitters [11] and reduction of these currents by THA may contribute to the observed inhibitory effect of THA on depolarization-induced release of γ-aminobutyric acid (GABA) in rat cerebral cortex [3]. THA's reduction of cardiac slow inward Ca^{2+} currents [13, 15] is likely mediated by L-type Ca^{2+} channels and Ca^{2+} current reduction in those studies may be qualitatively similar to the results found here.

THA had little effect on Ca^{2+} currents in the serum concentration range (20–300 nM) used in clinical trials in the treatment of Alzheimer's disease [21]. Although rodent in vivo levels of THA have been shown to be 10 times higher in brain than plasma [14], a log unit shift of the THA concentration-response curve in the present experiments would not have been significant since minimal current reduction was seen below ~ 10 μM. This result suggests that if THA has any therapeutic effect in Alzheimer's disease, it is not likely due to reduction of Ca^{2+} currents unless Ca^{2+} channels in the central ner-

dropyridine derivative and Ca^{2+} channel antagonist, in the management of primary degenerative dementia [22] and in animal models of aging and cognition [17].

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