Three Types of Depolarization-Activated Potassium Currents in Acutely Isolated Mouse Vestibular Neurons

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Chabbert, C., J. M. Chambard, A. Sans, and G. Desmadryl. Three types of depolarization-activated potassium currents in acutely isolated mouse vestibular neurons. J Neurophysiol 85: 1017–1026, 2001. The nature and electrophysiological properties of Ca2+-independent depolarization-activated potassium currents were investigated in vestibular primary neurons acutely isolated from postnatal mice using the whole cell configuration of the patch-clamp technique. Three types of currents were identified. The first current, sensitive to TEA (I_{TEA}) and insensitive to 4-aminopyridine (4-AP), activated at −40 mV and exhibited slow activation (τ_{ac}, 38.4 ± 7.8 ms at −30 mV, mean ± SD). I_{TEA} had a half activation potential [V_{ac(1/2)}] of −14.5 ± 2.6 mV and was inactivated by up to 84.5 ± 5.7% by 10-s conditioning prepulses with a half inactivation potential [V_{inac(1/2)}] of −62.4 ± 0.2 mV. The second current, sensitive to 4-AP (maximum block around 0.5 mM) and to α-dendrotoxin (I_{DTX}), appeared at −60 mV. Complete block of I_{DTX} was achieved using either 20 nM α-DTX or 50 nM marratoxin. This current activated 10 times faster than I_{TEA} (τ_{ac}, 3.5 ± 0.8 ms at −50 mV) with V_{ac(1/2)} of −51.2 ± 0.6 mV, and inactivated only slightly compared with I_{TEA} (maximum inactivation, 19.7 ± 3.2%). The third current, also sensitive to 4-AP (maximum block at 2 mM), was selectively blocked by application of blood depressing substance (BDS-I; maximum block at 250 nM). The BDS-I–sensitive current (I_{BDS-I}) activated around −60 mV. It displayed fast activation (τ_{ac}, 2.3 ± 0.4 ms at −50 mV) and fast and complete voltage-dependent inactivation. I_{BDS-I} had a V_{ac(1/2)} of −31.3 ± 0.4 mV and V_{inac(1/2)} of −65.8 ± 0.3 mV. It displayed faster time-dependent inactivation and recovery from inactivation than I_{TEA}. The three types of current were found in all the neurons investigated. Although I_{TEA} was the major current, the proportion of I_{DTX} and I_{BDS-I} varied considerably between neurons. The ratio of the density of I_{BDS-I} to that of I_{DTX} ranged from 0.02 to 2.90 without correlation with the cell capacitances. In conclusion, vestibular primary neurons differ by the proportion rather than the type of the depolarization-activated potassium currents they express.

INTRODUCTION

Vestibular primary neurons are involved in transmitting afferent information about accelerations from the inner ear vestibular mechanoreceptors to vestibular nuclei. Vestibular afferents are classified as regularly or irregularly discharging patterns (Smith and Goldberg 1986). Irregular afferents have phasic response dynamics, higher sensitivities to natural and to external galvanic stimulations, and larger axons than regular fibers. They also differ in their response to the activation of efferent pathway (Goldberg et al. 1984; Smith and Goldberg 1986). These authors suggest that discharge regularity as well as the other functional properties of vestibular primary afferents could be the consequence of differences in membrane conductances between each class of neurons. However, the intrinsic properties supporting these differences are unknown. We therefore developed a preparation of vestibular ganglion neurons acutely isolated from mice. Previous analysis of voltage-activated conductances showed the presence in all vestibular neurons of one sodium current (Chabbert et al. 1997) and five calcium currents (Chambard et al. 1999; Desmadryl et al. 1997), but potassium currents that might influence their discharge patterns were not investigated yet. Since a large variety of voltage-activated K+ currents have been described in various neuronal preparations, and since they have been reported to play an important role in shaping membrane electrical activity (Rudy 1988), we characterized these currents in vestibular primary neurons.

Over the last two decades many studies have been performed, characterizing the outward voltage-activated K+ conductances expressed in dorsal root ganglion (DRG) neurons (Everill et al. 1998; Gold et al. 1996; Kostyuk et al. 1981; Robertson and Taylor 1986), and other sensory neurons (Brew and Forsythe 1995; Garcia-Diaz 1999; Locke and Nerbonne 1997a; Manis and Marx 1991; McFarlane and Cooper 1991; Oyelese and Kocsis 1996; Rathouz and Trussell 1998; Stansfeld and Feltz 1988). Identification of the different current types according to their activation and inactivation properties, which often overlap, remains difficult (Gold et al. 1996; McFarlane and Cooper 1991). Pharmacological agents such as tetraethylammonium (TEA) and 4-aminopyridine (4-AP) have been widely used to separate different classes of voltage-activated K+ currents. However, separation of the different types of 4-AP–sensitive currents has often been impaired both by the overlap in the doses required to block each current (Gold et al. 1996; Hoshi and Aldrich 1988; McFarlane and Cooper 1991) and by the voltage sensitivity of the blocking effect (Yeh et al. 1976). Conversely, several peptide toxins that selectively block each type of 4-AP–sensitive K+ current are now available. α-Dendrotoxin (α-DTX), a toxin purified from snake venom, has been reported to selectively block a fast activating...
and partially inactivating 4-AP–sensitive K⁺ current (Stansfeld and Feltz 1988). More recently margatoxin (MgTX), a toxin purified from scorpion venom, has been shown to block a 4-AP–sensitive K⁺ current in human peripheral T-lymphocytes (Garcia-Calvo et al. 1993). Another peptide purified from sea anemone, blood depressing substance (BDS-I), has been reported to block a fast activating and inactivating K⁺ current (Diochot et al. 1998).

The aim of the present study was to identify the different types of depolarization-activated K⁺ currents present in vestibular primary neurons using these new pharmacological tools, and to characterize their respective kinetic properties. Records were obtained using the whole cell configuration of the patch-clamp technique applied to primary neurons acutely isolated from postnatal mice. Based on their pharmacological and electrophysiological properties, we report here for the first time that three distinct outward Ca²⁺-independent depolarization-activated K⁺ conductances are present in vestibular primary neurons. These three conductances are expressed in various proportions between neurons.

METHODS

Cell culture

Potassium currents were studied in neurons acutely isolated from the superior branch of the vestibular nerve innervating the utricular macula and the horizontal and lateral cristae using an isolation procedure previously described (Desmadryl et al. 1997). Ganglia were aseptically dissected from postnatal day 5 to 8 (P5 to P8) mice (CERJ, Le Genest, France) rapidly killed by decapitation (the day of birth was considered as postnatal day 0). About 20 ganglia for each experiment were collected in phosphate-buffered saline (PBS; Life Technologies). We tested different incubation times and trypsin concentrations without any difference in the amplitude or the shape of the recorded currents. We settled on protocols employing trypsin at 0.25% for 12 min at 37°C in PBS containing 0.25% EDTA-trypsin (Life Technologies). Ganglia were triturated with fire-polished Pasteur pipettes of three decreasing diameters in a cell culture medium containing Neurobasal medium (Life Technologies), 10% B27 (Life Technologies), 25 μM glutamine, and 0.25 mM glutamate. Neurons were plated onto 35-mm culture dishes (Nunc) coated with 10 μg/ml poly-D-ornithine (Sigma) in cell culture medium. Cells were used between 1 and 4 h after dissociation. Under phase contrast microscopy, dissociated neurons had a spherical shape and birefringent cytoplasm as previously reported (Desmadryl et al. 1997). Cell diameters ranged between 12.5 and 25 μm. Only isolated neurons exhibiting no processes were chosen for the electrophysiological studies. Their capacitances ranged from 9 to 32 pF (18.6 ± 4.4 pF, mean ± SD) in a sample of 80 neurons.

Electrophysiological recordings

Whole cell recordings of voltage-dependent potassium currents were obtained at room temperature (25°C) under conditions optimized to ensure their complete isolation from other voltage-dependent currents. Tetrodotoxin (1 μM, Sigma) was used to block Na⁺ currents, and extracellular Na⁺ was replaced by choline. Calcium was omitted from the extracellular medium, and 2 mM EGTA was added to block voltage-activated Ca²⁺ currents, as well as Ca²⁺-activated K⁺ currents. The standard extracellular solution contained (in mM) 135 cholineCl, 5 KCl, 10 HEPES, 10 glucose, 1 MgCl₂, 2 EGTA, and 0.001 TTX. For extracellular TEA solutions, cholineCl was replaced by equimolar TEACl. The pH of the recording solutions was adjusted to 7.35 and osmolality set at 300 mOsm/l. Recording pipettes pulled from hematocrit tubes (Modulohm I/S, Herlev, Denmark) were coated with ski wax to reduce capacitive transients. Pipettes 2–3 MΩ were filled with the following intracellular solution (in mM): 135 KCl, 10 EGTA, 25 HEPES, and 10 glucose. The pH was adjusted to 7.35 and the osmolality set at 300 mOsm/l. Whole cell currents were recorded using a Axopatch 200B (Axon Instruments, Foster City, CA) patch-clamp amplifier. After seal formation and membrane disruption, cell capacitance and series resistance were estimated from the decay of the capacitance transient induced by a ±10-mV pulse from a holding potential (HP) of −100 mV. Series resistance was in the range of 5–9 MΩ, and the membrane capacitance could be charged with a time constant of 100 μs. Series resistances were 85% compensated after cancellation of the capacitive transients. No linear leakage compensation was performed. Voltage errors resulting from uncompensated series resistances were corrected only for the high-threshold TEA-sensitive current when maximum voltage error exceeded 5 mV. The liquid junction potential between the internal and the extracellular solution, measured according to Neher (1992), was −6.8 mV at 25°C for control extracellular medium. Data presented are not corrected for junction potential unless specified. Current signals were filtered at 5 kHz, digitized, and stored.

Drugs

4-AP and TEA were obtained from Sigma-Aldrich and were dissolved directly in the extracellular medium. α-DTX (Latoxan), MgTX (Bachem), and BDS-I (a generous gift from S. Diochot, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, France) were dissolved as stock solutions at 10⁻⁴ M in bidistilled water and stored at −80°C. Drugs and peptides were applied to the bathing medium in the vicinity of the cell by a fast gravity perfusion system.

Analysis

All experimental parameters, such as the holding and test potentials, were controlled with an IBM PC equipped with a Tecmar Labmaster analog interface (Axon Instruments). Cell stimulation, data acquisition, and analysis were performed using Pclamp software (v 5.5 and v 6, Axon Instruments). The mean chord conductances were calculated, assuming a potassium equilibrium potential of −71 mV. For each component of the whole cell K⁺ current, activation and inactivation curves of pooled data were best fitted with single Boltzmann function of the form \( G_{max} = \frac{1}{1 + \exp(V - V_{1/2})} \), where \( V_{1/2} \) is half activation or inactivation potential \( V_{max/1/2} \) and \( k \) the limiting slope. Time constants (τ) were best fitted with single exponential function of the form \( A \cdot \exp(-t/\tau) \), where \( A \) is the current peak and \( \tau \) the exponential time constant. Except when specified, pooled data are given as means ± SD. Statistical significances were examined using ANOVA, corrected for unequal variances when necessary.

RESULTS

Whole cell voltage-activated outward K⁺ currents

K⁺ currents were evoked by depolarizing pulses at various voltages from HP −100 mV. Figure 1 illustrates activation (Fig. 1A) and inactivation (Fig. 1B) of currents recorded in representative neurons in control external solution. Examination of their general shape suggested that they were composed of at least two distinct components with an early transient component and a sustained component in most neurons (Fig. 1Aa, −10 mV trace). Analysis of the current-voltage (I-V) relationship for the sustained component confirmed the presence of two distinct currents. The first component of the sustained current activated at potentials just positive to −60
indicated that the early transient and sustained components inactivated with $V_{\text{inac}}(1/2)$ of $-76$ and $-61$ mV, respectively.

To determine that depolarization-activated whole cell currents are carried by K⁺, the reversal potential was estimated by stepping the HP from $-100$ to $+20$ mV for $20$ ms to activate the whole cell K⁺ current, followed by $100$-ms depolarizations from $-100$ to $+20$ mV in $10$ mV increments. Reversal potential $E_{\text{rev}}$ was estimated by measuring tail currents $2$ ms after the end of the depolarizing pulses in external solutions containing $5$ or $30$ mM K⁺. This procedure gave a mean $E_{\text{rev}}$ of $-71.2 \pm 1.4$ mV ($n = 8$) in standard external solution and $-32.7 \pm 1.2$ mV ($n = 8$) in $30$ mM K⁺ external solution (including the junction potential values). The calculated equilibrium potential in our recording conditions using the Nernst equation gave $-83.2$ and $-38$ mV, respectively. From our data it appears that the $E_{\text{rev}}$ of the whole cell K⁺ current was dependent on the K⁺ gradient, but less than that expected for an absolute K⁺ current, suggesting that K⁺ channels in our preparation are not absolutely selective to K⁺. Alternatively, these discrepancies could be the consequence of K⁺ accumulation in the extracellular space, as reported by Rathouz and Trussell (1998) in neurons of the avian nucleus magnocellularis.

**Pharmacological identification of the different components of the whole cell K⁺ current**

We first studied the effect of external applications of increasing concentrations of 4-AP and TEA on the whole cell K⁺

**FIG. 1.** Whole cell depolarization-activated K⁺ currents in vestibular primary neurons. Aa: representative whole cell K⁺ currents elicited in control external solution by $400$-ms depolarizing pulses ranging from $-80$ to $+30$ mV in $10$-mV increments from holding potential (HP) $-100$ mV. Ab: plot of current amplitudes taken either $5$ ms after the beginning (○) or before the end (●) of the test pulse, as a function of the test potential for the neuron shown in Aa. Dotted lines emphasize the increase in the slope of the current-voltage relation (I-V) for sustained component (arrow). Ac: plot of relative activation of the sustained component of whole cell K⁺ current as a function of test potentials for a sample of $4$ neurons. Dotted lines show the $2$ Boltzmann components that compose the data curve. B: steady-state inactivation of whole cell K⁺ currents elicited by $400$-ms depolarizing pulses to $+20$ mV preceded by $10$-s conditioning prepulses from $-110$ to $-20$ mV from HP $-100$ mV. Bb: plot of relative currents against conditioning voltages for the neuron shown in Ba. Note that in this neuron whole cell currents were inactivated by up to $60\%$. Bc: plot of relative inactivation of whole cell K⁺ currents as a function of test potentials for a sample of $5$ neurons. Currents were measured either $5$ ms after the beginning (○) or before the end (●) of the test pulses.

mV, and the second above $-30$ mV. This can be seen as an increase in the slope of the I-V curve (Fig. 1Ab, arrow). The presence of two components in the sustained current was further supported by plotting relative activation, expressed as $G/G_{\text{max}}$ versus test potential in a sample of four neurons (Fig. 1Ac). The curve was composed of two Boltzmann components (dotted lines) with $V_{\text{ac}}(1/2)$ of $-47$ and $-12$ mV, which contributed to $45$ and $55\%$ of the total current, respectively, in this cell. These two $V_{\text{ac}}(1/2)$ values matched those estimated below for the α-DTX and TEA-sensitive components.

Steady-state inactivation of the whole cell K⁺ current confirmed that it was composed of different components. Figure 1Ba illustrates inactivation elicited at $+20$ mV by $10$-s conditioning prepulses to various voltages. Plotting current amplitudes normalized to maximum current as a function of conditioning voltages revealed that a large part of the whole cell K⁺ current did not inactivate even for long conditioning prepulses (Fig. 1Bb), whereas the inactivating portion exhibited distinct voltage dependence when measured at the beginning (○), or the end (●) of the test pulse. Plotting relative inactivation against conditioning voltages in a sample of five neurons.

**FIG. 2.** Pharmacological separation of the different components of the whole cell K⁺ current using 4-aminopyridine (4-AP) and tetraethylammonium (TEA). Effect of subsequent external applications of 4-AP and TEA on whole cell K⁺ currents. A: representative traces elicited by depolarizing pulses to $-10$ mV from HP $-100$ mV (a) in control solution (1), presence of $0.1$ mM 4-AP (2), $2$ mM 4-AP (3), and $40$ mM TEA (4) in the same cell. Digital subtraction reveals the $2$ currents sensitive to 4-AP (b and c), and the current sensitive to TEA (d). B: corresponding I-V relationship of whole cell K⁺ currents evoked by $300$-ms depolarizing pulses from $-70$ to $0$ mV from HP $-100$ mV showing whole cell K⁺ current (● and ○), currents sensitive to $0.1$ and $2$ mM 4-AP (● and ○, respectively), and currents sensitive to $40$ mM TEA (○ and ●). Current amplitudes were measured either $5$ ms after the beginning (●, ○, ●, and ○) or $5$ ms before the end (●, ○, ●, and ○, Bb) of the test pulse. C: effect of subsequent applications of increasing concentrations of TEA (as indicated), followed by applications of $0.1$ and $2$ mM 4-AP on current traces evoked by $200$ ms pulses to $+20$ mV.
reported to selectively block distinct 4-AP-sensitive K⁺ currents. Figure 3 illustrates representative effects of these toxins on single traces of whole cell K⁺ currents. Application of α-DTX blocked a fast activating sustained component of the whole cell K⁺ current with noticeable effect at 2 nM and maximum blocking effect around 20 nM (Fig. 3A). MgTX blocked a component of the whole cell K⁺ current with an effect from 10 nM and maximum blocking effect around 50 nM (Fig. 3B). Applications of nanomolar concentrations of BDS-I blocked a fast activating transient component of the whole cell K⁺ current with noticeable effect from 20 nM and maximum blocking effect around 250 nM (Fig. 3C). When used at its maximum blocking concentration, α-DTX impeded the effect of subsequent application of 0.1 mM 4-AP (Fig. 3D). A similar result was obtained with MgTX (Fig. 3E). Each of the two toxins also prevented the effect of the other without preventing the effect of 2 mM 4-AP on the fast activating transient component (Fig. 3F). Presence of 250 nM BDS-I in the external solution did not prevent the blocking effect of α-DTX or TEA, whereas it prevented those of 2 mM of 4-AP (Fig. 3G).

**Fig. 3.** Effects α-dendrotoxin (α-DTX), margatoxin (MgTX), and blood depressing substance (BDS-I) on whole cell K⁺ currents. A: effect of subsequent applications of 2 and 20 nM of α-DTX on current traces evoked by 400-ms pulses to +20 mV. B: effect of subsequent applications of 10 and 50 nM of MgTX on current traces evoked by 700-ms pulses to +20 mV. C: effect of subsequent applications of 20 and 250 nM of BDS-I on current traces evoked by 200-ms depolarizing pulses to −10 mV. Note the complete block of the transient component, whereas the sustained one was not affected. D: effect of 0.1 mM of 4-AP following application of 20 nM of α-DTX on current traces evoked by 400-ms pulses to −10 mV. E: effect of 0.1 mM of 4-AP following application of 50 nM of MgTX on current traces evoked by 700-ms pulses to −10 mV. Note that the transient component was not affected. F: effect of subsequent applications of 20 nM α-DTX, 50 nM MgTX, and 2 mM 4-AP on current traces evoked by 200-ms pulses to +20 mV. Note that 4-AP blocks a transient component without affecting the sustained one. G: effect of subsequent applications of 20 nM α-DTX, 2 mM 4-AP, and 40 mM TEA on current traces evoked by 400-ms pulses to −10 mV in solution containing 250 nM BDS-I. All experiments were conducted from HP −100 mV.

**Fig. 4.** Pharmacological separation of the different components of the whole cell K⁺ currents using α-DTX, MgTX, and BDS-I. Aα: effect of external application of 20 nM α-DTX on whole cell K⁺ currents evoked by 150-ms depolarizing pulses to −20 mV from HP −100 mV. Representative traces elicited in control solution (1), and in presence of α-DTX (2). Digital subtraction (1) − (2) reveals currents sensitive to α-DTX (I_{TEA}). Ab: corresponding I-V relationship from HP −100 mV of whole cell K⁺ currents evoked by 150-ms pulses (●) and of I_{TEA} (□) measured 5 ms either after the beginning (□) or before the end (●) of the test pulse. Ba: effect of external application of 50 nM MgTX on whole cell K⁺ currents in similar conditions as in A. Bb: corresponding I-V relation showing the MgTX-sensitive current. Ca: effect of external application of 250 nM BDS-I on whole cell K⁺ currents evoked in control solution by 250-ms pulses at −10 mV following 150-ms prepulses to −100 mV from HP −50 mV. This protocol was used to reduce the large TEA-sensitive current. Cb: corresponding I-V relations showing the BDS-I-sensitive current.
Figure 4 illustrates the isolation of the three components of whole cell K⁺ current using α-DTX, MgTX, or BDS-I on depolarizing pulses to −10 mV (Fig. 4, Aα–Ca) and on the corresponding I-V relations (Fig. 4, Ab–Cb). Pharmacological properties of the toxins reported in Fig. 3, and the similarity in the I-V relations shown in Fig. 4 indicate that α-DTX, MgTX, and 4-AP (below 0.5 mM) affect the fast activating sustained component of the whole cell K⁺ current, thereafter referred as I₄TX, whereas BDS-I and 4-AP (at millimolar concentrations) affect its transient component, thereafter referred as I BDS-I.

Characterization of I₆TX

The high-threshold slow activating and inactivating TEA-sensitive current was isolated by subtracting traces evoked in 40 mM TEA external solution from those elicited in standard external solution (Fig. 5). Steady-state voltage-dependent activation of I₆TX was studied using depolarizing pulses at various voltages from HP −100 mV. I₆TX appeared from a threshold between −50 and −40 mV and exhibited slow activation and slow time-dependent inactivation (Fig. 5Aα). This current was fully activated around +20 mV. Figure 5Ab illustrates the plot of relative activation of I₆TX expressed as G/G max as a function of the test potential in a sample of nine neurons. Values of current amplitudes were taken 5 ms before the end of the test pulse. The data curve was best fitted with single Boltzmann function with Vₐc₅₀ of −14.5 ± 2.6 mV and k of 10.2 ± 0.6 mV (means ± SE). The time constant for activation (τ ac) of I₆TX was voltage dependent and ranged from 38.4 ± 7.8 ms at −30 mV, to 5.1 ± 1.7 ms at +25 mV for a sample of seven neurons.

The inactivation, not visible within the 150-ms pulse, was evident for longer depolarizations. Steady-state voltage-dependent inactivation of I₆TX was studied on currents elicited at +20 mV following 10-s prepulse protocol (Fig. 5Ba). Current amplitudes measured 5 ms before the end of the test pulse were normalized to maximum current and expressed as I/I max. In a sample of six neurons, the mean maximum inactivation of I₆TX was 84.5 ± 5.7%. Plot of relative inactivation as a function of the prepulse voltages was best fitted with single Boltzmann function (Fig. 5Bb), with V inac(1/2) of −62.4 ± 0.2 mV, with k of 11.2 ± 0.3 mV (means ± SE).

Steady-state time-dependent inactivation and recovery from inactivation of I₆TX were studied using two-step protocols. Inactivation of I₆TX elicited by depolarizing pulses to +20 mV was achieved by increasing the duration of conditioning pre-pulses to −20 mV (Fig. 5Ca). Recovery of I₆TX previously inactivated by holding the neuron at −20 mV for several minutes was achieved by increasing the duration of conditioning prepulses to −100 mV (Fig. 5Da). Relative time-dependent inactivation (Fig. 5Cb) and recovery from inactivation (Fig. 5Db) of I₆TX were plotted as a function of prepulse duration in a sample of seven neurons. Data points were fitted with single exponential functions of the form A exp(−t/τ) where τ is constant of 6.7 ± 1.0 s and 254.4 ± 14.1 ms (solid lines).

Steady-state voltage-dependent deactivation of I₆TX was determined using a protocol in which a 20-ms pulse to +10 mV was applied to fully activate the current, followed by a range of more negative potentials from −45 to −70 mV to shut it (not shown). The fit of tail currents revealed that they decayed with a single exponential time course. The mean time constant of deactivation (τ deac) at −50 mV was 26.4 ± 3.6 ms for a sample of four neurons. The presence of 20 nM α-DTX, 50 nM MgTX, or 2 mM 4-AP in the external solution did not change either the maximum amplitudes at +20 mV (I max) nor the kinetic characteristics of I₆TX (Table 1).

FIG. 5. Electrophysiological characterization of the high-threshold, slow activating and inactivating TEA-sensitive K⁺ current. Traces were obtained by subtracting those elicited in TEA 40 mM external solution from those elicited in standard external solution. Aα: family of I₆TX elicited by 150-ms depolarizing pulses ranging from −80 to +30 mV in 10-mV increments from HP −100 mV in a representative neuron. Ab: plot of relative activation of I₆TX as a function of the test potential in a sample of 9 neurons. Ba: steady-state inactivation of I₆TX elicited by 400-ms depolarizing pulses to +20 mV preceded by a 10-s conditioning prepulse from −120 to −20 mV from HP −100 mV. Bb: plot of relative inactivation of I₆TX as a function of the test potential in a sample of 6 neurons. Ca: steady-state time-dependent inactivation of I₆TX elicited by 250-ms test pulses to +20 mV preceded by a conditioning prepulse to −20 mV from HP −100 mV. Cb: plot of the relative time-dependent inactivation of I₆TX as a function of prepulse duration in a sample of 7 neurons. Cb: recovery from inactivation of K⁺ currents elicited in the same neuron as in Ca using similar protocol, except that HP and conditioning prepulses were changed to −20 and −100 mV, respectively. Da: recovery from inactivation of I₆TX as a function of prepulse durations in a sample of 6 neurons. Data points were fitted with single exponential function (solid line). In Ab, Bb, Cb, and Db, current amplitudes were measured 5 ms before the end of the test pulses.
very fast kinetics. On a sample of seven neurons, rise time of activation was voltage dependent and displayed lizing pulses. Plot of relative activation of very little time-dependent inactivation even for 10-s depolar-

\[ I_{\text{DTX}} \]

\[ \begin{array}{l|c|c|c|c|c}
 \text{Drugs} & I_{\text{max}} \text{, pA} & V_{\text{act}(1/2)} \text{, mV} & \tau_{\text{ac}, \text{ms}} & V_{\text{inac}(1/2)} \text{, mV} \\
\hline
 \text{TEA} & 3.634 \pm 1.029 (9) & -15.8 \pm 4.7 (9) & 38.4 \pm 7.8 (7) & 5.1 \pm 1.7 (7) & -63.9 \pm 5.9 (6) \\
 \text{TEA} + \text{DTX} & 3.116 \pm 1.118 (2) & -14.6 \pm 2.1 (2) & 31.5 \pm 4.7 (2) & 4.8 \pm 1.9 (2) & -60.3 \pm 3.2 (4) \\
 \text{TEA} + \text{MgTX} & 3.989 \pm 1.269 (3) & -16.7 \pm 6.5 (3) & 35.8 \pm 8.0 (3) & 5.2 \pm 2.4 (3) & -65.4 \pm 5.7 (3) \\
 \text{TEA} + 4-\text{AP} & 3.537 \pm 382 (4) & -15.9 \pm 5.4 (4) & 46.6 \pm 6.9 (2) & 5.0 \pm 1.6 (2) & -66.4 \pm 5.7 (3) \\
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\end{array} \]

value for the representative neuron and the relative inactivation for a sample of seven neurons (Fig. 7Aa). I_{\text{BDS-I}} was completely activated at 0 mV and almost totally inactivated after 150 ms.

Activation of I_{\text{BDS-I}} estimated at the peak of the current in a sample of 15 neurons (Fig. 7Ab) gave \( V_{\text{act}(1/2)} = -31.3 \pm 0.4 \) mV and \( k = 8.5 \pm 0.4 \) mV (means \pm SE). The rise time of activation was of the same order as that of I_{\text{DTX}}, \( \tau_{\text{ac}} \) and decay time (\( \tau_{\text{inac}} \)) were voltage dependent, ranging from 2.3 \pm 0.4 ms at -50 mV to 0.8 \pm 0.1 ms at +10 mV, and 53.5 \pm 11.7 ms at -50 mV to 12.2 \pm 3.1 ms at +10 mV, respectively.

Steady-state voltage-dependent inactivation of I_{\text{BDS-I}} was studied using a 200-ms pulse to -100 mV from HP -50 mV, followed by 100-ms conditioning steps between -120 and -10 mV, before a 200-ms test pulse to +10 mV. Traces for a representative neuron and the relative inactivation for a sample of seven neurons are shown in Fig. 7, Ba and Bb, respectively.
FIG. 6. Electrophysiological characterization of the low-threshold, fast activating α- and MgTX-sensitive K⁺ current. Traces for voltage-dependent activation (A) and inactivation (B) were obtained by subtracting traces elicited in the absence of 20 nM α- and 50 nM MgTX from control external solution from those elicited in their absence. Ab: family of IᵦTX elicited by 150-ms depolarizing pulses ranging from −80 to −10 mV in 10-mV increments from HP −100 mV. Bb: plot of relative activation of IᵦTX and MgTX-sensitive current as a function of the test potential in a sample of 7 and 9 neurons, respectively. Bc: steady-state inactivation of IᵦTX elicited by 200-ms depolarizing pulses to −10 mV preceded by a 10-s conditioning prepulse from −120 to −20 mV from HP −100 mV. D: plot of current amplitudes normalized to maximum current and expressed as Iₘax/Iₘ₀ for IᵦTX and MgTX-sensitive current as a function of the test potential in a sample of 5 and 4 neurons, respectively. In Ab and Bb, current amplitudes were measured 5 ms after the beginning of the prepulse.

IᵦBS-D had Vᵦ₀ of −65.84 ± 0.3 mV, and k of 5.51 ± 0.25 mV (means ± SE).

Time dependence of inactivation and recovery from inactivation of IᵦBS-D were studied using two step protocols in the presence of 20 nM α- and 20 mM TEA to reduce TEA- and α- independent depolarization-activated K⁺ currents. Under these experimental conditions, the Iₘax values and the kinetic characteristics of IᵦBS-D did not change (Table 1). IᵦBS-D evoked by 250-ms test pulses to 0 mV from HP −100 mV were progressively inactivated by increasing the duration of a conditioning prepulse to −50 mV (Fig. 7Ca). After complete inactivation was achieved by holding the membrane potential to −50 mV, IᵦBS-D progressively recovered when the duration of a conditioning prepulse to −100 mV was increased (Fig. 7Da). Time-dependent inactivation and recovery from inactivation of IᵦBS-D estimated in a sample of eight neurons had mean time constants of 25.4 ± 0.7 ms (Fig. 7Cb) and 95.8 ± 4.0 ms (Fig. 7Db), respectively.

Distribution of K⁺ currents among vestibular primary neurons

The three types of depolarization-activated K⁺ current were found in all neurons investigated, with substantial variations in their relative amplitudes, densities, and distributions (Table 2). I₀ was the predominant current making up to 56% of the total current. There was a large variability in the relative expression of the two fast activating currents. The ratio of the density of IᵦBS-D and IᵦTX varied from 0.02 to 2.90 (mean 0.90 ± 1.20; n = 38). No correlation between the size or the capacitance of the recorded neurons and the relative distribution of each current was noticed (data not shown).

Discussion

By using a combination of pharmacological and electrophysiological approaches, we identified and characterized for the first time, three different types of Ca²⁺-independent depolarization-activated K⁺ currents in vestibular primary neurons acutely isolated from postnatal mouse. Experimental condi-
TABLE 2. Comparison of the amplitude, density, and relative proportion of the three depolarization-activated K\(^+\) currents in vestibular neurons

<table>
<thead>
<tr>
<th>Current</th>
<th>Amplitude, pA</th>
<th>Density, nS/pF</th>
<th>Proportion, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I_{\text{TEA}})</td>
<td>3.748 ± 1.325</td>
<td>2.50 ± 1.14</td>
<td>56.4 ± 11.4</td>
</tr>
<tr>
<td>(I_{\text{DTX}})</td>
<td>1.776 ± 1.001</td>
<td>1.16 ± 0.65</td>
<td>25.7 ± 9.8</td>
</tr>
<tr>
<td>(I_{\text{BDS-I}})</td>
<td>1.253 ± 0.700</td>
<td>0.78 ± 0.47</td>
<td>17.8 ± 7.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. Maximum amplitudes of \(I_{\text{TEA}}\), \(I_{\text{DTX}}\), and \(I_{\text{BDS-I}}\) elicited at +20 mV (given in pA) were measured either 5 ms before the end of the test pulse (\(I_{\text{TEA}}\), \(I_{\text{DTX}}\)) or at the peak current (\(I_{\text{BDS-I}}\)). The density of each K\(^+\) current (given in nS/pF) was estimated as the ratio of the maximum slope conductance relative to the cell capacitance. Mean relative proportion of each current is given as a percentage of the total K\(^+\) current density in each neuron.

\(I_{\text{TEA}}\) is characterized by its sensitivity to TEA and its insensitivity to 4-AP in the same way that \(I_k\) described in a majority of DRG neurons (Gold et al. 1996; Kostyuk et al. 1981), in other sensory neurons (Garcia-Diaz 1999; Manis and Marx 1991; McFarlane and Cooper 1991), and in a number of cortical neurons (for review see Locke and Nerbonne 1997a).

\(I_{\text{TEA}}\) exhibits a high threshold of activation (between −30 and −40 mV), displays voltage-dependent activation, with a time course of activation 10 times slower than those of the 4-AP-sensitive currents. Although its half activation potential (−15 mV) is comparable to those found in adult rat DRG neurons (\(I_{\text{Kr}}\) in Gold et al. 1996) and in neurons from the auditory brain stem nuclei (Rathouz and Trussel 1998), it differs notably from several other sensory neurons. For example, its half activation potential is 30 mV more negative that in neonatal rat DRG neurons (McFarlane and Cooper 1991), and 20 mV more negative than in several cortical neurons (see Locke and Nerbonne 1997a). Such a property is of importance regarding its putative function in limiting firing by holding the membrane potential near \(E_k\) with the consequence of inducing rapid accommodation during sustained depolarization (Oyelere and Kocsis 1996).

Although the majority of \(I_k\) in other sensory neurons (Kostyuk et al. 1981; McFarlane and Cooper 1991; Rathouz and Trussel 1998), and other cell types (see Rudy 1988) are usually described as nonactivating currents, \(I_{\text{TEA}}\) displays voltage-dependent inactivation with a half inactivation potential around −65 mV. Similar observations were previously reported in rat visual cortical neurons (Locke and Nerbonne 1997a), and in adult rat DRG neurons (Gold et al. 1996). Gold et al. (1996) showed that two out of the three types of \(I_k\) found in these cells (termed \(I_{\text{Na}}\) and \(I_{\text{Ca}}\)) displayed such properties. In most respects, \(I_{\text{TEA}}\) found in vestibular neurons is identical to \(I_{\text{Kr}}\) described in those cells.

\(I_{\text{DTX}}\) This current shares most of the properties that define \(I_D\) (a \(\alpha\)-DTX–sensitive current) in sensory neurons (Everill et al. 1998; Stansfeld and Feltz 1988; Stansfeld et al. 1986, 1987), namely its low-voltage threshold, fast time course of activation and deactivation, voltage-dependent activation, and partial steady-state inactivation. K\(^+\) currents possessing these properties have been described in a wide variety of neurons (Brew and Forsythe 1995; Foehring and Surmeier 1993; Rathouz and Trussel 1998; Reid et al. 1999; Southan and Robertson 1998; Storm 1988). In some preparations, discrepancies can be noticed in the voltage dependence for activation (Locke and Nerbonne 1997a; McFarlane and Cooper 1991; Wu and Barish 1992) and in the sensitivity to \(\alpha\)-DTX (Everill et al. 1998; Wu and Barish 1992). Such discrepancies could be the consequence of a lack of selectivity of \(\alpha\)-DTX, when used over nanomolar concentration range, since other types of K\(^+\) channels have been reported to be sensitive to \(\alpha\)-DTX. For example, hippocampal neurons express a population of \(I_A\) channel (Ficker and Heinemann 1992) that possess a binding site for \(\alpha\)-DTX (Halliwell et al. 1986), whereas \(I_A\) channels are usually reported to be insensitive to \(\alpha\)-DTX. In our experimental conditions, no effect of \(\alpha\)-DTX on the kinetics or the voltage dependence of \(I_{\text{BDS}}\) was noticed. Similarly an \(\alpha\)-DTX sensitivity has been described for \(I_k\) in guinea pig DRG neurons (Penner et al. 1986). This was not the case in the present study. Another explanation for the variability is that several types of \(\alpha\)-DTX–sensitive K\(^+\) currents, distinct from \(I_A\) or \(I_k\) are expressed in different neurons, and sometimes within a single neuron as reported in human peripheral myelinated neurons (Reid et al. 1999). To determine whether a single type or several populations of \(I_{\text{DTX}}\) are present in vestibular primary neurons, it will be important to determine which \(\alpha\)-subunits form the \(I_{\text{DTX}}\) K\(^+\) channels. The coupled sensitivities of \(I_{\text{DTX}}\) to \(\alpha\)-DTX and MgTX we described here is a first step in this way. Only three types of \(\alpha\)-subunit, Kv1.1, Kv1.2, and Kv1.6, have been reported to be sensitive to \(\alpha\)-DTX, and only Kv1.2 is insensitive to TEA (Grissmer et al. 1994). Although this observation suggests that Kv1.2 is one of the \(\alpha\)-subunits that form the \(I_{\text{DTX}}\) channel in vestibular neurons, it cannot be excluded that Kv1.1 and Kv1.6 may also be involved in the structure of this channel, since they have been described as heteromeric structures with Kv1.2 in K\(^+\) channels of the rat cerebellum (Koch et al. 1997). The involvement of \(I_{\text{DTX}}\) in limiting repetitive firing both in sensory (Stansfeld et al. 1986) and cortical neurons (Brew and Forsythe 1995; Wu and Barish 1992) is widely accepted. However, the precise role of \(I_D\) in shaping a single action potential remains somewhat uncertain. By directly measuring the action potential–evoked Ca\(^{2+}\) rise in basket cell terminals, Tan and Llano (1999) indicated that 4-AP–sensitive K\(^+\) channels are involved in depolarizing the action potential, but not the \(\alpha\)-DTX–sensitive ones. Opposite observations were made in rat visual cortical neurons, where \(I_D\) plays an important role in action potential depolarization (Locke and Nerbonne 1997b). Other authors suggest that \(I_D\) might be a large component of the afterhyperpolarization of action potential in CA1 pyramidal neurons (Golding et al. 1999), or rather be involved in the control of resting membrane potential in basket cells terminals (Robertson and Southan 1999).
I_{BDS}

I_{BDS} shares most of the properties previously reported for I_A in a wide variety of neurons (Rudy 1988). I_A is thought to modulate the timing of repetitive action potential generation, the repolarization of single action potential, and the time required to reach the threshold to fire an action potential (Storm 1988; Tan and Llano 1999; Wu and Barish 1992). A threshold for activation around −60 mV, an absence of sensitivity to α-DTX, and time constants for activation and inactivation in the same range to those of I_{BDS-I} have been described for I_A (rise time <0.5 ms; decay time 10–25 ms at 0 mV) in DRG neurons (Stansfeld et al. 1987). Kinetics and voltage dependence for activation and inactivation in the same range as those found for I_{BDS-I} were reported for I_A in cochlear ganglion neurons (Garcia-Diaz 1999) [\tau_{ac} at 0 mV <0.2 ms; \tau_{ac(1/2)} = −38.4 mV; \tau_{inac(1/2)} = −75 mV], and in nodose neurons (McFarlane and Cooper 1991) [\tau_{ac} at 10 mV = 1–1.5 ms and decay time 10–30 ms; \tau_{ac(1/2)} = −21 mV; \tau_{inac(1/2)} = −73 mV]. I_{BDS-I} found in vestibular neurons displays a kinetic of recovery from inactivation slower than those reported in dorsal cochlear nucleus pyramidal cells (Kanold and Manis 1999), and in basal ganglia and basal forebrain neurons (Tkatch et al. 2000), but faster than those reported in hippocampal neurons (Martina et al. 1998; Wu and Barish 1992), and in striatal neurons (Song et al. 1998). Attempts to identify the subunits that form I_A-type channels in other neurons revealed that Kv 1.4, Kv 3.4 and Kv 4.2 and Kv 4.3 α-subunits underlie the transient K_+ current (Diochot et al. 1998; Ohya et al. 1997; Rudy et al. 1999). Analysis of the biophysical properties of the current indicated that the time course of inactivation of I_A tends to be voltage independent when carried by various Kv 4 channels, and highly voltage dependent, as is the case for I_{BDS-I}, when carried by other subunits (Everill et al. 1998). An interesting observation is the fact that in our preparation, I_{BDS-I} displays a sensitivity to nanomolar concentrations of BDS-I. This result is of interest first because it constitutes the first demonstration of a BDS-I sensitivity of a neuronal I_A, and second because it suggests the identity of at least one of the subunits that form the channel, since the toxin isolated from sea anemone is a selective blocker of the K_+ channels form with Kv3.4 α-subunit (Diochot et al. 1998). Diochot et al. (1998) also reported a small blocking effect of BDS-I on the Kv1.2 containing K_+ channel expressed in COS cells. It is unlikely, however, that I_{BDS-I} is in fact an inactivating component of the I_0 current since the concentration of BDS-I we used was 40 times smaller than those used in the COS-transfected cells. Again, alternative experimental approaches will be needed to determine which α-subunits form the I_{BDS-I} channels present in vestibular neurons, and whether they are assembled in heteromeric structures.

Our results demonstrate that the distribution of the three distinct depolarization-activated K_+ currents in vestibular neurons is heterogeneous. Although I_{TEA} is the major current, the proportion of I_{DTX} and I_{BDS-I} varies considerably from one neuron to another. Whether these observations reflect different functional populations of neurons remains uncertain since the current density ratios of I_{BDS-I}/I_{DTX} within neurons are distributed as a range rather than a distribution into distinct groups. Moreover, there was no obvious correlation between the size or the capacitance of the recorded cells and the respective densities of the two 4-AP–sensitive currents. Similar observations have been reported in rat DRG neurons (Everill et al. 1998), where I_K, I_D, and I_A are expressed with a large variability between cells, and as a range rather than in distinct sub-groups.

The role that each of the three K_+ currents plays in determining the electrophysiological properties of vestibular neurons now needs to be correlated with the different firing patterns (regular and irregular), exhibited by the primary afferent (Goldberg et al. 1984). The presence of the three types of K_+ currents within all the neurons investigated and the large variability in the expression of the two fast activating currents suggest that, if they are involved in shaping action potential, a variation in their relative expression would be sufficient to account for the diversity in the electrical activity recorded in these neurons. The use of specific toxins should permit determination of the involvement of each of the voltage-activated K_+ currents in the electrical activity of the vestibular neurons. However, such investigations should be carefully conducted, since these compounds could interact with other voltage-activated ionic conductances (e.g., BDS-I has a slight effect of on sodium current) (Diochot et al. 1998).

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