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Tetraethylammonium (TEA) increases the inactivation time constant of the transient K^+ current in suprachiasmatic nucleus neurons

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Abstract

Identifying the mechanisms that drive suprachiasmatic nucleus (SCN) neurons to fire action potentials with a higher frequency during the day than during the night is an important goal of circadian neurobiology. Selective chemical tools with defined mechanisms of action on specific ion channels are required for successful completion of these studies. The transient K^+ current (I_A) plays an active role in neuronal action potential firing and may contribute to modulating the circadian firing frequency. Tetraethylammonium (TEA) is frequently used to inhibit delayed rectifier K^+ currents (I_{DR}) during electrophysiological recordings of I_A. Depolarizing voltage-clamped hamster SCN neurons from a hyperpolarized holding potential activated both I_A and I_{DR}. Holding the membrane potential at depolarized values inactivated I_A and only the I_{DR} was activated during a voltage step. The identity of I_A was confirmed by applying 4-aminopyridine (5 mM), which significantly inhibited I_A. Reducing the TEA concentration from 40 mM to 1 mM significantly decreased the I_A inactivation time constant (τ_{inact}) from 9.8 ± 3.0 ms to 4.9 ± 1.2 ms. The changes in I_A τ_{inact} were unlikely to be due to a surface charge effect. The I_A amplitude was not affected by TEA at any concentration or membrane potential. The isosmotic replacement of NaCl with choline chloride had no effect in I_A kinetics demonstrating that the TEA effects were not due to a reduction of extracellular NaCl. We conclude that TEA modulates, in a concentration dependent manner, τ_{inact} but not I_A amplitude in hamster SCN neurons.

Section

Neurophysiology; Neuropharmacology and other forms of Intercellular Communication

Keywords

potassium current; suprachiasmatic nucleus; circadian rhythm; KV4 channel

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1. Introduction

Neurons in the hypothalamic suprachiasmatic nucleus (SCN) contain a molecular clock that drives circadian rhythms. The circadian clock determines many facets of SCN neuronal activity including the frequency of action potential firing and also the strength of afferent excitatory and inhibitory synapses (Gompf and Allen, 2004; Itri et al., 2005; Lundkvist et al., 2002; Pennartz et al., 2001). The mechanisms underlying the circadian regulation of cellular electrical activity remain largely unknown. Several ionic currents including a persistent Na^+ current, delayed rectifier K^+ channels (I_{DR}), large conductance Ca^{2+}-activated K^+ channels (BK), transient K^+ current (A-type, I_A), and voltage-dependent L- and T-type Ca^{2+} channels regulate action potential firing or show significant day-night differences in activity or both (Itri et al., 2004; Jackson et al., 2004; Kim et al., 2005; Kononenko et al., 2004; Kuhlman and McMahon, 2004; Meredith et al., 2006; Pennartz et al., 2002; Pitts et al., 2006). Identification of the contribution that each ion channel makes to the firing of SCN neurons requires selective chemical tools with defined mechanisms of action.

I_A is a rapidly activating, rapidly inactivating K^+ current that contributes to setting the timing between action potentials and the postsynaptic responses to synaptic inputs (Connor and Stevens, 1971). I_A is observed in the majority of SCN neurons and may play a role in setting the action potential firing frequency in these cells (Bouskila and Dudek, 1995; Huang, 1993; Huang et al., 1993). I_A is carried by ion channels composed of α subunits of the Kv4 family (*Shal*, (Jerng et al., 2004)). In neurons, the observed I_A activation and inactivation kinetics require the presence of Kv Channel Interacting Proteins (KChIP). KChIP are EF-hand Ca^{2+}-binding proteins that associate with the cytoplasmic tail of the Kv α subunits and alter the expression of the α subunits and I_A inactivation kinetics (An et al., 2000; Rhodes et al., 2004).

Activation of I_A increases the interspike interval and slows the action potential firing frequency by reducing the rate of membrane depolarization (Rudy et al., 1999). I_A is largely inactivated in the range of resting membrane potentials (−40 mV to −55 mV) recorded from SCN neurons during the daytime (Kuhlman et al., 2003; Schaap et al., 1999; Teshima et al., 2003). I_A rapidly recovers from inactivation during the afterhyperpolarization that follows the upstroke an action potential. The membrane hyperpolarization moves the Kv4 channels to closed states from which I_A can be activated during a subsequent subthreshold depolarization (Bardoni and Belluzzi, 1993; Campbell et al., 1993). Therefore, a careful characterization of I_A properties is required to understand the physiological properties of SCN neurons and for the development of accurate computational models of neuronal function and circuitry in the SCN.

In addition to I_A, SCN neurons have both fast and slow delayed rectifier K^+ currents (I_{DR}) and depolarization of an SCN neuron will activate both I_A and I_{DR} (Bouskila and Dudek, 1995; Itri et al., 2005; Kuhlman and McMahon, 2004). Two experimental strategies are usually used individually or together to isolate I_A from the I_{DR}. The first takes advantage of the fact that I_A and I_{DR} have different rates of inactivation at depolarizing voltages. Holding the membrane potential at relatively depolarized levels inactivates I_A and a subsequent membrane depolarization only activates I_{DR}. Alternately, tetraethylammonium (TEA) is used to separate I_A from the I_{DR} because I_A is much less sensitive to TEA block than I_{DR} (Andreasen and Hablitz, 1992). To produce a significant reduction in the I_{DR} requires a TEA concentration of 20 mM to 60 mM (Bardoni and Belluzzi, 1993; Zhou and Hablitz, 1996). Together, the low TEA sensitivity of I_A and the voltage-dependence of I_{DR} activation and I_A inactivation, allow accurate recording of I_A. However, some authors have found that TEA alters I_A amplitude but not the kinetics (Bardoni and Belluzzi, 1993; Sanchez et al., 1998). While studying the kinetics of I_A in hamster SCN neurons we observed that the TEA concentrations required to block I_{DR} significantly increased I_A inactivation time constant.

2. Results

The first experiments were designed to determine I_A inactivation time constant (τ_{inact}) in hamster SCN neurons. In the presence of TEA (40 mM), SCN neurons were voltage-clamped at −100 mV then sequentially stepped in 10 mV increments to membrane potentials ranging from −30 mV and +60 mV (Fig. 1A). The I_{DR} recorded under these conditions was only a small fraction of the total I_{DR} due to TEA inhibition (Fig. 1B). The membrane holding potential was then set at −40 mV to inactivate I_A and a second set of depolarizing pulses applied (range −30 mV to +60 mV). I_A was isolated by digitally subtracting the currents recorded at the −40 mV holding potential from those recorded using the −100 mV holding potential (Fig. 1C). The identity of I_A was confirmed by an 8 min bath application of 4-aminopyridine (4-AP, 5 mM), which significantly reduced the I_A amplitude 83 ± 10% (Fig. 1D). The mean I_A amplitude recorded at +60 mV was 964 ± 231 pA during control recordings and 162 ± 64 pA (n = 4, $p < 0.04$) after 4-AP application. I_A amplitude was not affected by TEA (1 mM and 40 mM) when the experiments were performed at 36°C (Fig. 1E). The mean maximal I_A amplitudes, recorded in 1 mM and 40 mM TEA with a voltage step to +60 mV were not significantly different ($p > 0.21$); 1.4 ± 0.6 nA (n = 14) and 1.6 ± 0.4 nA (n = 10), respectively. Recently it was demonstrated that I_A activation kinetics are modified in preparations maintained at room temperature (Campbell et al., 1993). Therefore, experiments were performed at 22 °C to determine whether the TEA effects on I_A were temperature dependent. However, neither 1 mM nor 40 mM TEA had an effect on I_A amplitude when the experiments were performed at 22°C (n = 3).

I_A inactivation progressively slowed during the 8 min TEA (40 mM) perfusion at both 36 °C and 22 °C (Fig. 1F). To further examine the effect of TEA on I_A kinetics, a sequence of voltage steps were used to activate I_A in each SCN neuron. A τ_{inact} was calculated for I_A activated by each voltage step, the τ_{inact} plotted versus membrane potential (Vmemb), and the data fit with a line, $\tau_{inact} = a*V_{memb} + b$ (Fig. 2A, B). The τ_{inact} for each SCN neuron was estimated from the linear fit at Vmem = 0 (parameter b). The τ_{inact}, estimated in the presence of 40 mM TEA, ranged from 5.4 ms to 13.6 ms with mean of 9.8 ± 3.0 ms (n = 12). Reducing the TEA concentration from 40 mM to 1 mM decreased the mean estimated τ_{inact} to 4.9 ± 1.2 ms with a range of 2.6 ms to 6.3 ms (n = 15). The τ_{inact} recorded in 40 mM TEA was significantly longer than that recorded in 1 mM TEA ($p < 0.0001$) and the individual τ_{inact} fell into two distinct populations (Fig. 2C, D). The slope of the linear fit (parameter a) in 1 mM and 40 mM TEA were similar (−0.26 ± 0.01 vs. −0.07 ± 0.02 ms/10 mV, n = 15 and n = 12, respectively, $p < 0.30$). The shallowness of the τ_{inact} voltage in the presence of both low and high TEA concentrations suggests that the TEA induced lengthening of τ_{inact} was not due to a surface charge effect.

In *Helix aspersa* neurons, TEA in the extracellular solution shifts the voltage-dependence of I_A activation by altering the membrane potential near the voltage sensing domains of the Kv4 channels (Denton and Leiter, 2002). Experiments were performed using an initial holding potential of −100 mV followed by three holding potentials, −50 mV, −40 mV, and −30 mV to determine whether the holding potential used to inactivate I_A altered the TEA effect on τ_{inact}. The τ_{inact} in the presence of 1 mM TEA at the three different holding potentials were not significantly different: 4.6 ± 0.3 at −50 mV, 4.8 ± 0.3 at −40 mV and 4.9 ± 0.4 at −30 mV (n = 8, $p > 0.22$, Fig. 3). In the presence of 40 mM TEA, the τ_{inact} were 8.5 ± 0.7 at −50 mV, 8.8 ± 0.7 at −40 mV and 7.9 ± 0.6 at −30 mV (n = 8, $p > 0.29$). These data demonstrate that the holding potential used to inactivate I_A did not alter the lengthening τ_{inact} by 1 mM or 40 mM TEA. It further demonstrated that the subtraction procedure used to isolate I_A produced similar results at each holding voltage.

During the experiments described above, NaCl (39 mM) was replaced isosmotically by TEA to increase the TEA concentration from 1 mM to 40 mM. Therefore, additional experiments

were performed to insure that the effect of TEA on I_A τ_{inact} was not due to the reduction of extracellular NaCl. I_A τ_{inact} was similar in normal ACSF (1 mM TEA) and when NaCl was isosmotically replaced with choline chloride (39 mM, 8 min perfusion): 2.7 ± 0.9 ms vs. 4.0 ± 1.5 ms (n = 8, $p > 0.06$).

3. Discussion

Identification of the mechanisms that drive SCN neurons to fire action potentials with a higher frequency during the day than during the night is an important topic in circadian neurobiology. I_A plays an active role in neuronal action potential repolarization and may contribute to setting the circadian firing frequency (Bouskila and Dudek, 1995; Huang, 1993; Huang et al., 1993). I_A may also modulate the response of SCN neurons to retinohypothalamic tract excitatory input. Thus, the accurate determination of I_A kinetics is very important for the development of realistic models of SCN neurons that faithfully reproduce the spiking activity. SCN neurons express many types of K^+ channels that may also determine the membrane potential and firing frequency (Itri et al., 2004; Kuhlman and McMahon, 2004; Pitts et al., 2006; Teshima et al., 2003). It is an experimental challenge to isolate and characterize these diverse K^+ currents. A frequently used strategy to isolate I_A is to block I_{DR} with TEA. Unfortunately, in hamster SCN neurons application of TEA at concentrations sufficient to inhibit the I_{DR} altered I_A τ_{inact} that was shown in our experiments.

TEA has variable effects on I_A amplitude and kinetics depending on the concentration and type of the cell. In, TEA had no effect on I_A guinea pig laterodorsal tegmental neurons (40 mM), sensormotor cortical neurons, or cerebellar granule cells (20 mM) (Bardoni and Belluzzi, 1993; Sanchez et al., 1998; Zhou and Hablitz, 1996). In contrast, 60 mM TEA reduces I_A amplitude by more than 40% in layer 1 neurons and layer II/III pyramidal cells of the neocortex. In these neurons the τ_{inact} was found to be approximately 15 ms (Zhou and Hablitz, 1996). In rat dorsal nucleus neurons, a TEA concentration of 10 mM reduced the peak I_A by 23% but did not alter the τ_{inact} value of 12.3 ms (Fu et al., 1996). In hamster SCN neurons, we found that while I_A amplitude was insensitive to TEA, I_A τ_{inact} was significantly increased from 4.9 ms in 1 mM of TEA to 9.8 ms in 40 mM TEA. The last value is within the range of I_A τ_{inact} (10 – 20 ms) observed in other neurons (Bardoni and Belluzzi, 1993; Fu et al., 1996; Zhou and Hablitz, 1996).

TEA is a quaternary cation that may alter ion channel kinetics by multiple mechanisms. One possibility is a surface charge effect where the electrical potential across the membrane is reduced by cations neutralizing negatively charged membrane components. For example, TEA in the extracellular solution shifts the voltage-dependence of I_A activation, in *Helix aspersa* neurons, by altering the membrane potential near the voltage sensing domains of the Kv4 channels (Denton and Leiter, 2002). It is unlikely that the TEA alteration of I_A τ_{inact} was due to a surface charge effect since there was a very shallow voltage dependence of τ_{inact} of hamster SCN neurons in either high or low TEA concentrations. We therefore conclude that TEA is not a useful blocker for the evaluation of I_A kinetics in hamster suprachiasmatic nucleus neurons because it alters I_A τ_{inact}.

4. Experimental Procedure

Golden hamsters (*Mesocricetus auratus*) were housed under a 14:10 hr light–dark cycle for at least a week. The hamsters were deeply anesthetized with halothane at least 1 hour before the beginning of the dark period, decapitated and the brain removed (Gillette, 1986). The Oregon Health & Science University Institutional Animal Care and Use Committee approved in advance all procedures involving animals. Thin slices containing the SCN (250 μm) were cut

with a vibrating blade microtome (Leica, VT1000S, Nussloch, Germany) and incubated in a storage chamber with a physiological solution at 33°C saturated with 95% O_2 - 5% CO_2.

After 2–3 hr, SCN slices were placed in a small recording chamber and perfused with a solution containing in mM: NaCl 139, KCl 3, NaH_2PO_4 1.2, $MgCl_2$ 3.3, $CaCl_2$ 0.2, $NaHCO_3$ 20, glucose 9 saturated with 95% O_2 - 5% CO_2 at 36°C. Recording electrodes were pulled using a PP-83 electrode puller (Narishige, Japan) and had resistances of 8–10 MΩ when filled with a solution composed of (in mM): K-gluconate 125, KCl 10, EGTA 5, HEPES 10, MgATP 2 and Tris GTP 0.2. Individual SCN neurons were visualized using infrared illumination and differential interference contrast optics on a Leica DFLMS microscope (Nussloch, Germany). Currents were recorded in the whole-cell patch-clamp configuration using an Axopatch-1D amplifier (Axon Instruments, USA). Series resistances were compensated a minimum of 70%. The evoked currents were low-pass filtered at 2 KHz and acquired at a sampling rate of 20 KHz with an ITC16 interface (Instrutech, USA) and Pulse+PulseFit software (HEKA, Lambrecht, Germany). The recorded currents were analyzed with Excel (Microsoft, USA) and plotted with Igor Pro (WaveMetrics, USA). The reported voltages were not corrected for a liquid junction potential of approximately −13 mV.

The experimental strategy used to study I_A in neurons takes advantage of the observation that I_A is inactivated at depolarized membrane potentials. I_A was isolated by subtracting the currents activated from a holding potential of −100 mV from those activated by depolarizing pulses from a holding potential of −40 mV. I_A inactivation time constant (τ_{inact}) was determined by fitting the subtracted currents with the equation:

$$i(t)=i_0+Ke^{-\frac{t}{\tau}}$$

where τ is the inactivation time constant, i_o is the baseline current and K is the maximal I_A amplitude. The data are presented as the mean ± standard deviation.

Voltage-dependent Na^+ channels were blocked with tetrodotoxin (TTX, 0.5 μM) and spontaneous $GABA_A$ receptor-mediated currents were inhibited with picrotoxin (50 μM). Voltage-dependent Ca^{2+} currents were insignificant at the external Ca^{2+} concentration of 0.2 mM. The minimal concentration of TEA used was 1 mM, which inhibited the Ca^{2+}-activated K^+ currents and the fast I_{DR} (Wong and Adler, 1986).

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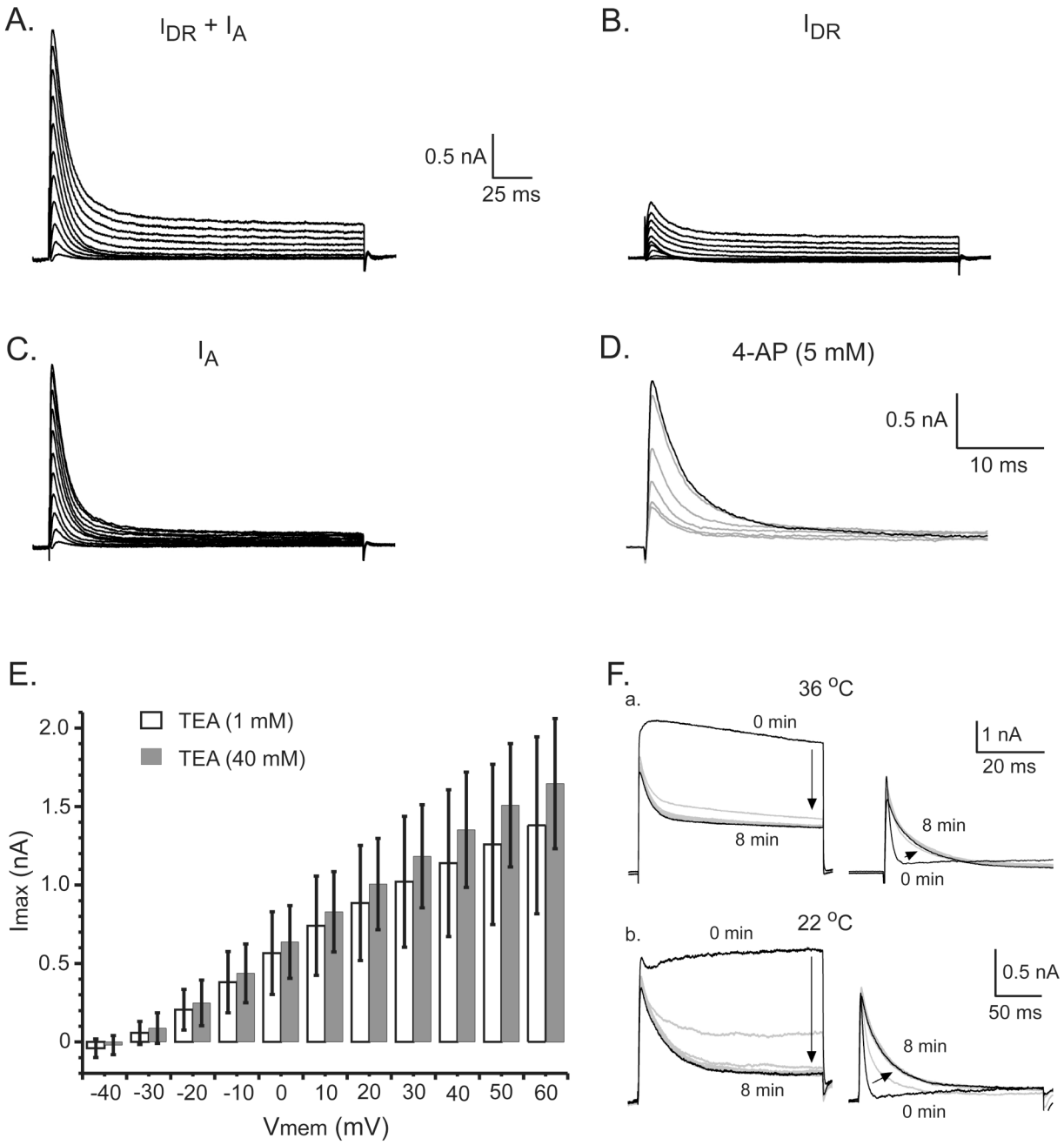

Figure 1.

Effects of TEA and 4-AP on I_A of hamster SCN neurons. The bath solution contained TTX (0.5 μM), picrotoxin (50 μM), TEA (40 mM) and a low extracellular Ca^{2+} concentration (0.2 mM) to reduce Ca^{2+} currents. A. Currents were activated by sequence of voltage steps to potentials between −30 mV and +60 mV from a holding potential of −100 mV. This depolarization protocol activated both I_{DR} and I_A. B. Holding the membrane potential at −40 mV and applying a sequence of voltage steps to depolarized values between −30 mV to +60 mV activated the I_{DR}. C. Digital subtraction of the currents recorded in A and B. D. Example of the inhibition of I_A by 4-AP (5 mM). The currents shown were recorded each minute during an 8 min 4-AP application. E. A high concentration of TEA (40 mM) did not alter I_A amplitude.

Each bar represents the mean I_A amplitude at each step potential (mean ± standard deviation, n = 10). F. a. TEA (40 mM) added to the recording chamber (0 min) produced a progressive decrease in the I_{DR} activated from a holding potential of −80 mV by a voltage step to +60 mV. Note that the remaining current shows a rapid activation followed by inactivation. I_A recorded during the same experiment. I_A τ_{inact} increased from 3.3 ms to 28 ms during the 8 min TEA application (36 °C). b. A similar experiment performed at 22°C. TEA (40 mM) increased I_A τ_{inact} from 1.3 ms to 10.4 ms.

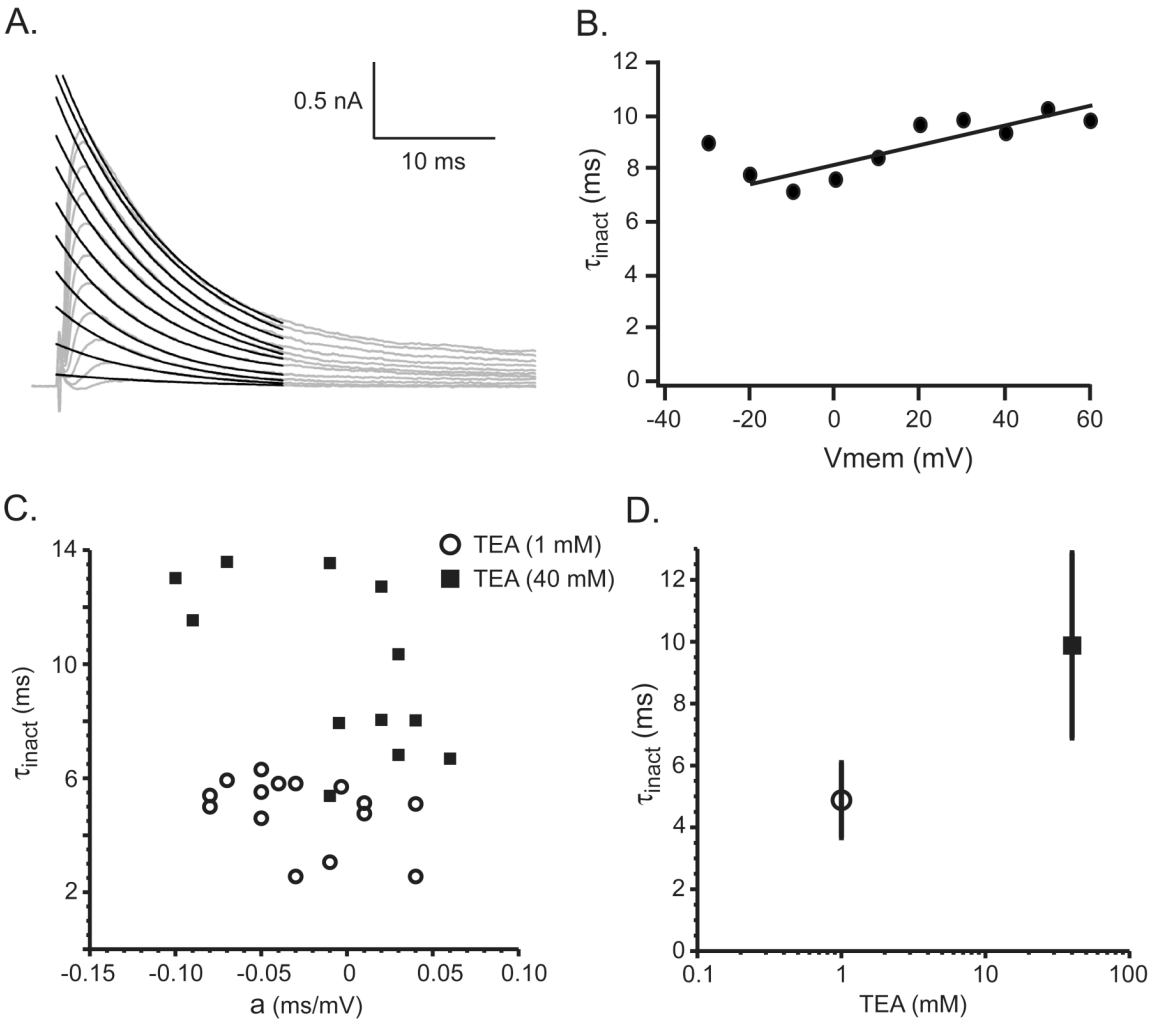

Figure 2.

Sensitivity of I_A τ_{inact} to TEA (40 mM). A, B. Example of the calculated I_A τ_{inact}. I_A (gray lines) and the black lines the fit to the equation $i(t)=i_0+Ke^{-\frac{t}{\tau}}$ (see Experimental Procedures). B. The value of the I_A τ_{inact} was directly related to the value of the membrane potential (Vmem). C. The effect of high (40 mM) and low (1 mM) TEA concentrations on parameters of I_A τ_{inact}. Distribution of the values for the parameter (a) and I_A τ_{inact} calculated from the linear fits to the data from each neuron. The parameter (a) represents the voltage dependence of I_A τ_{inact} while parameter (b) represents the I_A τ_{inact} at 0 mV. D. The mean ± standard deviation of I_A τ_{inact} in the presence of either 40 mM or 1 mM TEA shows the strong effect of the TEA on I_A inactivation kinetics.

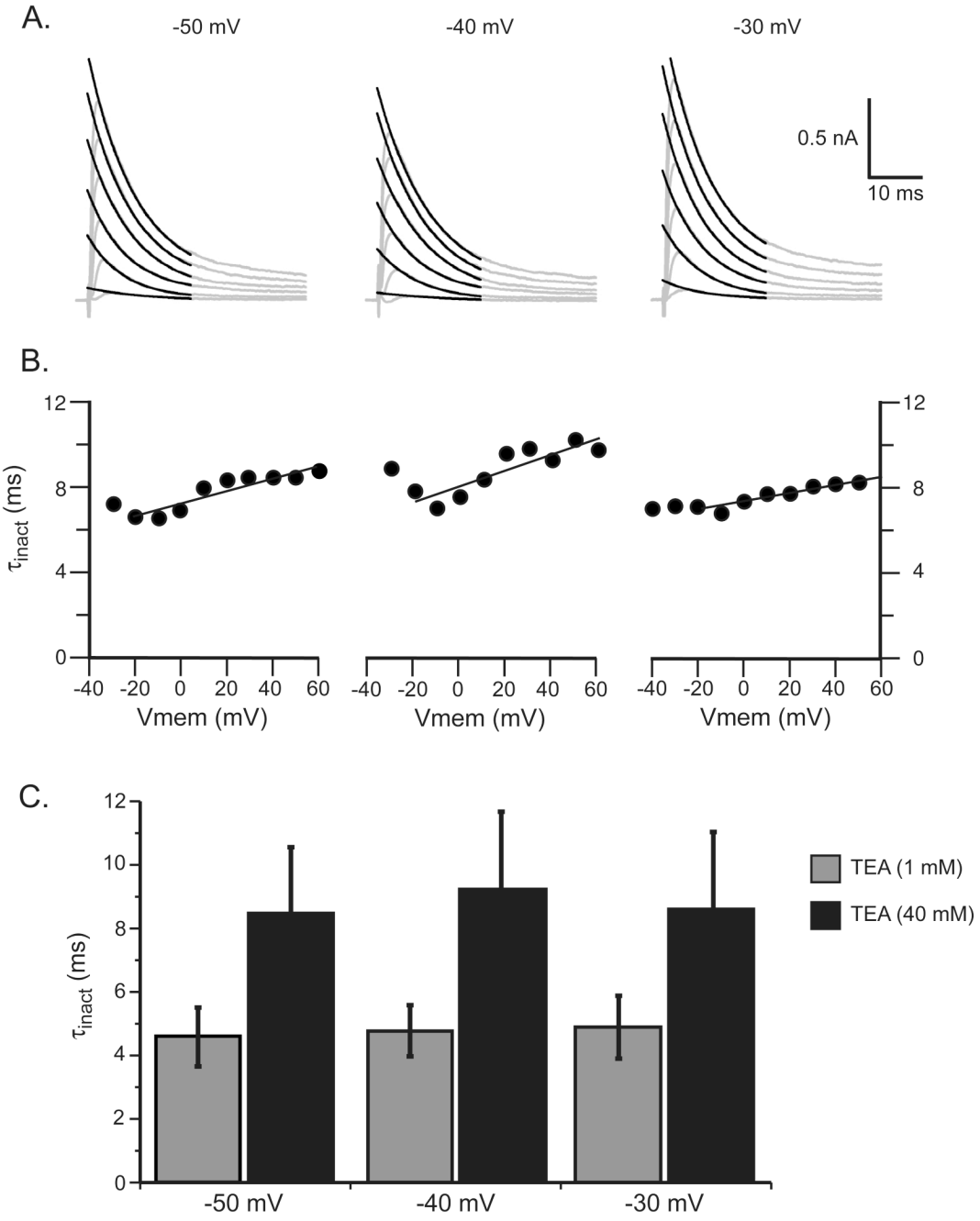

Figure 3.

Determination of I_A τ_{inact} at holding potentials of −50 mV, −40 mV and −30 mV. A. The gray lines were I_A and the black lines the fit to the equation $i(t)=i_0+Ke^{-\frac{t}{\tau}}$ B. The calculated I_A τ_{inact} was not affected by the holding potential. C. The mean ± standard deviation of I_A τ_{inact} recorded at three holding potentials in the presence of high (40 mM) and low (1 mM) TEA concentration.