Characterization of Outward Currents in Neurons of the Avian Nucleus Magnocellularis

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Rathouz, Margaret and Laurence Trussell. Characterization of outward currents in neurons of the avian nucleus magnocellularis. J. Neurophysiol. 80: 2824–2835, 1998. Neurons of the nucleus magnocellularis (NM) preserve the timing of auditory signals through the convergence of a variety of voltage- and ligand-gated ion channels. To understand better how these channels interact, we have characterized the kinetics, voltage sensitivity, and pharmacology of outward currents of NM neurons in brain slices. The reversal potential (E_rev) of outward currents varied with potassium concentration as expected for currents carried by potassium. However, E_sat was consistently more positive than the Nernst potential for potassium (E_K). Deviation of E_sat from the calculated E_K most likely arose from potassium accumulation in extracellular spaces by potassium conductances active at rest and during depolarizing steps. Three outward potassium currents were studied that varied in voltage and pharmacological sensitivity. A tetrodylammonium (TEA)-sensitive, high-threshold current was activated within 1–5 ms of the onset of depolarization, with a half-maximal activation voltage (V_1/2) of ~19 mV. It was blocked partially by 4-aminopyridine (4-AP) and was the dominant ionic conductance of NM neurons. A dendrotoxin-I (DTX) and 4-AP-sensitive, low-threshold current had a V_1/2 of ~58 mV, rapid activation kinetics, and only partial inactivation, with decay time constants between 20 and 100 ms. A rapidly inactivating current was observed that was resistant to TEA and DTX and was blocked by intracellular Cs^+. The transient current was inactivated almost completely at the resting potential. The onset of inactivation was fastest at potentials negative to those that caused activation. When intracellular K^+ was replaced by Cs^+, large inward and outward currents were obtained that corresponded respectively to the above-mentioned DTX- and TEA-sensitive currents. Outward, TEA-sensitive current was carried by Cs^+, with a P_Cs/P_K of ~0.1. In current-clamped neurons, DTX induced repetitive firing and increased membrane time constant near rest but had little effect on action potential duration. These studies indicate that a low-threshold, DTX-sensitive current plays a key role in making NM neurons highly responsive to the onset and offset of synaptic stimuli.

INTRODUCTION

Auditory brain stem nuclei can extract distinct elements of acoustic signals by means of their refined circuitry and a special combination of synaptic and membrane properties (Carr 1993; Oertel 1991; Trussell 1997). In neurons of nucleus magnocellularis (NM), the avian homologues of mammalian spherical bushy cells, slight depolarization above the resting potential markedly enhances membrane conductance (Reyes et al. 1994; Zhang and Trussell 1994a). The attendant shortening in membrane time constant is proposed to allow the membrane potential to follow rapid synaptic currents accurately (Zhang and Trussell 1994a) and so generate brief, well-timed excitatory post-synaptic potentials (EPSPs). Reyes et al. (1994) and Koyano et al. (1996) have described a low threshold, 4-amino-pyridine (4-AP)-sensitive outward current in NM that may account for this property.

Mammalian bushy cells and neurons of the medial nucleus of the trapezoid body (MNTB) exhibit firing properties apparently identical to those of NM (Banks and Smith 1992; Oertel 1983). Voltage-clamp analyses of these neurons have revealed two distinct currents that differ in voltage and drug sensitivity (Brew and Forsythe 1995; Manis and Marx 1991). In MNTB, one of these currents is sensitive to low concentrations of dendrotoxin-I (DTX) and appears to be a key element in assuring a one-to-one relationship between pre- and postsynaptic firing (Brew and Forsythe 1995). We have examined NM neurons under voltage-clamp conditions to determine if this DTX-sensitive current is present, to identify what other outward currents are expressed, and to determine which aspects of these currents might be important to the firing properties of the cells.

METHODS

Electrophysiology

Recordings were made from NM neurons in brain slices (250–300 μm) taken from embryonic day 17–20 chicks (Sunnyside Hatcheries, Beaver Dam, WI). Previous studies have indicated that the chick cochlear nucleus is physiologically mature by the time of hatching (Zhang and Trussell 1994a,b); moreover, as noted below, the cellular properties we describe here are quite similar to those observed in cochlear nuclear neurons of rodents >2 wk after birth. Slices were prepared as described previously (Zhang and Trussell 1994a,b) and bathed in an oxygenated solution containing (in mM) 140 NaCl, 5 KCl, 1 MgCl_2, 3 CaCl_2, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 20 glucose, and 0.001 tetrodotoxin at pH 7.25. In experiments using 10 mM tetrathylenammonium Cl (TEA), NaCl was replaced by 10 mM to maintain osmolarity (290–320 mOsm). Patch pipettes contained one of three solutions: a high K^+ solution [which contained (in mM) 140 K-gluconate, 5 NaCl, 1 MgCl_2, 10 HEPES, 1 ethylene glycol-bis(β-aminopropyl ether))-N,N,N’,N’-tetraacetic acid (EGTA), and 1 ATP, pH to 7.25 with KOH], a reduced K^+ solution (which contained, in mM, 28 K-glucuronate, 112 N-methyl-d-glucamine acid, 1 MgCl_2, 10 HEPES, 5 NaCl, 1 EGTA, and 1 ATP, pH to 7.25 with NaOH), and a Cs^+-based solution [which contained (in mM) 70 CsSO_4, 85 sucrose, 5 bis-(o-aminophenoxy)-N,N,N’,N’-tetraacetic acid (BAPTA), and 10 HEPES, 4 NaCl, 1 MgCl_2, and 1 ATP, pH to 7.25 with CsOH]. In the reduced intracellular K^+ solution, the bath potassium also was reduced by 2.4 mM with compensation by NaCl. All reported holding potentials are corrected for liquid junction potentials measured for each experimental solution. Recordings were made at room temperature (22–24°C); currents
**TABLE 1.** Percent block of low- and high-threshold current components by TEA, 4-AP, and DTX

<table>
<thead>
<tr>
<th></th>
<th>−50 mV</th>
<th>+7 mV</th>
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</thead>
<tbody>
<tr>
<td>TEA 1 mM</td>
<td>15 ± 19</td>
<td>52 ± 18 (4)</td>
</tr>
<tr>
<td>10 mM</td>
<td>38 ± 20</td>
<td>69 ± 12 (12)</td>
</tr>
<tr>
<td>0.2–1 mM 4-AP</td>
<td>54 ± 12</td>
<td>60 ± 12 (11)</td>
</tr>
<tr>
<td>0.1 µM DTX</td>
<td>78 ± 18</td>
<td>5 ± 5 (4)</td>
</tr>
<tr>
<td>0.1 µM DTX plus 10 mM TEA</td>
<td>98 ± 10</td>
<td>91 ± 2 (6)</td>
</tr>
</tbody>
</table>

Shown is 100 × (1 − ratio of leak-subtracted current in drug and control); values are percent means ± SD. TEA, tetraethylammonium; 4-AP, 4-aminopyridine; DTX, dendrotoxin-I.

recorded at higher temperatures were significantly more difficult to voltage clamp (as explained later).

Drug solutions such as TEA or 4-AP were bath applied; using a flow rate of 3–4 ml/min, solutions equilibrated within 5 min, as determined by the stability of test responses delivered at 30-s intervals. DTX was pressure applied from a patch pipette with 2–5 psi directly to the cell body of the recorded neuron. We could not reverse fully the effects of this drug. Because NM neurons are predominantly adendritic, pressure ejection effectively exposed the entire nonaxonal membrane to DTX. Recordings were made using borosilicate patch pipettes and an Axopatch 200A amplifier (Axon Instruments). Using values read from calibrated trimpots on the Axopatch amplifier, the average membrane capacitance measured in 141 recordings was 14.0 ± 2.4 (SD) pF, whereas the average series resistance was 6.1 ± 2.7 MΩ. Series resistance was compensated by 92 ± 6%, yielding an uncompensated resistance of 502 KΩ or a 5-mV error for a 10-nA current. The output of the amplifier was filtered at 5 kHz and digitized at 10 kHz by a Digidata 1200 run by pClamp software (Axon Instruments). In some instances, a linear “leak” was removed by subtracting a line based on the slope of the current-voltage relationship between −80 and −100 mV. The resulting “leak-subtracted” current-voltage relationship then was used to measure the fraction of the conductance that was blocked under different pharmacological conditions, as shown in Table 1.

**Analysis**

Reversal potentials were determined by delivering a conditioning voltage step to +10 mV followed by a step to potentials from −104 to −24 in 10-mV increments, which generated tail currents, as shown in Fig. 2. The *I-V* relations for tail current 0.5 and 20 ms after the conditioning step ended were plotted, and the reversal potential of the current generating the tail was taken as the crossing point of the two curves. This method, which avoids the use of leak subtraction, should be valid if the leak current is linear. Activation and deactivation phases of currents were fit with single exponential curves. In some of the traces for the low-threshold current, a slight sigmoidal onset was apparent at some potentials, but a single exponential fit was used in these cases as well to describe simply the overall population of cells. To determine rate constants of channel activation (opening) and deactivation (closing), terms were adopted from a simple Hodgkin-Huxley model in which the two states are related by

\[
\text{CLOSED} \rightleftharpoons \text{OPEN}
\]

with a Hodgkin-Huxley activation variable *m* given by

\[
\frac{dm}{dt} = \frac{m_{\text{max}} - m}{\tau_m}
\]

where

\[
\tau_m = (\alpha + \beta)^{-1}
\]

Values for rates *α* and *β* then were determined from measured time constants at each voltage as follows

\[
\alpha(V) = \frac{SS(V)}{\tau(V)}
\]

\[
\beta = [1 - SS(V)]/\tau(V)
\]

where *SS(V)* is the value for *m* determined from the steady-state conductance/voltage curve obtained from tail current measurements. The peak amplitude of tails at each potential were normalized to the maximal value in each experiment and plotted against voltage. The data points were then fitted with a sum of two Boltzmann curves each having the form

\[
G/G_{\text{max}} = \frac{1}{[1 + e^{-(V - V_0)/\sigma}]},
\]

where *E* is the holding potential during the conditioning step, *V* is the potential at half-maximal activation, and *K* is a slope factor in millivolts. Once values for rate constants were determined from Eqs. 2–4, the rate-voltage relation was fitted with equations of the form

\[
\alpha(V) = \alpha_0 + e^{(E - V)/\sigma}
\]

\[
\beta(V) = \beta_0 + e^{(E - V)/\sigma}
\]

where *α₀* and *β₀* are the values at *E* = 0 mV. These expressions were then used with Eq. 1 to describe the relation between time constant and potential.

All measurements are given as means ± SD, unless otherwise indicated. Reagents were obtained from Sigma (St. Louis, MO), except for DTX (Alomone Labs, Jerusalem).

**RESULTS**

**Outward currents activated from rest**

The mean resting potential of neurons recorded with 140 mM K-glucuronate-filled electrodes was −66 ± 7 mV (*n* = 29 neurons). Neurons were stepped to a variety of potentials from a holding potential of between −65 and −60 mV, as indicated in Fig. 1A, to determine what currents were activatable from potentials near rest. Examination of the current records and current-voltage (*I-V*) relations in Fig. 1 reveal that membrane conductance increased at potentials just positive to −70 mV (Fig. 1B, →). Accordingly, steps from −60 to −110 mV showed deactivation of this conductance (Fig. 1A, inset, □). Depolarization positive to −25 mV produced a second increase in slope of the *I-V*, indicating activation of a second type of conductance (Fig. 1B, →). The magnitude of these outward currents varied between cells but averaged 7.6 ± 2.8 nA at +7 mV (*n* = 47). A third conductance increase was seen with hyperpolarizing steps more negative than −90 mV (Fig. 1A, inset, *_; Fig. 1B, inset, →). This slowly activating inward current resembled the h current observed in other auditory neurons (Banks et al., 1993), was present inconsistently (e.g., compare Figs. 3 and 4) and so will not be described in detail in this report.

To determine whether the outward currents described above are carried by K⁺, reversal potentials were measured by stepping membrane potential from −60 to +20 mV for 200–250 ms and then back to various more negative potentials. Current reversal potential was determined by the intersection of *I-V* relations obtained 0.5 and 20 ms after the step back to more negative potentials (see METHODS). This procedure gave a mean reversal potential of −65.7 ± 2.7 mV (*n* = 10 cells). In cells with both reduced internal K⁺ (28 mM, see METHODS) and reduced bath K⁺ (2.6 mM),
reversal potentials were $-39.1 \pm 7.5$ mV. For these normal and low K$^+$ solutions, the calculated Nernst potentials for K$^+$ were $-83$ and $-60$ mV, respectively. Thus reversal potentials were dependent on the K$^+$ gradient but less than expected for a “pure” K$^+$ current. Although this result may occur if the ion channels are not strongly selective for K$^+$, it also would be expected if the K$^+$ gradient was collapsed partially by K$^+$ accumulation in narrow extracellular spaces during the depolarizing voltage step. To test for this latter possibility, the reversal potential was estimated after steps to 20 mV for only 2 or 25 ms. In normal and reduced potassium solutions, these reversal potentials were, respectively, $11.7 \pm 3.6$ mV ($n = 6$) and $10.6 \pm 3.6$ mV ($n = 6$) more negative than reversal potentials after 200 and 250 ms steps. Figure 2 shows examples of these experiments in two cells with short (i) and long (ii) conditioning steps, and in high (Fig. 2A) and low (Fig. 2B) internal K$^+$ solutions, as well as the estimates made of reversal potentials (iii). The results indicate that K$^+$ may accumulate in extracellular spaces during the steps and perhaps also during normal electrical activity. Indeed, given that some current is activated even at the holding potential (Fig. 1), higher-than-expected [K$^+$] near the membrane surface could in principle be maintained at rest. Taken together, these effects would not only compromise estimates of reversal potential but also confound measurement of “inactivation” of K$^+$ currents by gradually reducing the potassium driving force during a depolarization. However, given the rough dependence of the currents on [K$^+$], and the effect of known K$^+$ channel blockers described in a later section, these outward currents are most likely carried by K$^+$. In a later section, these blockers are used to minimize the possible effects of K$^+$ accumulation on the inactivation time course.

**Pharmacology of outward current**

Reyes et al. (1994) showed that outward current in NM was reduced by 0.2 mM 4-AP and, to a lesser extent, by 10 mM TEA, but did not separate the current into distinct components based on voltage sensitivity. Koyano et al. (1996) suggested that 4-AP and TEA distinguish between distinct high- and low-threshold components of NM outward current. We have examined the sensitivity of the components of outward current to bath application of these compounds. Figure 3A shows examples of current responses from one cell in control, in 1 mM TEA, and in 0.2 mM 4-AP solutions. I-V relations (Fig. 3B) show that 1 mM TEA preferentially, but partially, blocked a higher threshold current, whereas 4-AP reduced both this and a lower threshold current. Table
1 summarizes the extent of block at −50 and +7 mV for several concentrations of 4-AP and TEA. The differences in sensitivity at the two potentials provide evidence that at least two distinct potassium currents are activated on depolarization but also that 4-AP does not selectively block the lower threshold current (see DISCUSSION), similar to observations made in mammalian bushy cells (Manis and Marx 1991). The low- and high-threshold currents will be referred to here as LTC and HTC, respectively.

Selective blockade of LTC was obtained by pressure ejection of 0.1 μM DTX directly onto the cell body. Figure 4 shows a sequence of current responses and I-V relations before and during application of 10 mM TEA and then 0.1 μM DTX plus TEA. Shown in insets are digital subtractions of currents made to isolate the components blocked by each drug, as indicated. These subtracted records, and their I-V relations (below), indicate that TEA, even at 10 mM, primarily blocks HTC. In the presence of TEA, the more transient LTC remains, which itself is removed by DTX. The differential sensitivity of LTC and HTC to 10 mM TEA and 0.1 μM DTX is expressed in Table 1 as the percent block of current at −50 mV and +7 mV. These data indicate that DTX blocks selectively the low-threshold current, that TEA is weakly active on LTC, and that 4-AP at the concentrations used here (0.2–1 mM) blocks both current components. In the presence of TEA and DTX, a small, residual current had a higher threshold than even the HTC and was not studied further (Fig. 4C).

**LTC**

Using TEA and DTX, we examined the activation and deactivation kinetics for LTC and HTC at different potentials. LTC was defined by the subtraction digital protocol shown above (current in TEA minus current in TEA plus DTX). In Fig. 5A, peak LTC currents were normalized to the current at +35 mV and averaged for six cells. We could not derive a conductance-voltage curve for LTC using tail currents in K+-filled cells because the tails in the region negative to −70 were often too small and rapid to measure accurately. However, in a later section we describe experiments in which the reversal potential was shifted sufficiently positive to resolve tail currents adequately.

The activation phase (Fig. 5A, inset) of LTC currents was fitted with an exponential curve and time constants were determined from the fits (see Fig. 5 legend) (Hodgkin and Huxley 1952; Manis and Marx 1991). Because deactivation tails were not always clearly detectable in potassium-filled cells (see preceding text), we also used deactivation tails of LTC in cells filled with 140 mM Cs+ in which outward currents were smaller but inward tails were prominent. The use of cesium for analysis of these currents is described justly in a later section. Deactivation of the current following steps from +20 mV to various more negative potentials was measured using single exponential fits. Data for activation (●) and deactivation (○ for K+, ▲ for Cs+) kinetics are shown in Fig. 5B. As described in METHODS, values for rates of opening (α) and closing (β), plotted in Fig. 5C, were calculated using these time constants and the conductance-voltage curve obtained in the Cs+ experiments presented later. Exponential fits to the calculated rates constants (Eqs. 5 and 6) provided a convenient descriptor for the relation between time constant and voltage (Eq. 1, Fig. 5B, ———). This curve had a peak at −60 mV with a value of just under 3 ms and declined to <1 ms at −20 mV. Assuming a Q10 of ≥2, it is likely that at physiological temperature (40–41°C) LTC would activate with a time constant of <1 ms at most potentials. These data indicate that LTC could become activated and deactivated by rapid voltage excursions during individual action potentials.

Inactivation of LTC was studied by fitting its decay phase with a sum of two exponential curves. The average of these fits for four neurons is shown in Fig. 6. These data show that inactivation of LTC is relatively independent of voltage, with ~25–40% inactivation observed at all potentials and with slow and fast time constants of 20 and 90 ms (Fig.
FIG. 4. Sensitivity of outward currents to 10 mM TEA and 0.1 μM dendrotoxin-I (DTX). A–C: responses from 1 cell to 250-ms steps from −96 to +14 mV in 10-mV increments. Holding potential −66 mV. Control bath solution (A) changed to indicated drug solutions in B and C. Insets: digital subtractions of panels as indicated. D: I-V’s for peak currents in A, left inset (A and B) and right inset (B and C). These latter 2 curves define the TEA-sensitive high-threshold current (HTC) and the DTX-sensitive low-threshold current (LTC), respectively.

6A) at roughly equal proportions (Fig. 6B). Thus even at the physiological voltage range at which steady depolarizations might be evident during repetitive synaptic activity (−65 to −45 mM) (Zhang and Trussell 1994b), inactivation of LTC would not likely be significant.

HTC

HTC was defined for analysis as either the current blocked by TEA (Fig. 4B, inset) or as the current remaining in the presence of 0.1 μM DTX, without TEA, (Fig. 7A, inset) for voltage steps delivered from holding potentials between −60 and −50 mV. Figure 7A shows the I-V relation for averaged peak HTC current. In addition to its more depolarized threshold potential, and little apparent inactivation (Figs. 3 and 4), HTC exhibited activation rates somewhat slower than those for LTC, as illustrated by the activation and deactivation time constants obtained in potassium-filled neurons (Fig. 7B; see METHODS). Rate constants determined using these data and the HTC Boltzmann relation derived later are plotted in Fig. 7C; the resulting time constant plot (from Eq. 1) is shown in Fig. 7B. Overall, this relation peaked at 5 ms at a potential of −25 mV.

Transient outward current

In the presence of both 10 mM TEA and 0.1 μM DTX, little current was activated by steps from −60 mV up to 0 mV (Fig. 4C). However, 200-ms pulses to between −110 and −120 mV revealed a rapidly inactivating outward current on subsequent depolarizing steps positive to −50 mV. A similar transient current was observed in cells bathed in TEA plus 0.2 mM 4-AP (n = 3; not shown). Current responses without the negative prepulse were subtracted from responses with the prepulse to further isolate the transient outward component, as illustrated in Fig. 8A. The averaged I-V relation for peak transient current and the conductance/voltage curve derived from the I-V are shown in Fig. 8, B and D, respectively. A Boltzmann relation fit to this curve showed a V1/2 of −11 mV and a slope factor of 19 mV. These Boltzmann parameters, along with the persistence of the transient current in TEA and DTX, show that the transient current is not simply an inactivating component of one of the other currents described above. Conditioning prepulses to potentials between −100 and −50 progressively reduced the response to a test step to 0 mV, as seen in Fig. 8C. Figure 8D plots the voltage sensitivity of inactivation (●), which had a V1/2 of −83 mV and a slope factor of 6 mV.

The rate of inactivation of transient current after activation was estimated by fitting single exponential curves to the decay phase of currents like those in Fig. 8A. Figure 8B, ●, shows that the exponential rate of inactivation after activation of the transient current was relatively insensitive to voltage. As significant inactivation can occur at potentials between −80 and −50 mV (Fig. 8D), where no activation has occurred, we measured the rate of inactivation at these potentials using the protocol illustrated in Fig. 9A. Steps were first made to −113 mV to reprime the transient current. Then a step was made to an intermediate potential for 0 to 50 ms, followed by a step to +8 mV to assay the amplitude of transient current. The amplitude of the transient current was measured as the current amplitude 2 ms after the onset of the test step, which corresponds roughly to the time to peak of the transient current in the absence of the intermediate step. This approach was necessary as a subtraction protocol was not employed in this experiment; it should be valid if the kinetics of the current are not dependent on their history of activation. The peak amplitude of the transient


K CURRENTS IN AUDITORY NEURONS 2829

Figure 5. Characterization of LTC. A: average I-V for LTC current defined as current in 10 mM TEA minus current in 0.1 μM DTX and TEA. Current activation phase (inset shows examples with steps from −54 to +6 mV) were fit with the function $N(1 - \exp(-t/\tau))$, where $N$ is the peak of the current and $\tau$ is the exponential time constant. Parameter $N$ then was averaged from 4 to 7 neurons and plotted in A. Data normalized to current at +35 mV. B: values for $\tau$ of exponential current activation (●, 11 cells), deactivation (○, 6 cells), and deactivation in cells filled with Cs $(\square, 4$ cells). Curve drawn through the points derives from Eq. 1. C: rate constants of channel opening ($\alpha$) and closing ($\beta$) derived from Eqs. 2 and 3 as a function of voltage. $\alpha$, fits to Eqs. 5 and 6, with $\alpha_0 = 0.2, V_{1/2} = -60$ mV, and $K = 21.8$ mV for $\alpha(V)$ and $\beta_0 = 0.17, V_{1/2} = -60$ mV, and $K = 14$ mV for $\beta(V)$. 

Current declined exponentially with increasing duration of the intermediate step (Fig. 9A, inset, ■). Surprisingly, the rate of inactivation of transient current was faster with this protocol than when inactivation occurred after activation. Figure 9B, ●, plots the rate of inactivation obtained with four different intermediate steps to potentials where activation did not occur. This analysis indicates that inactivation of the transient current from a closed state is highly voltage sensitive, such that inactivation is faster with more positive intermediate steps. The rate of recovery from inactivation (Fig. 9C) estimated at −113 mV varied widely among cells but was comparable with the rates of inactivation after channel opening ($27 \pm 19$ ms, $n = 4$).

Cesium-filled neurons

In an effort to block outward potassium currents by replacement of intracellular potassium with cesium ions, we observed that, despite the absence of intracellular potassium, NM neurons exhibited substantial outward current components on depolarizations from −70 mV. For example, using cesium sulfate-filled patch pipettes (see METHODS), cells produced peak currents of $+2.6 \pm 1.2$ nA at +7 mV ($n = 30$). We have characterized these currents to see if they were produced by the same ion channels as those generating the potassium currents described earlier. Figure 10A shows a profile of current responses on depolarization. Voltage steps between −60 and −40 mV evoked steady inward current (see inset), whereas more positive steps produced a net outward current with only gradual inactivation. After repolarization to −70 mV, large inward tail currents were observed (Fig. 10, A, →, and B, inset). These tail currents were most likely carried by extracellular $K^+$ (5 mM), as reduction in bath $K^+$ to 0.5 mM eliminated most of the tail current in four neurons (not shown), whereas removal of bath Ca$^{2+}$ or application of 2 mM Co$^{2+}$ did not block the tail currents ($n = 3$). These experiments indicate that these channels have negligible permeability to Na$^+$ ions as no inward current was observed in low K$^+$ solution despite the presence of 140 mM Na$^+$. Reversal potential analysis, performed as described earlier for potassium current tails, indicated that ionic current in cesium-filled cells reversed at $-27 \pm 6$ mV. The measured reversal potential of $-27$ mV indicates a permeability ratio $P_C/P_K$ of 0.1, as estimated using the Goldman-Hodgkin-Katz voltage equation, neglecting a contribution from Na$^+$. 

Figure 6. Kinetics of LTC inactivation. A: variation of inactivation time constants with clamp potential. Decay phase of traces, such as in Fig. 4C, inset, were fit with a sum of 2 exponentially decaying curves plus a constant $A_{fast} \exp(-t/\tau_{fast}) + A_{slow} \exp(-t/\tau_{slow}) + A_{steady-state}$. B: variation with potential of relative magnitude of exponential and steady-state components.
Conductance/voltage curves constructed from the tail currents are shown in Fig. 10B (see METHODS). These curves illustrate that the tails are composed of two Boltzmann components, with $V_{1/2}$S of −58 and −19 mV, reminiscent of LTC and HTC. The proportions of the Boltzmann fit contributed equally by low- and high-threshold components were 18 and 82%, respectively. Consistent with LTC and HTC generating the large tail currents in Cs+-filled cells, the low and high-threshold components of the tails were blocked by 0.1 μM DTX and 10 mM TEA, respectively ($n = 6$), as illustrated in Fig. 11 for one cell. We then determined whether or not LTC and HTC could account for the inward and outward ionic current observed during the depolarizing steps in Cs+-filled neurons. Steady inward current evoked by depolarization to −50 mV was fully blocked by DTX but not by TEA ($n = 6$) as shown in Fig. 12. A and C. As with the tail currents, eliminating bath Ca$^{2+}$ (i.e., no added calcium, $n = 3$) or applying of 2 mM Co$^{2+}$ ($n = 3$) did not alter the low-threshold inward current, whereas it was eliminated by reducing K$^+$ from 5 to 0.5 mM ($n = 4$ cells). These data indicate that the inward current is not a calcium current but is carried by extracellular K$^+$ through LTC channels. Indeed, we did not observe any steady Ca$^{2+}$ current in NM neurons, unlike the reports of Koyano et al. (1996) and Sivaramakrishnan and Laurent (1995). These differences may result from rapid washout of calcium current after cell dialysis in our low series resistance recordings.

Finally, we observed that DTX, although it blocked inward current during and after depolarizing steps in cesium-filled cells, usually had little effect on current evoked by steps >0 mV (Fig. 12, B and C). By contrast, the outward current evoked by depolarization to +40 mV was reduced markedly by 10 mM TEA (Fig. 12, B and C). This result may indicate that although Ca$^{2+}$ is not very permeant through LTC channels, it does not prevent inward K$^+$ flux. Thus the permeability ratio given above most likely reflects the properties of HTC channels. In the presence of both TEA and DTX, negligible inward or outward current was obtained up to +20 mV even with prepulses to −110 mV, indicating that the transient outward current described earlier is blocked by internal Cs$^+$.

### Effects of DTX on firing properties

In previous studies of NM, it was shown that 1 mM 4-AP, but not 1 mM TEA, induces repetitive firing in NM neurons (Zhang and Trussell 1994b). Reyes et al. (1994) showed that while lower doses of 4-AP did not induce repetitive firing, 4-AP broadened action potentials and decreased the ability of the cell to “phase-lock” to regular, short current pulses. We examined the action of the more selective blocker DTX on firing properties of NM neurons. Figure 13A shows that 0.1 μM DTX converted neurons from single to multiple spiking in response to a prolonged suprathreshold current step, as observed in eight of eight neurons. Thus the effects of higher concentrations of 4-AP on firing properties are probably largely due to block of LTC. Brew and Forsythe (1995) observed a similar phenomenon in MNTB neurons and suggested that LTC may serve to prevent repetitive spiking in response to brief current stimuli, such as occurs during synaptic stimuli. We tested this hypothesis by injection of brief (1 ms) current steps in the presence of DTX. In only one of six cells did suprathreshold stimuli result in more than one spike, even though repetitive firing was produced by longer steps. Moreover, DTX produced little broadening of the action potential half-width (increasing it by 0.25 ± 0.35 ms, $n = 5$ cells), in spite of the high likelihood that LTC is activated during the spike. However, block of LTC clearly altered the membrane time constant. Time constants determined by the exponential decay time of voltage at the termination of 50-pA current pulses ranged from 2.3 to 7.4 ms, declining with increasing amplitude of the current step. Figure 13B shows exponential time constants of decay of current after 50-, 100-, 150-, and 200-pA stimuli from rest, normalized to the time constant obtained with a 50-pA pulse, in the presence and absence of DTX. Whereas time constants fell by half in control solutions, over this same range of currents steps, no significant change in time constant was observed in the presence of DTX. These data indicate that activation of LTC at potentials just positive to rest could control the duration of rapid synaptic stimuli.

### DISCUSSION

#### Role of outward currents in the control of firing properties

Neurons in the auditory pathway meet several requirements to transmit action potential timing accurately (Trussell 1997). Such neurons transmit signals one for one, and their...
output occurs with a short and uniform latency. Action potentials have a brief refractory period, a property that sets limits on the duration of both the channels generating the action potentials and of the underlying synaptic currents. Finally, EPSPs in these cells show little temporal summation. Among the currents described here, LTC appears to play a

![Image of Fig. 8](http://example.com/figure8.png)

**Fig. 8.** Transient outward current. **A:** protocol for revealing transient current, requiring digitally subtracting responses to a series of depolarizing voltage steps (10-mV intervals) with a holding potential of −69 mV from a similar series obtained with a holding potential of −119 mV. All recordings were made in the presence of 0.1 μM DTX and 10 mM TEA. Resulting currents showed more rapid and complete inactivation than the LTC. **B:** average peak transient currents from 5 neurons (normalized to current at +7 mV). **C:** protocol for determining effect of prepulse potential on amplitude of transient current. **D:** voltage dependence of activation and inactivation of transient current. For activation (○), peak currents were obtained from data in **B** and adjusted for driving force, assuming an $E_{rev}$ of −67 mV. Data expressed as $G/G_{MAX}$. Inactivation (●) was measured using protocol of **C**. Subtraction paradigm was not used here, so time to peak of the largest response (with −129-mV prepulse) was determined and the amplitude of the other responses at that same time point was measured. Peak amplitudes for the prepulse experiments are expressed relative to the difference in amplitude between the largest and smallest responses, $(I - I_{min})/(I_{max} - I_{min})$. Boltzmann parameters (Eq. 4) for activation and inactivation are indicated in the panel.

![Image of Fig. 9](http://example.com/figure9.png)

**Fig. 9.** Kinetics and voltage dependence of inactivation of transient current. **A:** protocol for determining rates of transient current inactivation at potentials that do not activate channels. No leak subtraction was employed. Amplitude of responses to steps to +8 mV were measured 2 ms into the voltage jump (● in inset). Note that currents obtained after intermediate step to −63 mV never reach the amplitude of current obtained without the intermediate step. Thus the number of activatable channels decreases faster at −63 mV than at +8 mV. **B:** rate of inactivation at subactivating potentials (●) obtained by exponential fits to curves described by connected symbols A, inset, plotted as a function of the intermediate step potential. ○, single exponential time constants of fits to current decay, such as in **8A**, on jumps to potentials that activate transient current. **C:** protocol for estimating rates of recovery of transient current from inactivation. Recovery was estimated as the rate of increase in peak responses to the second of a pair of steps to +8 mV. Scale bar in **C** applies also to **A**. All recordings made in presence of 10 mM TEA and 0.1 μM DTX.
significant part in endowing many auditory neurons with these properties. The low threshold and rapid kinetics of LTC ensure that spike threshold is raised quickly and membrane time constant quickly lowered after sudden depolarizations. That the current rapidly deactivates is also adaptive in that it permits the cell to return suddenly to a “ready state” after a transient stimulus. HTC is presumably a key player in repolarization of the action potential because NM spikes in TEA or 4-AP are broadened (Koyano et al. 1996; Reyes et al. 1994; Zhang and Trussell 1994a). In this regard it also would be expected to play a significant role in setting the spike refractory period.

It is perhaps surprising, therefore, that LTC has so little role in determining the shape of the action potential as determined by the failure of DTX to produce significant broadening. Indeed, the low threshold and rapid activation kinetics of LTC almost guarantee that it would be fully activated during a spike. Previous studies have suggested that a LTC plays a role in shaping the action potential, as a blocker of the current, 4-AP, widens the spike (Koyano et al. 1996; Reyes et al. 1994; Zhang and Trussell 1994a). However, as the data in Table 1 indicate, 4-AP is not selective for this current at the concentrations used and blocks both LTC and HTC to a significant extent. At potentials at which only LTC would be active, 4-AP at 0.2–1 mM blocks more than half the current, whereas DTX blocks nearly 80%. By contrast, at +7 mV, well above the activation potential for HTC, 4-AP blocks half the current, whereas DTX blocks only 5%, in keeping with the fact that HTC is a much larger conductance. Thus it is likely that LTC is activated during the action potential yet is of low enough conductance that HTC essentially controls the time course of the voltage change. However, at potentials closer to threshold, where HTC is not activated, LTC is the dominant outward current and so critically controls the time constant of synaptic potentials that cause the cell to fire. Because LTC has a $V_{1/2}$ ($\approx -58$ mV) close to the resting membrane potential, it has a significant part in endowing many auditory neurons with these properties.
potential of NM neurons (−66 mV), it may be that this current plays a large role in determining the resting potential of the cell.

Reversal potential shifts

During large depolarizing steps, we observed a positive shift in reversal potential consistent with accumulation of K⁺ in the extracellular space. Buildup of K⁺ has been well documented in other preparations, particularly in myelinated axon, where it is thought to influence the shape of the action potential and limit responsiveness during repetitive activity (Chiu 1991). Of particular concern in this report is whether the decline in driving force that parallels the change in $E_{\text{rev}}$ could complicate analysis of current inactivation. However, the magnitude of this effect should increase with the size of the current, and we analyzed inactivating currents only in cases in which channel blockers were used such as TEA, for LTC, and both TEA and DTX, for the transient outward current. Indeed, given that the greatest inactivation was observed for the small residual current in both TEA and DTX, and for both this current and LTC was rather insensitive to the amplitude of the voltage step, it seems likely that such inactivation was genuine. Nevertheless, the accumulation of K⁺ in nMAG deserves further analysis: because these neurons receive large somatic end-bulb synapses, somatic K⁺ channels could alter the resting potential around preterminal membrane and therefore influence synaptic strength.

Identification of LTC and HTC

Low-threshold potassium currents like the LTC described here have been observed in a variety of auditory (Banks and Smith 1992; Brew and Forsythe 1995; Koyano et al. 1996; Manis and Marx 1991; Oertel 1983; Reyes et al. 1994; Smith 1995; Zhang and Trussell 1994a) and nonauditory cell types (Brau et al. 1990; Dubois 1981; Stansfeld et al. 1987; Storm 1988). For example, a low-threshold, gradually inactivating current has been described in hippocampus and called $I_D$ (Storm 1988). This term also was used by Brew and Forsythe (1995) to describe a current very much like LTC in the MNTB. However, hippocampal $I_D$, named for its ability to permit action potentials to occur only after a delay, is distinct from LTC in auditory neurons in that the latter cell type fires only at the onset of the stimulus and virtually never at later times. The sensitivity of single-spike firing to DTX also has been observed in sensory ganglia (Stansfeld et al. 1987). The LTC may be homologous to the low-threshold, DTX-sensitive current of frog node of Ranvier (Brau et al. 1990; Dubois 1981). Whether $I_D$ of hippocampal neurons (Storm 1988; Wu and Barish 1992) corresponds to a different channel than LTC remains unclear. Possibly, differences in its density in the membrane, combined with differences in the relative strength of physiological stimuli, could alter whether the channel limits the cell to early or late spikes.

Several voltage-gated potassium channels have been localized to auditory neurons on the basis of immunocytochemistry and in situ hybridization. Kv1.1 and 1.2 are found at high levels in cochlear nucleus and MNTB (Wang et al. 1994) and when expressed in cell lines produce a DTX-sensitive current of rather low threshold (Grissmer et al. 1994; Hopkins et al. 1994). Kv3.1 also is expressed in cochlear nucleus and MNTB and encodes a high-threshold, TEA-sensitive current (Kanemasa et al. 1995; Perney et al. 1992). It is likely that these genes code for some of the channels found in NM, as suggested for MNTB by Brew and Forsythe (1995) and by Wang et al. (1998). However, clear differences in activation ranges, kinetics and absolute drug sensitivity remain (see Grissmer et al. 1994; Hopkins et al. 1994; Kanemasa et al. 1995), which suggest that other channel subunits, or structural modifications of Kvs 1 and 3, are needed to produce the channels found in NM.

Two components of HTC

We have described HTC, a TEA-sensitive, DTX-resistant current as a single, high-threshold conductance. Several lines
of the two components of HTC in voltage dependence (as judged by comparing peak and steady-state currents) indicates that HTC may operationally be considered one current. Additional pharmacological analysis will be needed to separate these components more fully.

**Transient outward current**

This is the first report of an A-like current, i.e., a transient outward current with a very negative potential range for inactivation, in avian cochlear nuclear neurons. It is possible that this current also is expressed in mammalian bushy cells (Fitzackerly et al. 1995; Heck et al. 1997); this could be tested in voltage-clamp experiments on identified cells. The role of this current in spike firing remains uncertain, however, because the channel appears to inactivate at potentials so far negative to rest that little would be active physiologically. Nevertheless, in terms of its kinetics and activation/inactivation potentials, the current is similar to transient currents of rodent hippocampus (Ficker and Heinemann 1992; Numann et al. 1987). It is possible that, at physiological temperatures, the $V_{1/2}$ of the inactivation curve (Fig. 8D) could be more positive than we have measured and so favor activatable transient current at rest. We observed that inactivation was faster and more voltage sensitive at potentials negative to the activation range than at more positive potentials. This result suggests that inactivation of the transient current may occur via open or closed states with rates that have different voltage sensitivities. Therefore if this current is activatable from the resting potential, the differences we observed between inactivation rates from open and closed states might be of significance: slight changes in resting potential would be expected to produce rapid changes in the fraction of ‘‘A-current’’ channels available for activation. However, as with $I_{D}/LTC$, such a current likely would play a different role from that of the classical A current in delaying spike onset (Connor and Stevens 1971), possibly instead helping to terminate action potentials and to prevent weak inputs or slower membrane potential changes from reaching threshold.

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