Voltage-Clamp Analysis and Computer Simulation of a Novel Cesium-Resistant A-Current in Guinea Pig Laterodorsal Tegmental Neurons

RUSSELL M. SANCHEZ, ALISA SURKIS, AND CHRISTOPHER S. LEONARD
Department of Physiology, New York Medical College, Valhalla, New York 10595

Sanchez, Russell M., Alisa Surkis, and Christopher S. Leonard. Voltage-clamp analysis and computer simulation of a novel cesium-resistant A-current in guinea pig laterodorsal tegmental neurons. J. Neurophysiol. 79: 3111–3126, 1998. Increased firing of cholinergic neurons of the laterodorsal tegmental nucleus (LDT) plays a critical role in generating the behavioral states of arousal and rapid eye movement sleep. The majority of these neurons exhibit a prominent transient potassium current ($I_A$) that shapes firing but the properties of which have not been examined in detail. Although $I_A$ has been reported to be blocked by intracellular cesium, the $I_A$ in LDT neurons appeared resistant to intracellular cesium. The present study compared the properties of this cesium-resistant current to those typically ascribed to $I_A$. Whole cell recordings were obtained from LDT neurons ($n = 6/1$) in brain slices with potassium- or cesium-containing pipette solutions. A transient current was observed in cells dialyzed with each solution (KGluc-85%; CGluc-79%). However, in cesium-dialyzed neurons, the transient current was inward at test potentials negative to about $-35$ mV. Extracellular 4-aminopyridine (4-AP; 2–5 mM) blocked both inward and outward current, suggesting the inward current was reversed $I_A$ rather than an unmasked transient calcium current as previously suggested. This conclusion was supported by increasing $[K]_i$ from 5 to 15 mM, which shifted the reversal potential positively for both inward and outward current ($+17.89 \pm 0.41$ mV; mean $\pm$ SE). Moreover, recovery from inactivation was rapid ($\tau = 15.5 \pm 4$ ms; $n = 4$), as reported for $I_A$, and both inward and outward transient current persisted in calcium-free solution ($[Ca^{2+}]/4$ mM ethylene glycol-bis(β-aminoethyl ether)-$N,N,N',N'$-tetraacetic acid; $n = 4$) and during cadmium-blockade of calcium currents ($n = 3$). Finally, the transient current was blocked by intracellular 4-AP, indicating that adequate dialysis occurred during the recordings. Thus the Cs-resistant current is a subthreshold $I_A$. We also estimated the voltage-dependence of activation ($V_{1/2} = -45.8 \pm 2$ mV, $k = 5.21 \pm 0.62$ mV, $n = 6$) and inactivation ($V_{1/2} = -59.0 \pm 2.38$ mV, $k = -5.4 \pm 0.49$ mV, $n = 3$) of this current. Computer simulations using a morphologically accurate model cell indicated that except for the extreme case of only distal A-channels and a high intracellular resistivity, our parameter estimates were good approximations. In conclusion, guinea pig LDT neurons express subthreshold A-channels that are resistant to intracellular cesium ions. This suggests that these channels differ fundamentally in their ion permeation mechanism from those previously studied. It remains to be determined if Cs’ resistance is common among brain A-channels or if this property is conferred by known A-channel subunits.

INTRODUCTION

The A current ($I_A$) is a voltage-dependent potassium current first identified in invertebrate neurons by Hagiwara et al. (1961) and subsequently characterized by Connor and Stevens (1971a) and Neher (1971). Similar potassium currents, distinguished by their rapid inactivation at depolarized membrane potentials, have since been described in neurons and cardiac muscle from a variety of invertebrate and vertebrate species (Rogawski 1985; Rudy 1988). Although similarly categorized, currents that have been called $I_A$ may have varied functional roles as they display a diversity of voltage ranges of activation and inactivation, channel kinetics, and sensitivity to neuromodulators and pharmacological antagonists (Rudy 1988). In general, $I_A$s that activate at subthreshold membrane potentials serve to bias a cell’s firing toward a pattern of widely spaced single spikes by delaying the return of the membrane potential to firing threshold after an action potential (Connor 1975; Connor and Stevens 1971b; Hille 1992a; McCormick and Huguenard 1992). Neurons of the laterodorsal tegmental nucleus (LDT) exhibit various firing patterns that depend upon the animal’s sleep-waking state (Kayama et al. 1992; Steriade et al. 1990). They are believed to initiate the transition from slow-wave sleep to rapid eye movement sleep (REM) (for review see Steriade and McCarley 1990). In vitro studies aimed at identifying the intrinsic membrane properties that contribute to the state-dependent alterations in excitability of these cells have identified a transient outward current similar to $I_A$ in the vast majority of cholinergic neurons of the guinea pig and rat LDT (Kamondi et al. 1992; Leonard and Llinás 1990). Although this current is hypothesized to contribute significantly to their firing patterns in vivo, it has not been examined in detail in LDT neurons, and thus its functional significance for these cells remains unclear.

$I_A$ has been reported to be blocked by intracellular cesium ions in various mammalian cells (Bardoni and Belluzzi 1993; Cull-Candy et al. 1989; Kamondi et al. 1992; Rennie and Ashmore 1991; Ueda et al. 1992), and this cesium-blockade has been presumed to be characteristic of A-channels, similar to most other potassium channels (Hille 1992a). However, in a recent study of excitatory synaptic currents in guinea pig LDT neurons (Sanchez and Leonard 1996), we noted a prominent transient current similar to $I_A$ that persisted despite intracellular dialysis with Cs”, thus raising the possibility that blockade by intracellular Cs” may not be a property shared by all rapidly inactivating potassium channels. As $I_A$ was reported previously to be blocked by intracellular cesium in neonatal rat LDT neurons (Kamondi et al. 1992) and because we could find no previous report of a native $I_A$ that is not blocked by intracellular cesium, we have further analyzed properties of this current in cesium-dialyzed guinea pig LDT neurons to confirm that it is an A-type potassium current. In addition, we have simulated this...
current using a morphologically realistic model cell to examine the influence of channel distribution and cable properties on our estimates of the steady-state voltage-dependence of this current under our experimental conditions.

**METHODS**

**Preparation of brain stem slices**

Coronal brain stem slices containing LDT were prepared using standard methods (Leonard and Llinás 1994). Female Hartley guinea pigs (170–290 g) were anesthetized with barbiturate (50–75 mg/kg ip) and decapitated, and a block of brain containing the pontomesencephalic junction was removed rapidly and immersed in ice-cold oxygenated Ringer. The block was mounted with cyanoacrylate (KrazyGlue) on a vibratome stage and 350-μm coronal slices were cut while the block remained submerged in cold oxygenated Ringer. Two or three slices containing LDT bilaterally were obtained from each animal. Slices were incubated in continuously oxygenated Ringer at room temperature for 1–2 h before recording.

**Solutions and drugs**

The Ringer solution for slice preparation and recording contained (in mM) 124 NaCl, 5 KCl, 1.2 NaH2PO4, 2.7 CaCl2, 1.2 MgSO4, 26 NaHCO3, and 10 glucose and was bubbled with 95% O2-5% CO2. To study currents in calcium-free Ringer, CaCl2 was replaced by 4 mM ethylene glycol-bis (N,N,N*,N*-tetraacetic acid) (EGTA). To block Ca currents, Ringer was prepared by adding CdCl2 (0.5 mM) and NiCl2 (0.5 mM) and by replacing NaH2PO4 and MgSO4 with equimolar amounts of NaCl and MgCl2, respectively, to prevent precipitation. In all voltage-clamp experiments, tetrodotoxin (TTX; 1 μM) was added to the Ringer to block fast sodium currents upon establishing the whole cell configuration. In experiments designed to measure the time course of equilibration, TTX was added to the recording solution before recording. The primary patch solution contained (in mM) 125 Cs-gluconate, 5 KCl, 2 MgCl2, 10 N-2-hydroxyethylpipеразине-N'-2-этилентриазетоновый кислота, 2 Na-ATP, 0.3 GTP, and 10 EGTA, pH = 7.2. A potassium-based patch solution used for some experiments was the same, except that Cs-gluconate was replaced with 125 mM potassium gluconate. All drugs used in these experiments were obtained from Sigma. All drugs were dissolved at high concentration in ultrafiltered and de-ionized water or recording solution, diluted in standard recording solution to a known concentration, and applied by superfusion over the slice.

**Recording**

Experiments were carried out with submerged slices continuously superfused with oxygenated recording solution at room temperature (20–22°C). The gigaseal whole cell patch-clamp technique (Blanton et al. 1989; Hammersmith et al. 1981) was used to record from neurons in the compact region of LDT (Leonard et al. 1995). The bath ground was a silver-silver chloride pellet located downstream from the slice near the outflow port of the chamber. Recording electrodes were pulled from borosilicate glass pipettes (1.5 mm OD, 0.86 mm ID; No. BF150-86-10, Sutter Instrument, Novato, CA) on a Flaming-Brown micropipette puller (Model P-87, Sutter Instrument) using a multiple-step protocol. The tips were heat-polished using a Narishige microforge (Model MF-83, Narishige Instruments, Tokyo, Japan). Electrodes filled with patch solution had resistances of 4–8 MΩ. Calculated junction potentials for each patch solution differed by only 1.5 mV, and the data were not corrected for this difference. Seal resistances were always >1 GΩ and were often >10 GΩ. Series resistances after establishing the whole cell configuration ranged from 15 to 33 MΩ.

Recordings were obtained using an Axoclamp-2A amplifier (Axon Instruments, Burlington, CA) in continuous single-electrode voltage-clamp mode with gains of 90–100 nA/mV without series resistance compensation. Data were analog low-pass filtered at 3 kHz. Series resistance was estimated at the start and at irregular intervals during each experiment by switching the amplifier to bridge mode and balancing the bridge response to a small current pulse (10–50 pA). Input resistances were estimated by measuring the current response to a small voltage step (−5 or −10 mV) from a holding potential of −60 mV. Apparent input resistances ranged from 140 to >2,000 MΩ, but were typically 400–800 MΩ at the start of the experiment.

Data were displayed simultaneously on an oscilloscope and collected by computer using the program Clampex (Axon Instruments). Clampex also controlled and implemented command current and voltage protocols. Subtraction of leakage currents was used for analyzing the transient currents as described in RESULTS. Traces shown in all figures represent raw, unsubtracted data, unless otherwise indicated. Data were analyzed off-line on a computer using Clampfit (Axon Instruments) and on a Macintosh computer using Axograph (Axon Instruments) and Igor Pro (WaveMetrics). Curve fits were done in Igor Pro, which uses a Levenberg-Marquardt algorithm for minimizing χ2. Pooled data are reported as means ± SE. Differences between means were compared by Student’s t-test using DataDesk software on a Macintosh computer.

**Computer simulation of the transient current**

The NEURON software package (Hines 1989) was used to simulate single-electrode voltage-clamp experiments on LDT neurons. A typical LDT cell that had been previously intracellularly labeled, recovered histologically, and traced (Surkis et al. 1996) using the Neuron Tracing System (Sun Technologies) (Capowski 1985) was imported into NEURON. The cell was given a uniform membrane capacitance of 1 μF/cm2 and a uniform membrane conductance of 3 × 10−5 S/cm2. The value for membrane conductance was chosen to produce an input resistance of ~250 MΩ. The A-current kinetics were modeled using a 2-ms time constant of activation and 50-ms time constant of inactivation. These values were chosen to produce a time course for current responses that was roughly comparable with that seen in the experimental data. Experimentally determined values for the voltage-dependence of activation and inactivation and reversal potential of the transient current were used in the model.

Intracellular resistivity was set at either 100, 400, or 2,000 Ω-cm. The 100 and 400 Ω-cm values were chosen as typical of the range of values reported in the literature for mammalian central neurons (Major et al. 1994; Rapp et al. 1994; Shelton 1985; Spruston and Johnston 1992), whereas the 2,000 Ω-cm case was tested because preliminary studies of the cable properties of LDT cells have indicated that these cells may have an unusually high intracellular resistivity (Surkis et al. 1995). Simulations were run with a series resistance of either 20 or 0.0001 MΩ, the former being representative of the values obtained in these experiments and the latter chosen for the purposes of comparison with an ideal voltage-clamp. Finally, the A-conductance was distributed over the model dendrites. The NEURON software package (Hines 1989) was used to simulate single-electrode voltage-clamp experiments on LDT neurons. A typical LDT cell that had been previously intracellularly labeled, recovered histologically, and traced (Surkis et al. 1996) using the Neuron Tracing System (Sun Technologies) (Capowski 1985) was imported into NEURON. The cell was given a uniform membrane capacitance of 1 μF/cm2 and a uniform membrane conductance of 3 × 10−5 S/cm2. The value for membrane conductance was chosen to produce an input resistance of ~250 MΩ. The A-current kinetics were modeled using a 2-ms time constant of activation and 50-ms time constant of inactivation. These values were chosen to produce a time course for current responses that was roughly comparable with that seen in the experimental data. Experimentally determined values for the voltage-dependence of activation and inactivation and reversal potential of the transient current were used in the model.

Intracellular resistivity was set at either 100, 400, or 2,000 Ω-cm. The 100 and 400 Ω-cm values were chosen as typical of the range of values reported in the literature for mammalian central neurons (Major et al. 1994; Rapp et al. 1994; Shelton 1985; Spruston and Johnston 1992), whereas the 2,000 Ω-cm case was tested because preliminary studies of the cable properties of LDT cells have indicated that these cells may have an unusually high intracellular resistivity (Surkis et al. 1995). Simulations were run with a series resistance of either 20 or 0.0001 MΩ, the former being representative of the values obtained in these experiments and the latter chosen for the purposes of comparison with an ideal voltage-clamp. Finally, the A-conductance was distributed over the model cell in one of five configurations: uniformly over the whole cell; in the soma only; or in the distal, proximal, or middle dendrites only. Distal dendritic segments were defined as those that initiated at a distance of >70 μm from the soma, middle dendritic segments as those that initiated between 30 and 70 μm from the soma, and proximal dendrites as those that initiated <30 μm from the soma.

**RESULTS**

Previous studies of the guinea pig LDT and pedunculopontine tegmental nucleus using sharp microelectrodes have
found that the majority of neurons exhibit subthreshold voltage-responses indicative of an early, transient outward current termed $I_A$ (Leonard and Llinás 1990, 1994; Sanchez and Leonard 1994). Such characteristic voltage-responses also were observed in the majority of LDT neurons (15/16) recorded under current-clamp conditions in this study with patch pipettes containing potassium gluconate. The influence of such a transient outward current was clearly manifest in the membrane potential trajectory after the termination of brief hyperpolarizing current pulses. Under these conditions, a characteristic “anode-break hyperpolarization” occurred, prolonging the decay of membrane potential back toward baseline (Fig. 1A). Functionally, this current strongly shapes the subthreshold and firing behavior of these neurons. For example, membrane depolarization elicited from potentials negative to rest, activated the current, which then delayed the onset of spiking (Fig. 1B). In the following experiments, we have investigated the current underlying this subthreshold membrane behavior of guinea pig LDT neurons.

\textbf{Identification of transient currents}

Voltage-clamp data were obtained from 20 LDT neurons recorded with the potassium gluconate pipette solution and 42 LDT neurons recorded with the cesium gluconate solution. A transient current was elicited in both groups of cells by voltage steps positive to $-50$ mV after a hyperpolarizing voltage command (typically to $-90$ or $-100$ mV for 1 s). Figure 2 shows representative recordings from a cell of each group. The traces in A were elicited by stepping to a series of test potentials to demonstrate the voltage-dependence of activation, and those in B were elicited by stepping to a fixed test potential after a 1-s prepulse of variable amplitude to demonstrate the voltage-dependence of inactivation removal of the transient current. The transient outward current evoked in cesium-loaded cells by voltage steps positive to $-35$ mV was qualitatively similar to that in cells recorded with potassium gluconate (Fig. 2A, 1 and 2), and required prior hyperpolarization to remove inactivation (Fig. 2B, 1 and 2). However, in Cs-loaded cells, the transient current was inward for test potentials negative to $-35$ mV. Nevertheless, a plot of the normalized current-voltage relation for the transient current in these two cells showed the voltage-dependence of activation was nearly identical (Fig. 2A3). The relation between $I/I_{\text{max}}$ of the transient current and the prepulse potential for both cells was also similar (Fig. 2B3).

These data suggested that a similar conductance was activated in both cells even though the current was inward between $-50$ and $-35$ mV for the Cs-dialyzed neuron. This was also suggested by the prevalence of these transient currents among the recorded cells. A transient outward current was observed in 85% (17/20) of the cells recorded using potassium gluconate, whereas 79% (33/42) of cells recorded with cesium gluconate exhibited a transient inward current that became outward positive to about $-35$ mV.

To determine if the appearance of the transient inward current was related to dialysis of the cells by Cs$^+$, we measured the time course of equilibration of the currents in individual cells after breakthrough with pipettes containing cesium gluconate (Fig. 3). A family of depolarizing voltage steps delivered within 2 min after establishing the whole cell configuration evoked transient outward current but no inward current for steps positive to $-50$ mV (Fig. 3A, left). After 18 min of dialysis, however, the same family of voltage steps elicited a transient inward current at test potentials positive to $-50$ mV. This transient inward current then decreased and reversed for potentials between $-30$ and $-35$ mV and grew outward at more depolarized potentials (Fig. 3A, middle). After 20 min, the currents had stabilized (Fig. 3A, right). A similar pattern was observed for the transient currents that were elicited at the end of the protocol during voltage steps back to the holding potential of $-40$ mV, which followed the test pulses (Fig. 3A, right). These currents, which were produced by the removal of inactivation during the most negative of the test pulses, were initially outward but became inward concurrently with the appearance of transient inward currents during the test pulses (Fig. 3A, middle and right). The time course of the emergence of these transient inward currents also matched the time course of the decrease in the peak outward current, which was tracked with higher temporal resolution (Fig. 3B). These data were fit well with a single exponential having a time constant of $\sim 6$ min, indicating that the exchange of Cs$^+$ for K$^+$ was $\sim 95\%$ complete within 20 min.

The change in the $I$-$V$ relation produced by Cs$^+$ dialysis is summarized in the leak-corrected $I$-$V$ plots obtained just after breakthrough and after 28 min of dialysis (Fig. 3C). These curves were qualitatively similar to those obtained from the two groups of cells recorded with potassium and cesium-patch solutions and support the conclusion that the transient currents were generated by the same conductance before and after Cs$^+$...
dialysis because the threshold for activation and the slope conductances between −35 and −20 mV were virtually identical.

Because a positive shift of the potassium equilibrium potential would be expected with substitution of Cs⁺ for intracellular K⁺, we hypothesized that the transient inward current was a reversed A-type potassium current. However, we could not immediately exclude a possible contribution of other currents, particularly, given that a low-voltage–activated (T-type) calcium current, with an overlapping voltage-dependence to the transient current in those cells resulted entirely from the peak transient outward current in every cell tested (4-AP), an A-channel blocker with no known effect on calcium channels, would be expected to block the outward transient current while leaving the inward transient current intact. For cesium-dialyzed LDT cells recorded in our standard Ringer, superfusion of 4-AP (2–5 mM) reduced the peak transient outward current in every cell tested (n = 10) and reduced the inward current in 9 of 10 cells (Fig. 4). In addition, Thompson (1977, 1982) has previously shown that the blockade of IA by 4-AP is state-dependent and relieved in a voltage-dependent manner. This effect is manifest as a reduction in the early transient current and an emergence of a later, more slowly inactivating component. As can be seen in Fig. 4, evidence for such voltage-dependent unblocking was observed for both the inward and outward transient currents after the application of 3 mM 4-AP to the slice. This concentration of 4-AP inhibited the early inward and outward currents and introduced a late, slowly inactivating component that was larger at depolarized levels. This action of 4-AP was observed easily as a “crossover” in the current traces obtained before and during 4-AP application (Fig 4, right). Thus the action of 4-AP on the transient, Cs⁺-resistant current was consistent with previously described actions of this compound on IA.

Superfusion of lower concentrations of 4-AP (50–100 µM; n = 2) or tetraethylammonium (TEA, 20–40 mM; n = 7) had no effect on the fast transient currents. In only one cell (of 10), a larger transient inward current was observed in the presence of 4-AP at the more positive activation potentials (data not shown), consistent with activation of a low-threshold calcium current in addition to IA. Moreover, for six cells exposed to 5 mM 4-AP, both the inward and outward transient currents were abolished, suggesting that the transient current in those cells resulted entirely from the activation of IA. Thus these data support our initial interpretation that the inward transient current is carried through A-channels rather than T-type calcium channels.
Reversal potential

As the contribution of a T current could be excluded in these six cells, we chose these to estimate the reversal potential of the transient current. Under our recording condition of equimolar (5 mM) internal and external K\(^+\), a pure potassium current would be expected to reverse at 0 mV. However, we estimated the mean reversal potential of the transient current to be \(-34.7 \pm 2.6\) mV (n = 6). The outward transient current seen at potentials negative to the presumed E\(_K\) suggested that Cs\(^+\) ions were permeating the A channels.

To confirm that the 4-AP-sensitive conductance in these cesium-dialyzed LDT neurons was yet selective for K\(^+\), we analyzed the tail-currents evoked during activation of the transient current in two concentrations of extracellular potassium ([K\(^+\)]\(_i\)). Data for one cell in which the tail-currents were measured after activation of both inward (activation potential = \(-50\) mV) and outward (activation potential = \(-10\) mV) transient currents are shown in Fig. 5, A and B. The reversal potentials for the transient currents were extrapolated from straight line fits to the I-V curves for the tail currents (Fig 5C). If the activated conductance were primarily selective for K\(^+\), increasing [K\(^+\)]\(_i\) from 5 to 15 mM would be expected to shift the reversal potential to a more positive value. Indeed, the data revealed a positive shift in reversal potential by 17.89 ± 0.41 mV (n = 3). Although the shift in reversal potential was less than that predicted from the Nernst equation for a purely potassium-selective conductance (+27 mV), such a significant shift suggested nonetheless that the activated conductance was highly selective for K\(^+\). This shift was similar for tail currents measured following activation steps to potentials at

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** Transient outward currents shift inward after breakthrough with Cs-glucamine patch solutions. A: transient currents were evoked by an activation voltage protocol (inset). Membrane potential was held at \(-90\) mV for 1 s before a 400 ms test pulse that varied in amplitude between \(-70\) and \(-20\) mV in 5-mV steps. Currents are displayed during the period from just before the test pulse until the end of the protocol. Left: data were obtained within 2 min after establishing the whole cell configuration and all transient currents were outward. Center and right: data were obtained by 17.8 and 28.75 min after breakthrough, respectively. Transient outward current decreased concurrently with the emergence of transient inward current. Inward shift of the transient currents was nearly complete by 17.8 min of dialysis suggesting dialysis was nearly complete. This shift is consistent with a positive shift in the potassium equilibrium potential. Arrows indicate the current resulting from voltage jumps to the holding potential of \(-40\) mV after removal of inactivation during the most negative test pulses. These currents also become inward during dialysis and are consistent with a reversed \(I_h\). B: time course of the dialysis was tracked by measuring the peak outward current evoked by a voltage step to \(-25\) mV after a 1-s prepulse to \(-90\) mV delivered every 10 s in 2 cells. Slow phase of the decay was well fit with a single exponential having a time constant of \(~6\) min, indicating that dialysis was complete in \(~20\) min. C: leak-corrected current-voltage relations for data obtained at 1.88 min and at 28.75 min after breakthrough. Raw currents were leak-corrected by subtracting currents evoked by identical test pulses without a negative prepulse. Voltage dependence of the current before and after dialysis was nearly identical in threshold and slope conductance between \(-35\) and \(-20\) mV, suggesting the same channels were responsible for both the inward and outward current.

![Figure 4](http://jn.physiology.org/)

**FIG. 4.** Application of 4-aminopyridine (4-AP; 3 mM) inhibited the transient component of both the outward and inward voltage-dependent currents. Protocol (inset) consisted of hyperpolarizing prepulses to \(-100\) mV for 1 s followed by depolarizing pulses ranging from \(-65\) to \(-25\) mV in 5-mV increments. In addition to inhibiting the transient current, 4-AP (<5 mM) also increased the late outward and inward current. This “crossover” is shown for currents evoked by test pulses to \(-45\) and \(-35\) mV (right). Control recordings (——) are superimposed on recordings during 4-AP application (····). This behavior is characteristic of the voltage-dependent unblocking of A current (\(I_h\)) by 4-AP and becomes more prominent at depolarized test potentials. See text for the details.
Fig. 5. Reversal potential of the transient current shifts with changes in extracellular potassium and cesium. Reversal potential for the transient current was measured by analyzing tail currents produced by a protocol consisting of a prepulse to $-100 \text{ mV}$ for 1 s to remove inactivation followed by a 40-ms step to $-10 \text{ mV}$ to activate the outward current (top) or to $-50 \text{ mV}$ to activate the inward current (bottom) and then stepping to a series of potentials from $-50$ to $-10$ or $-15 \text{ mV}$ in 5-mV increments. Tail currents were elicited in this manner first in the standard Ringer containing 5 mM $[\text{K}^-]_o$ (A) and then in 15 mM $[\text{K}^-]_o$ (B). I-V curves for the tail-currents shown for this cell (C) showed a positive shift in the reversal potential for both the inward and outward transient currents after increasing $[\text{K}^-]_o$, further suggesting that both currents were carried by potassium. Reversal potential of the transient current also shifted to more positive values upon increasing $[\text{Cs}^-]_o$ to 5 mM. Outward $I_o$ from another cell was elicited by a step to $-20 \text{ mV}$ for 40 ms after a 1-s prepulse to $-90 \text{ mV}$ to remove inactivation. After activation, tails were evoked by a variable test step ranging from $-50$ to $-15 \text{ mV}$ in 5-mV steps. Currents illustrated in D and E are leak-corrected by subtracting currents evoked with the same protocol but without the prepulse from the raw currents. Holding potential was $-40 \text{ mV}$. Currents in D were recorded in normal solution, and those in E were recorded in solution containing 5 mM $\text{Cs}^+$. D and E: $\rightarrow$ 0 current. Extracellular $\text{Cs}^+$ reduced the leakage current as indicated by the reduced current during the prepulse in E and shifted the tail currents inward. Tail current amplitudes are plotted as a function of voltage for normal- and $\text{Cs}^+$-containing solutions in F demonstrating that the reversal potential shifted positively by 8.2 mV. Collectively, these data indicate the channels underlying the transient current are permeable to both potassium and cesium.

which the current was inward or outward, further suggesting the activation of a single conductance.

To confirm that $\text{Cs}^+$ ions permeated the channels, we measured tail currents before and after adding 5 mM $\text{Cs}^+$ to the extracellular solution (Fig. 5, D and E). Although the addition of cesium immediately reduced the leakage current flowing during the prepulse to $-90 \text{ mV}$ (Fig. 5, D and E), it did not block the transient current. Rather, the tail currents shifted inwardly and the reversal potential shifted positively, as expected for a permeant ion (Fig. 5F). Increasing extracellular cesium to 5 mM resulted in an average change in reversal potential of $7.25 \pm 0.47 \text{ mV}$ ($n = 3$), which suggested the channels are substantially permeable to $\text{Cs}^+$. Assuming they are only permeable to $\text{Cs}^+$ and $\text{K}^+$ and that these ions permeate independently, we estimated the permeability ratio ($P_{\text{Cs}^+}/P_{\text{K}^+}$) to be 0.33 using the Goldman-Hodg-
The removal of inactivation upon hyperpolarization is rapid compared to T currents, having a time constant in the range of 12–20 ms (Kamondi et al. 1992). We investigated this property of the cesium-resistant current in guinea pig LDT cells using voltage protocols that consisted of hyperpolarizing prepulses of increasing duration followed by a fixed depolarizing test step. Figure 6 shows data from two cells, one recorded with potassium gluconate and the other with cesium gluconate. The fraction of peak current as a function of prepulse duration is shown in Fig. 6C for these two cells (peak current was taken to be the peak current elicited with a 500-ms or 1-s prepulse), and single exponential fits to the data (2 solid lines) showed that the time course of inactivation removal was similar under these two conditions. The mean time constant for cesium-dialyzed cells was 15.5 ± 4 ms (n = 4), which is very similar to that reported for IA in neonatal rat LDT cells (Kamondi et al. 1992). Additionally, this time constant was not significantly different when the test potential was negative enough to evoke inward transient currents (mean = 29.6 ms, n = 3, P > 0.1), further consistent with the activation of an inward IA.

Transient current in the absence of calcium currents

To examine properties of the A-like current in isolation, we recorded from cesium-dialyzed LDT neurons in Ringer containing no added Ca2+, 4 mM EGTA, and 20 mM TEA (to block other potassium currents). These data demonstrated that neither the outward nor the inward transient currents were blocked under this condition suggesting a negligible contribution of Ca2+ currents (n = 4; Fig. 7A). As expected for an IA, the transient current isolated in this manner was blocked by 4-5 mM 4-AP in each cell tested (n = 3; Fig. 7A). These data strongly supported our hypothesis that the inward transient current was purely IA under this condition. Current-clamp recordings under these conditions further showed a transient rebound excitation after hyperpolarizing current pulses when the cell membrane potential held near −50 mV. This was expected from the measured voltage-dependence of activation, and the underlying current was again confirmed to be 4-AP sensitive (Fig. 7B). These data initially indicated that IA could be examined in isolation under these conditions. However, analysis of the voltage-dependence of activation showed a negative shift in the conductance-voltage curve (Fig. 7C). The solid lines in Fig. 7C are Boltzmann equations fitted to the normalized conductance-voltage plot for this cell in the control and Ca2+-free solutions (see figure legend for equation). Parameters obtained from Boltzmann fits indicated that the membrane potential at which activation was half-maximal was shifted on average by −10.6 mV (n = 3) in Ca2+-free Ringer. This result was not surprising as such an effect of extracellular calcium removal has been known since the demonstration by Frankenhaeuser and Hodgkin (1957) that reducing extracellular calcium shifted the thresholds for activation of Na+ and K+ conductances in squid axon to more negative values. Indeed, the effects of surface charge screening by divalent ions on voltage-gated channels have been well documented (D’Arrigo 1973; Frankenhaeuser and Hodgkin 1957; Gilbert and Ehrenstein 1969; Hille et al. 1975; McLaughlin et al. 1971).

Mayer and Sugiyama (1988) previously reported that the voltage-dependence of activation for IA in cultured rat dorsal root ganglion neurons was shifted positively by increasing the extracellular concentration of Ca2+. Although these authors did not report such a large negative shift in Ca2+-free solution, the direction of this shift in our cells is consistent with such a dependence of IA on extracellular divalent cations, and the large magnitude of the shift may have been
FIG. 7. Transient current in Cs\(^+\)-dialyzed neurons was not blocked after removal of extracellular calcium or the blockade of calcium channels with cadmium. A: raw currents in response to a standard activation protocol are shown for 1 cell in control Ringer, in Ringer containing 0 mM calcium, 4 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 20 mM tetraethylammonium (TEA) and in the calcium-free solution with 5 mM 4-AP added. Rather than decrease, the inward current increased in the calcium-free and TEA-containing solution. Application of 5 mM 4-AP completely abolished both the inward and outward currents. B: current-clamp recordings from the same cell under the calcium-free condition showed a rebound transient depolarization evoked by protocols expected to elicit an inward \(I_A\). Rebound excitation was also blocked by 4-AP. C: Ca\(^{2+}\)-free condition produced a negative shift in the voltage-dependence of activation as indicated in normalized conductance-voltage curves. Conductance was measured as the chord conductance from the reversal potential of the transient current. C: fitted Boltzmann functions of the form \(G/G_{\text{max}} = 1/1 + \exp\left[\left(V - V_{1/2}\right)/k\right]\), where \(G\) is conductance, \(V_{1/2}\) is the potential at which the conductance is half-maximum, and \(k\) is a factor inversely proportional to the maximum slope of the activation curve. D: raw currents elicited with an activation protocol (bottom right) after Cs\(^+\) dialysis in a standard extracellular solution (control) and after superfusion with that solution containing CdCl\(_2\) (0.5 mM Cd\(^{2+}\)) was performed to rule out the possibility that the transient inward current resulted from current flow through Ca channels. In control conditions, breakaway Ca spikes and large tail currents were evoked by voltage pulses to +5 mV (Fig. 7D, right). Superfusion with a solution containing 0.5 mM Cd\(^{2+}\), abolished the high-voltage-activated Ca\(^{2+}\) currents but did not block the transient inward current (Fig. 7D, right) supporting the conclusions that the transient inward current was indeed reversed \(I_A\) and that \(I_A\) was not blocked by intracellular cesium ions.

Intracellular dialysis with 4-AP blocked \(I_A\)

To verify that the observed insensitivity of the transient current to intracellular cesium was not simply due to inadequate dialysis of the recorded neurons, we attempted to block the current from the inside using 4-AP (Thompson 1982). We reasoned that if the A current was insensitive to intracellular Cs\(^+\) because of poor solution exchange, then intracellular application of 4-AP also should be ineffective in blocking the Ca\(^{2+}\) currents. We tested this by comparing leak-corrected \(I_A\)s obtained with a potassium gluconate pipette solution (\(n = 5\)) to those obtained with a 4-AP-containing potassium gluconate pipette solution (10 mM 4-AP; \(n = 4\)). All currents for this comparison were recorded in an extracellular solution containing 0.5 mM Cd\(^{2+}\) and Ni\(^{2+}\) to block calcium currents. First we examined the effects of extracellular 4-AP (5 mM) on \(I_A\) under these conditions (Fig. 8A). Extra-
FIG. 8. Transient current was blocked by both extracellular and intracellular solutions containing 4-AP. A: extracellular 4-AP blocked by depressing the early component and by augmenting the late component of the current. A1: leak-corrected control currents recorded with the KGluc patch solution. Currents were evoked with test pulses (−70 to −5 mV in 5-mV increments) after a 1-s prepulse to −90 mV (see inset). Leak-correction was accomplished by subtracting currents evoked with identical test pulses but after a 1-s prepulse to −20 mV to inactivate the transient component. Extracellular solution contained 0.5 mM Cd²⁺ and 0.5 mM Ni²⁺ in this and all subsequent panels. A2: currents before and during wash-in of the extracellular solution containing 5 mM 4-AP. Currents were evoked by a test pulse to −10 mV after a 1-s prepulse to −90 mV. Note the characteristic “crossover” in the current records as 4-AP blocked the current (Thompson 1982). A3: leak-corrected during 4-AP blockade. Note the decrease in the difference between the early and late components compared with control (A1). B: intracellular application of 4-AP also blocked the transient current. Leak-corrected currents recorded from a different cell with a KGluc pipette solution containing 4-AP (10 mM). Note the smaller difference between the early and late components compared to control recordings (A1). C: summary indicating that neurons recorded with 4-AP in the pipette solution (10–25 mM) had smaller transient-currents than controls. Means ± SEs for transient current vs. test potential are illustrated for cells recorded with control solution (●; n = 5), with 10 mM 4-AP in the patch solution (▲; n = 3) and with 5 mM 4-AP in the extracellular solution (▲; n = 3). After either the intracellular or extracellular application of 4-AP, the transient current was significantly smaller (P < 0.05) than controls at each potential. There were no significant differences (P > 0.1) between the 4-AP groups at any potential. Thus dialysis was adequate to effectively depress Iₐ in LDT neurons. Transient currents were measured as the difference between the early and late components in the leak-corrected records. Calibration bars in A3 apply to A and B.

Cellular 4-AP depressed control currents (Fig. 8A1) by suppressing the early phase and by augmenting the later phase of the current (Fig. 8A3). This resulted in the characteristic “crossover” in the current traces (Fig. 8A2) as the Iₐ became progressively more blocked by 4-AP (Thompson 1982). After ~5 min of 4-AP application, the leak-corrected currents (Fig. 8A3) reached their steady-state values. The blocked currents were smaller than controls near the onset of the test pulse but were larger than controls by the end of the test pulse (cf. Fig. 8A, 3 to 1). Indeed at this concentration of 4-AP, the rapidly decaying transient current was abolished leaving only a much more slowly decaying late component.

To test if 4-AP also blocked the current from the inside, recordings were made with a pipette solution containing 4-AP. Under these conditions, the leak-corrected currents were small and appeared remarkably similar to those currents obtained after extracellular application of 4-AP (Fig. 8B). Indeed, subtraction currents from three of the four cells dialyzed with 4-AP had early currents that were smaller and late currents that were larger than controls. The fourth cell displayed no evidence of a transient current and was ex-
cluded from further analysis. Inhibited I_A currents were observed at even the earliest times after establishing a whole cell recording (within ~3 min), indicating that intracellular dialysis with this concentration of 4-AP rapidly blocked the transient current. These data are summarized in Fig. 8C, which compares the magnitude of the transient currents in control recordings (○; n = 5) to those obtained with 4-AP applied intracellularly (▲; n = 3) and extracellularly (△; n = 3). At each potential measured, the transient current was significantly smaller (P < 0.05) in the presence of 4-AP. Moreover, there was no significant difference (P > 0.1), at any potential studied, between the inhibition produced by extracellularly and intracellularly applied 4-AP. These data indicate that the I_A in LDT neurons is blocked effectively by intracellular application of 4-AP and that dialysis was adequate for intracellular blockade. Hence the resistance of the I_A to blockade by intracellular cesium did not arise from inadequate dialysis of the cells.

Voltage-dependence of I_A in cesium-dialyzed cells

Because the voltage-dependence of activation was sensitive to divalents, we estimated the steady-state voltage-dependence of activation and inactivation for this current in control Ringer (n = 6). We chose only those cells for which 4-AP was applied and blocked the inward current for this analysis to minimize any possible contamination by low voltage-activated calcium currents. The protocols used were the same as those described in Fig. 2. The amplitude of the transient current was measured by subtracting the responses to the same activation potentials after a 1-s prepulse to −40 mV (to completely inactivate the current). Simple leak subtraction of the passive current response (estimated from the apparent input resistance) gave similar measurements of the peak transient current, suggesting that in these cells, other voltage-dependent currents did not significantly activate within the time to peak of the transient current. For this reason, leak-subtracted measurements were used for some cells for which the subtraction protocol was not available.

Boltzmann functions (see Fig. 9 legend for equations) were fitted to the normalized conductance-voltage data for analysis of activation. Conductance was calculated as the chord conductance from the reversal potential for the transient current. The reversal potential was estimated by extrapolation of a straight-line fit of the points on the I-V curve at voltages just positive to the zero-crossing value (analysis of tail-currents in all three cells gave similar values for the reversal potential), and the mean reversal potential (as stated above) was −34.7 ± 2.6 mV (n = 6). The voltage-dependence of inactivation was analyzed by plotting the normalized peak current as a function of prepulse potential, and fitting the same Boltzmann function with I/I_{max} substituted for G/G_{max}. The parameters obtained from these fits, V_{1/2} and k, represent the membrane voltage at which the current or conductance is equal to half-maximum and a factor inversely proportional to the maximum slope of the curve, respectively.

Figure 9A shows data for a typical cell with the best-fitting Boltzmann functions superimposed (—) to show the goodness of fit, and Fig. 9B shows averaged data. The superimposed solid lines in Fig. 9B are Boltzmann curves generated from the average parameters obtained from the individual fits for these cells. These average values were V_{1/2} = −45.8 ± 2 mV and k = 5.21 ± 0.62 mV for activation of I_A (n = 6), and V_{1/2} = −59.0 ± 2.38 mV and k = −5.4 ± 0.49 mV for inactivation (n = 3). The overlap of the activation and inactivation curves indicated the presence of a “window” current, which peaked at potentials slightly positive to normal rest for these cells. This suggested that I_A contributes more to the membrane behavior at subthreshold potentials than had been previously thought (see Kamondi et al. 1992).

Simulation of the I_A in a typical LDT neuron

Data from whole cell voltage-clamp experiments are subject to numerous sources of error, particularly in cells with extended dendritic processes as is the case for guinea pig LDT neurons (Surkis et al. 1996). We therefore sought to determine the sensitivity of our measured activation and inactivation parameters to the cable structure of the neuron.

![Figure 9](http://jn.physiology.org/)

**Figure 9.** Activation and inactivation characteristics of I_A in cesium-loaded LDT neurons. A: data for a single cell showing the voltage-dependence of the activated conductance (●) and inactivated current (○). Best-fitting Boltzmann functions of the form \( G_{max} / (G_{max} - G_{rest}) = 1 / [1 + \exp((V - V_{1/2})/k)] \) for the activation curve and \( I/I_{max} = 1 / [1 + \exp((V - V_{1/2})/k)] \) for the inactivation curve. Fitted parameter \( V_{1/2} \) gives the potential at which the conductance is half-maximum, and \( k \) is a factor inversely proportional to the maximum slope of the curve. B: Boltzmann curves for activation and inactivation generated from the mean parameters obtained from fits to the data from individual cells, indicating a window current at subthreshold membrane potentials.
and to nonnegligible access resistances. Our approach was to simulate the $I_a$ using the average steady-state activation and inactivation parameters determined from the whole cell recordings and to vary the access resistance, channel distribution and intracellular resistivity in a compartment model based on a morphologically realistic cell (see METHODS; Fig. 10A). For each case, we estimated the error in our measured parameters by comparing the actual activation and inactivation parameters to those recovered from the model in response to a simulated somatic whole cell clamp. The simulation voltage protocols were largely the same as our experimental protocols and resulted in transient currents that were qualitatively similar to our experimental currents (Fig. 10, B and C). The reversal potential for simulated $I_a$ was determined by linear extrapolation of the points just above the zero-crossing value on the $I$-$V$ curve. To recover the voltage-dependence of activation and inactivation from these simulated data, chord-conductance was computed as a function of voltage, and a Boltzmann function was fit using the same procedure as for the experimental data.

Results from the simulations demonstrated that errors were introduced in the process of activation and inactivation parameter recovery both by nonnegligible access resistance and by imperfect voltage clamp due to the cable structure of the cell (Fig. 11). For both activation and inactivation, the errors introduced resulted in a negative shift in the recovered value of $V_{1/2}$, with the shift in $V_{1/2}$ for activation significantly larger than that for inactivation. The error in recovery of $V_{1/2}$ of inactivation was typically small (a few millivolts or less), except in the case where the intracellular resistivity was high (2,000 Ω-cm), and the channels were only present in the more distal dendritic processes. In contrast, the recovered $V_{1/2}$ of activation was shifted negatively by ~10 mV, or more in the case of high intracellular resistivity and distal dendritic distribution of channels.

The recovered values for $k$ (slope factor) of inactivation were also seen to shift to more negative values. For the 20 MΩ access resistance case, this shift was ~10% for intracellular resistivities of 100 or 400 Ω-cm, but could be much larger for the 2,000 Ω-cm case. The errors in the recovered values of the $k$ for activation were more complicated. Although these errors were typically smaller than those for $k$ of inactivation, this appeared to result from an offset of the errors due to access resistance and cable structure by errors due to imperfect determination of the reversal potential.

In general, errors introduced by increased access resistance were larger than errors due to the cable structure of the neuron for intracellular resistivities of 100 and 400 Ω-cm. However, for the case of more distal channel distributions and high intracellular resistivity (2,000 Ω-cm), the cable structure could produce large errors in the recovered voltage-dependence.

Figure 12 shows the Boltzmann curves that were obtained upon fitting the experimental data, along with two sets of corrected Boltzmann curves. The corrected Boltzmann curves were estimated by subtracting the errors in
FIG. 11. $V_{1/2}$ and $k$ of activation ($A$ and $B$) and inactivation ($C$ and $D$) recovered from each simulation were compared to experimentally determined values. $\circ$, average experimentally determined parameter used to simulate the $I_A$. $\bullet$, $\triangle$, and $\square$, values for each parameter recovered from a simulated somatic whole cell clamp having negligible access resistance; $\blacklozenge$, $\blacktriangle$, and $\blacksquare$, values resulting from an access resistance of 20 M$\Omega$. $A$: $V_{1/2}$ of activation was recovered more accurately when access resistance is low and when channels were close to or in the soma as would be expected. $B$: errors in $k$ of activation were small and distributed around the experimentally determined $k$. $C$: $V_{1/2}$ of inactivation follows the same pattern of errors as $V_{1/2}$ of activation ($A$) but with smaller errors. $D$: errors in $k$ of inactivation were small except for the most extreme cases of a very high intracellular resistivity and with channels distal to the soma. Recovered $V_{1/2}$ and $k$ of inactivation were reasonably accurate except in the most extreme cases of a very high intracellular resistivity and channels distal to the soma.

$V_{1/2}$ and $k$ from their experimentally determined values. This estimate for correcting the Boltzmann curves assumed that the magnitude of the detected errors is not strongly voltage-dependent, so that the errors in detecting $V_{1/2}$ and $k$ are not strongly affected if the underlying voltage-dependence is shifted by $\pm 20$ mV. The two corrected cases shown are a 400 $\Omega$-cm intracellular resistivity and uniform channel distribution throughout the cell or a 2,000 $\Omega$-cm intracellular resistivity and channels in the distal dendrites only, both with 20 M$\Omega$ access resistance. In both cases, a nonnegligible window current was still evident, although the magnitude was less than that indicated by the experimentally obtained Boltzmann curves, and the range in which the window current would be active was shifted to a more depolarized potential.

**DISCUSSION**

The data reported in this study demonstrate that guinea pig LDT neurons exhibit a subthreshold A-type potassium current that has the novel feature of not being blocked by intracellular cesium. Replacement of intracellular potassium with cesium shifted the reversal potential for this current toward a more positive value, resulting in both inward and outward transient currents. However, the reversal potential did not reach 0 mV under conditions of nominally equimolar intra- and extracellular potassium, suggesting that cesium ions also permeated the channels under this condition. As expected for an $I_A$ (Thompson 1982), the transient current was blocked by millimolar concentrations of 4-AP when applied externally or internally. Although it is generally as-
sumed that 4-AP blocks \( I_A \) from the inside, we have found no previous demonstration of an intracellular action of 4-AP on \( I_A \) in mammalian central neurons. Moreover, the \( I_A \) in LDT neurons showed clear evidence of a voltage-dependent reduction of the 4-AP block. This was described first in molluscan neurons (Thompson 1982) and is characteristic of \( I_A \) in cardiac myocytes (Castle and Slawsky 1993; Tseng et al. 1996) but does not appear to have been illustrated previously for mammalian central neurons.

A similar voltage-dependent and 4-AP-sensitive transient potassium current called \( I_A \) has been observed in hippocampal neurons, but this current was blocked by externally applied 4-AP at micromolar concentrations (Storm 1988). The \( I_A \) observed in LDT was unaffected by the external application of 100 \( \mu \)M 4-AP. Additionally, \( I_A \) required seconds of hyperpolarization to significantly remove steady-state inactivation. The mean time constant for inactivation removal of 15.5 ms observed for the transient current in LDT neurons distinguished it from both \( I_A \) and the T-type calcium current.

The reversal potential for the transient current was sensitive to changes in the extracellular potassium concentration, further confirming that the activated conductance was highly selective for potassium. Tail current measurements indicated that the channels have a relatively high permeability to \( \text{Cs}^- \) \((P_{\text{Cs}}/P_K = 0.33)\) compared to that reported for snail neurons \((0.14)\) (Taylor 1987). Additionally, this current was not blocked by the removal of calcium from the extracellular solution or by blocking \( \text{Ca}^{2+} \) currents with extracellular \( \text{Cd}^{2+} \). Taken together, these data indicate that the majority of LDT neurons in the guinea pig exhibit an A-type potassium current that shares properties with other identified \( I_A \)s but is not blocked by intracellular cesium.

Considering that \( I_A \) was shown to become inward at some potentials in our cesium-loaded cells, it is possible that an unblocked inward \( I_A \) was mistakenly identified as \( I_T \) in the previous study on neonatal rat LDT neurons (Kamondi et al. 1992). The range of activation potentials reported in that study (shown for 1 cesium-dialyzed cell) did not go positive to \(-35 \text{ mV}\), which was near the reversal potential observed for \( I_A \) in our experiments. Griguer et al. (1993) reported a novel potassium current in guinea pig vestibular hair cells that was resistant to internal cesium block, and that displayed a similar positive shift in the reversal potential, resulting in an inward potassium current, when internal potassium was replaced with cesium. These authors concluded that the inward currents in their experiments were a mixture of potassium and calcium currents. Our data further indicate that transient potassium currents may contribute to “isolated” inward currents in cesium-dialyzed cells if no other control measures are taken to confirm that they are blocked under this condition.

**Novel \( I_A \)?**

\( I_A \) has been reported to be blocked by intracellular cesium ions in various mammalian neurons, including rat cerebellar granule cells in slices (Bardoni and Belluzzi 1993) and culture (Cull-Candy et al. 1989), cat retinal horizontal cells (Ueda et al. 1992), guinea pig vestibular hair cells (Rennie and Ashmore 1991), and neonatal rat LDT neurons (Kamondi et al. 1992). Additionally, \( I_A \) was reported to be blocked by cesium in membrane “blebs” excised from the somata of cultured hippocampal, striatal, and spinal cord neurons of embryonic rat (Rizzo and Nonner 1992). Our finding that \( I_A \) was not blocked by intracellular cesium in guinea pig LDT neurons was further unexpected because different potassium channel types are thought to be very similar in their permeation mechanism (see Hille 1992a). Nevertheless, our data demonstrate that \( I_A \) expressed in guinea pig LDT neurons are resistant to blockade by internal cesium, indicating previously undocumented diversity in the patterns of ionic selectivity among channels displaying A-type properties.

The cloning of potassium channels has provided information on the molecular structure underlying some of their functional properties, and mutation studies have revealed that small specific changes in the amino acid sequence can significantly alter their ionic selectivity (Kukuljan et al. 1995; Pongs 1992). In particular, point mutations in the fifth hydrophobic region (H5) (Yool and Schwarz 1991) and in the internal loop connecting the fourth and fifth putative membrane-spanning segments (S4–S5 loop) (Slesinger et al. 1997) have been reported to affect ionic selectivity.

**FIG. 12.** Comparison of the average experimentally determined Boltzmann curves (---) with Boltzmann curves corrected for 2 model neurons differing in channel distribution and intracellular resistivity (broken lines). One set of curves was corrected for a case having a 400 \( \Omega \)-cm intracellular resistivity and a uniform channel distribution (---). Other was corrected for the extreme case having a 2,000 \( \Omega \)-cm intracellular resistivity and channels only in distal dendritic segments (---); both cases with 20 M\( \Omega \) access resistance. Corrected Boltzmann curves were more depolarized and the magnitude of the window current was smaller than for the experimentally measured case. Decreased window current resulted from the larger errors in \( V_{\text{h}} \) of activation compared with \( V_{\text{h}} \) of inactivation.
al. 1993) dramatically altered ionic selectivity of cloned Shaker K⁺ channels with little or no effect on their kinetics or voltage-dependence. Cloned Shaker channels have been shown to conduct a measurable Cs⁺ current when overexpressed and recorded in patches under bi-ionic conditions (Heginbotham and MacKinnon 1993), and substitution of a single amino acid in a cloned mammalian delayed rectifier channel dramatically increased the cesium to potassium permeability ratio (De Biasi et al. 1993). Although we are unaware of a previous report of native Iₐ channels that are not blocked by internal cesium, this feature of Iₐ in guinea pig LDT neurons may not be surprising, given the functional diversity that can result from small changes in channel sequence and may simply indicate variation among A-type channels in a property that has not been studied in great detail.

Comparison with cloned A-type channels

Five cloned potassium channels from mammalian brain and heart are known to exhibit A-like physiological properties in heterologous expression systems (for review see Chandy and Gutman 1995), and recently, a sixth mammalian channel was cloned which has A-type properties (Serodio et al. 1996). Two of these, Kv3.3 and Kv3.4 (using the nomenclature of Chandy et al. 1991), are significantly activated only in the suprathreshold voltage range, and are blocked completely by 1 mM TEA (Rudy et al. 1991; Schroter et al. 1991; Vega-Saenz de Miera et al. 1992; Weiser et al. 1994), and thus are unlikely to comprise a major constituent of the native channels carrying Iₐ in guinea pig LDT neurons. On the other hand, Iₐ in these neurons displays voltage-dependence of activation similar to expressed Kv1.4, Kv4.1, Kv4.2, and Kv4.3 channels, as well as similar pharmacological sensitivity to those in which this was examined (Baldwin et al. 1991; Blair et al. 1991; Pak et al. 1991; Serodio et al. 1996; Tseng-Crank et al. 1990). Expressed Kv4.1 channels display an inactivation rate constant that is strongly voltage-dependent with inactivation becoming faster at more positive potentials (Blair et al. 1991). Inspection of our transient currents in calcium-free and TEA-containing Ringer indicates that the decay time constant does not strongly decrease at more positive potentials, suggesting that native LDT A channels behave more similarly to channels of the Kv4 (Shal-related) subfamily. Moreover, the effect of 4-AP on the time course of current through homomorphic Kv1.4 and 4.2 channels is different. 4-AP reduces the peak current and accelerates the decay of current through Kv1.4 channels while it reduces the peak and decelerates the decay of current through Kv4.2 channels (Tseng et al. 1996). This later effect is similar to the action of 4-AP on Iₐ in LDT neurons and further suggests that native LDT A-channels behave more similarly to the Kv4 subfamily. Further study of the cloned channel proteins may reveal that a lack of cesium blockade from the intracellular side is diagnostic of this or an as yet unidentified potassium channel subfamily or specific subunit. A preliminary study of immunoreactivity to antibodies for Kv1.4 and Kv4.2 showed no labeling of cholinergic LDT neurons (K. Semba, personal communication). Recent in situ hybridization studies of Kv4.3 (Serodio and Rudy 1997; Serodio et al. 1996) indicate that it is present in an area containing the LDT but definite cellular localization studies remain to be done. It is also possible the constituent subunits comprising A-channels in LDT neurons may have yet to be identified by cloning techniques.

Comparison with native Iₐ

Analysis of the voltage-dependence of the cesium-resistant Iₐ in guinea pig LDT neurons revealed that this current activates at membrane potentials subthreshold for firing and that the steady-state activation and inactivation curves overlap in this range, indicating a window current that peaks near −50 mV. This current is thus qualitatively similar to Iₐ recorded in various mammalian central neurons, including neurons of the thalamus (Huguenard et al. 1991), hippocampus (Segal and Barker 1984), neostriatum (Surmeier et al. 1989), and cerebellum (Bardoni and Belluzzi 1993). However, the Vₐ/₂ for activation and inactivation for these other neurons differed by 35–50 mV, while this difference was only ~14 mV for Iₐ in LDT neurons, largely due to a Vₐ/₂ for inactivation removal that is relatively positive (~59 mV). This difference resulted in a larger window current, but the peak of the window was still only ~20% of the maximum conductance because the slopes of the activation and inactivation curves were relatively steep. These results suggested that Iₐ in guinea pig LDT neurons contributes substantially to the subthreshold membrane behavior because there is a small, but significant window current and also that this current is more finely tuned to changes in membrane potential across a physiologically relevant range. Indeed, such a window current could interact with persistent sodium currents to generate the subthreshold membrane potential oscillations previously observed in a class of LDT neurons (Leonard and Kumar 1993; Leonard and Llinás 1989) and in neighboring cholinergic pedunculopontine tegmental neurons (Takakusaki and Kitai 1997).

Precise quantitative voltage-clamp measurements are not feasible with single-electrode whole cell recordings from cells with complex dendritic branching as observed for guinea pig LDT neurons (Surkis et al. 1996), and thus the voltage analysis of Iₐ in this study was subject to various sources of error. For example, at the more negative activation potentials, it was possible that the inward Iₐ served to depolarize insufficiently clamped dendritic membrane, thus regeneratively activating more Iₐ and resulting in an overestimate of the conductance at threshold for activation and a steeper rise in the activation curve or negative shift in the Vₐ/₂ for activation. We therefore simulated Iₐ to estimate the influence of imperfect voltage clamping on the accuracy of our experimental measurements. The simulations indeed indicated that sources of experimental error (dendritic filtering and series resistance) resulted in estimates of the steady-state Vₐ/₂ of activation that were as much as 10 mV more negative than the true values. However, these simulations also indicated that the estimated Vₐ/₂ for inactivation removal was relatively unaffected by experimental error. Thus while the window current in LDT neurons may have been slightly overestimated, the data accurately reflect the large degree of inactivation removal at relatively positive membrane potentials, thus indicating that more Iₐ are available to be activated.
when the steady-state membrane potential is only slightly negative to rest.

Conclusion

The experiments reported herein indicate that the transient current observed in mature guinea pig LDT neurons is indeed a potassium current with properties characteristic of those termed $I_h$, except that the channels mediating this current are not blocked by internal Cs$^+$. Additionally, the data suggest that a window $I_h$ contributes to the membrane behavior at potentials near rest. Although resistance to internal cesium blockade may not be physiologically relevant for $A$-channel function, this feature indicates a previously unidentified fundamental heterogeneity in the permeation properties of native $A$-channels. Recent progress in the cloning of voltage-gated potassium channels has provided insight into their functional structure and assembly in heterologous expression systems. An uncommon feature such as resistance to blockade by internal cesium may yet be diagnostic of a particular potassium channel gene or gene subfamily and may be correlated with other functional properties such as sensitivity to neuromodulators as has been described for some $I_h$ (Aghajanian 1985; Akins et al. 1990; Leonard and Llinás 1994).

We thank Dr. Bernardo Rudy for helpful discussions about this work and for reading an earlier version of the manuscript.

This research was supported by National Institute of Neurological Disorders and Stroke Grant NS-27881 and by National Science Foundation Grant MH-10509 to A. Surkis from the National Institute of Mental Health. Shaker K.260), Boston, MA 02115.

REFERENCES


