Low-Voltage-Activated Potassium Channels Underlie the Regulation of Intrinsic Firing Properties of Rat Vestibular Ganglion Cells

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1Department of Otolaryngology, University of Tokyo School of Medicine, Tokyo; 2Department of Biochemistry and Cellular Biology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo; 3Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, Chiba; and 4Department of Physiology, Tsurumi University School of Dentistry, Yokohama, Japan

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Iwasaki S, Chihara Y, Komuta Y, Ito K, Sahara Y. Low-voltage-activated potassium channels underlie the regulation of intrinsic firing properties of rat vestibular ganglion cells. J Neurophysiol 100: 2192–2204, 2008. First published July 16, 2008; doi:10.1152/jn.01240.2007. Individual primary vestibular afferents exhibit spontaneous activity the regularity of which can vary from regular to irregular. Different aspects of vestibular responsiveness have been associated with this dimension of regularity of resting discharge. Isolated rat vestibular ganglion cells (VGCs) showed heterogeneous intrinsic firing properties during sustained membrane depolarization: some neurons exhibited a strong adaptation generating just a single or a few spikes (phasic type), whereas other neurons showed moderate adaptation or tonic firing (tonic type). Tonic discharging VGCs were rare at postnatal days 5–7 and increased up to ~60% of neurons during postnatal 2–3 wk. To explore the major factors responsible for the discharge regularity of primary vestibular afferents, we investigated the contribution of K+ channels to the firing properties of isolated rat VGCs. Phasic firing became tonic firing in the presence of 4-aminopyridine or α-dendrotoxin, indicating that Kv1 potassium channels control the firing pattern of the phasic VGCs. Tetraethylammonium decreased the number of spikes during step current stimuli in all types. Blockade of Ca2+-activated K+ channels decreased the number of spikes in tonic VGCs. Our results suggest that Kv1 channels are critical both in determining the pattern of spike discharge in rat vestibular ganglion neurons and in their proportional change during maturation.

INTRODUCTION

Primary vestibular neurons convey afferent information from the inner ear vestibular mechanoreceptors to vestibular nuclei and cerebellum. The three semicircular canals in the inner ear sense angular acceleration, whereas the two otolith organs (saccular and utricular maculae) sense linear acceleration and static tilt of the head (Baloh and Honrubia 2001). The sensory axons in the somatosensory and visual pathways are known to differ in their peripheral terminations, discharge properties, and central projections. Several studies have investigated whether the vestibular afferents innervating individual endorgans show a similar diversity of discharge properties. Three classes of firing properties of vestibular afferents have been characterized in recordings from in vivo preparations (Baird et al. 1988; Goldberg 2000; Goldberg et al. 1990a,b; for a review, see Goldberg 1991). The vestibular afferents show diverse spontaneous firing rates ranging from regular to irregular. Irregular neurons, which show large variability in interspike intervals of spontaneous firing, have greater sensitivity and phasic response dynamics to angular and linear accelerations acting on the head, large responses to externally applied currents, and larger axons with calyx terminals. Regular neurons, which show smaller variability in interspike intervals, have lower sensitivity and tonic response dynamics to angular and linear accelerations, and smaller axons with bouton terminals. Afferents with dimorphic terminals show intermediate properties between the irregular and regular neurons. Physiological properties of vestibular afferents undergo marked developmental changes. In rats, Curthoys (1979) showed that frequency of the spontaneous activity of primary vestibular afferents, which are already present at birth, increase through postnatal periods and that most neurons are irregularly discharging at birth, but the number of regular discharging neurons increase during the first 10 postnatal days. Responses of the vestibular system to angular acceleration stimulations also showed an increase in sensitivity during the postnatal periods (Curthoys 1979). Similar developmental changes of the firing properties of the vestibular afferents have been reported in mice using explants containing the inner ear and the vestibular ganglion cells (Desmedryl 1991; Desmedryl et al. 1986).

What are the factors that determine these differences in firing regularity and dynamic responses of vestibular neurons? Neither the types of hair cells they innervate nor types of synaptic inputs provide a conclusive answer to this question because vestibular neurons of bullfrogs, which lack type I hair cells and calyceal synapses, can still be grouped into irregular and regular discharging types (Honrubia et al. 1989). To answer the question, it is necessary to understand the cellular mechanism that determines neuronal excitability and the time between successive action potentials. In this regard, potassium channels are crucial regulators of neuronal excitability, setting resting membrane potentials and firing thresholds, repolarizing action potentials and limiting excitability. In the central auditory nuclei (Banks and Smith 1992; Brew and Forsythe 1995; Manis and Marx 1991) and vestibular nuclei (Gamkrelidze et al. 1998), low-voltage-activated potassium currents play essential roles in determining whether these neurons are capable of firing trains of action potentials or show accommodation. Smith and Goldberg (1986) suggested that the slope of the afterhyperpolarization, which is partly dependent on calcium-activated potassium...
channels, can account for the differences in firing regularity and sensitivity to galvanic currents.

To understand the control mechanism governing the discharge regularity of primary vestibular afferents, we explored the intrinsic firing properties of isolated rat vestibular ganglion cells (VGCs), by which we could examine the intrinsic membrane properties of neuronal cell bodies free from synaptic inputs. We also explored the role of potassium channels on their firing properties. The results showed that VGCs have heterogeneous intrinsic firing properties in response to sustained membrane depolarization with a striking difference in firing accommodation. Analysis of the firing properties of the VGCs on different postnatal days showed that the firing properties change during early postnatal period. Heterogeneous potassium currents such as voltage-activated potassium current and calcium-activated potassium currents have been reported in VGCs (Chabbert et al. 2001; Limón et al. 2005; Risner and Holt 2006). In this study, analysis of the correlation between differential expression of low-voltage-activated potassium currents and intrinsic firing properties of VGCs showed that the Kv1 channel, which is sensitive to α-dendrotoxin, influences the patterns of spike discharges of rat VGCs. Some of the results presented here have appeared in an abstract form (Iwasaki et al. 2007).

METHODS

Cell isolation

Rats were killed by decapitation in accordance with the Japanese Animal guidelines of the National Center of Neurology and Psychiatry. Superior vestibular ganglia, which innervate the utricular macula and the horizontal and anterior cristae, were isolated from neonatal [postnatal days (PD) 5–7] or juvenile (PD12–16 and PD23–27) Sprague-Dawley rats. The dissected vestibular ganglia were incubated in Hank’s solution (Gibco, Gaithersburg, MD) with papain (20 U/ml; Worthington Biochemical, Freehold, NJ) at 37°C for 20 min. Cells were dissociated by trituration using a sterile Pasteur pipette, and subsequently plated onto poly-l-lysine-precoated 35-mm culture dishes. The plating medium contained Leibovitz’s L-15 solution (Gibco BRL, Grand Island, NY), 10% fetal calf serum, 26 mM NaHCO3 and 30 mM glucose. Cells were maintained in a humidified atmosphere of 95% air-5% CO2 at 37°C. The cells were used for electrophysiological measurements after 3 h after plating to ensure that VGCs did not show any action potentials within 3 h after plating; however, we were able to record stable action potentials and K+ currents between 6 and 12 h after plating.

Electrophysiological recordings

Whole cell recording was carried out with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) at room temperature (23–27°C). Cells were visualized under phase contrast on an inverted microscope (Olympus IX-70, Tokyo, Japan) or under a ×40 water-immersion lens (Olympus Optical) attached to an upright microscope (Axioskop, Zeiss, Oberkochen, Germany). VGCs were identified by the morphological features described by Desmadril et al. (1997), i.e., spherical shape with refringent cytoplasmin, and had a larger diameter. Pipettes for whole cell recording contained (in mM) 105 potassium gluconate, 30 KCl, 1 CaCl2, 2 MgCl2, 5 ethylene diaminetetraacetic acid (EDTA), and 10 N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (HEPES; pH = 7.3); osmolality was adjusted to 300 mOsm/l. The extracellular solution for current-clamp recording contained (mM) 150 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, and 10 glucose (pH = 7.3, osmolality: 310 mOsm/l). Pipette resistance of the whole cell pipettes was 5–7 MΩ. The junction potential between internal and external solutions was +8 mV and was not corrected for. The capacitance cancellation was made to minimize the slowest component of capacitative currents elicited by a 5-mV voltage step; whole cell membrane capacitance (Cm) was read from the corresponding dial. The series resistance was compensated (60–80%) in the voltage-clamp experiments. Signal recordings and acquisition were controlled by pClamp 9.0 software (Axon Instruments) using a 12-bit data acquisition system (Digidata1332, Axon Instruments). For current-clamp recording, I-clamp fast mode was always used. For voltage-clamp recordings, VGCs were held at –90 mV, and depolarizing voltage steps were applied every 15 s. Tetrodotoxin (TTX; 1 μM) and CdCl2 (100 μM) were added to block the Na+ and Ca2+ currents, respectively. Leakage and capacitative currents were subtracted by using the P/4 procedure of the pClamp (Axon Instruments).

Data analysis

Signals were low-pass filtered at 5 kHz (Bessel 8 pole, 48 dB/octave) and digitized at 10 kHz. The digitized records were analyzed off-line using Axograph (Axon Instruments). Current amplitudes were averages of five consecutive data points around the measurement time, usually 10 ms after the start of voltage steps. K+ conductance (Gk) was calculated from the equation Gk = I/(E – EK), where (E) is the amplitude of the K+ current, E is the membrane potential, and EK is the reversal potential for potassium (–83 mV). The normalized conductance was fitted to the Boltzmann function Gmax = l/[1 + exp ((Em – EK)/k)], where Em is the membrane potential, EK is the half-activation potential, and k is the slope factor. Statistical comparisons were performed with SigmaStat software (version 3.0, SPSS, Chicago, IL). Data are presented as means ± SE unless otherwise noted. Group data with normal distribution (Shapiro Wilk W test) were compared using the t-test, while those showing skewed distribution were compared by the Mann-Whitney U-test. A P value of <0.05 was considered significant.

Morphological measurements

The cell diameters and the cross-sectional areas of VGCs used in the electrophysiological measurements were measured. For each VGC, an image was acquired with a CCD camera (Sony) mounted on the microscope (Axioskop, Zeiss, Oberkochen, Germany) equipped with a ×40 water-immersion lens (Olympus Optical) and differential interference contrast optics, digitized at a flatbed scanner (Canon) at a resolution of 500 dpi, and the cell diameters and the area within the outline were measured with National Institutes of Health image software (version 1.61, National Institute of Health).

RESULTS

Heterogeneous intrinsic firing properties of dissociated vestibular ganglion cells

We recorded the voltage responses of rat VGCs to depolarizing current stimuli in the current-clamp mode. Data were obtained from VGCs that fired spikes in response to depolarizing current stimuli and had resting membrane potentials of less than –50 mV. There was no spontaneous firing of action potentials observed in any VGCs.
Marked differences in firing properties were noted among the VGCs obtained from neonatal rats (PD5–7) with some VGCs showing sustained spike trains lasting throughout the stimulation period, whereas others exhibited a strong adaptation generating just a single spike or a short burst of spikes (Fig. 1A). Based on their firing patterns in response to depolarizing current pulses, the VGCs were categorized into three types (Fig. 1A). The first type of neurons showed strong firing adaptation, a single action potential at onset or a few spikes in response to strong stimulation (phasic type). The second type of neurons showed moderate firing adaptation (intermediate type). The third type of neurons showed no sign of spike frequency adaptation during prolonged depolarizing stimuli (tonic type). The latter type of neurons fired a burst of action potentials at frequency of ~60 Hz.

For quantification of the firing properties of VGCs, we used the maximal duration of the spike train in response to 480 ms current step stimuli ($D_{\text{max}}$; Fig. 1B) and defined $D_{\text{max}} < 50$ ms as “phasic,” $D_{\text{max}} > 400$ ms as “tonic,” and $D_{\text{max}}$ between 50 and 400 ms as “intermediate.” At PD5–7, the majority (66%, 173 of 261 cells) of VGCs belonged to the phasic type. The intermediate type and tonic type comprised 18% (47 of 261 cells) and 16% (41 of 261 cells) of VGCs tested, respectively (Fig. 1B). Figure 1C shows the relationship between current intensity and the number of action potentials (APs) during depolarization in each cell type.

We measured the cross-sectional area and the cell capacitance of VGCs to relate the electrophysiological properties of neurons with their morphological features. The mean cross-sectional areas of the phasic, intermediate, and tonic VGCs were 394.2 ± 10.3 µm² ($n = 163$), 408.3 ± 19.5 µm² ($n = 45$), and 404 ± 16.1 µm² ($n = 40$), respectively (Fig. 1D). There were no significant differences in the cross-sectional area among the three types of VGCs ($P > 0.5$: ANOVA). The mean value of cell capacitance was 11.5 ± 0.4 pF ($n = 163$) in the phasic, 11.7 ± 0.6 pF ($n = 45$) in the intermediate, and 12.2 ± 0.6 pF ($n = 39$) in the tonic type. There were no significant differences in cell capacitance among the three types of VGCs ($P > 0.5$: ANOVA). However, VGCs with

![Fig. 1](https://www.jn.org/article-pdf/100/10/2194/2194-iwasaki.pdf)
extraordinarily large cross-sectional area (>650 μm²) and large capacitance (>22 pF) tended to be the phasic type.

### Developmental changes in intrinsic firing properties of VGCs

To examine whether the firing properties of VGCs change with development, we also recorded the voltage responses to depolarizing current stimuli in VGCs obtained from PD12–16 and PD20–22 rats. In our experimental conditions, the number of living VGCs decreased markedly as rats matured. We could record about two to three neurons in each preparation obtained from 20 ganglia of rats older than PD12, whereas we could record about five to seven neurons in each preparation from 20 ganglia of PD5–7 rats. The width of APs and duration of afterhyperpolarization became smaller as the rats matured in the phasic VGCs, whereas those developmental changes in the waveforms were not apparent in the intermediate and tonic VGCs (Fig. 2A). At each postnatal day, all three types of firing patterns were seen (Fig. 2A). However, the distribution of firing patterns markedly changed with development (Fig. 2B). Although the phasic type formed the majority (66%) of VGCs at PD5–7, the proportion decreased to only 23% (8 of 35 cells) at PD12–16. In contrast, the tonic type increased from 14 to 60% (21 of 35 cells) during the period between PD5–7 and PD12–16. The percentages of the intermediate type changed little during the same period (20% at PD5–7 and 17% at PD12–16). The distributions of the firing patterns were similar between PD12–16 and PD20–22: the phasic type formed 28% of the cells (9 of 32 cells), intermediate type 22% (7 of 32 cells), and tonic type 50% (16 of 32 cells) at PD20–22. These results indicate that the firing patterns of VGCs change between PD5–7 and PD12–16. Figure 2C shows the relationship between the current intensity and the number of APs during depolarization at each developmental stage, suggesting developmental increase in gain of the responses to depolarizing stimuli occur between PD5–7 and PD12–16.

We compared morphology of the VGCs with DIC optics at different postnatal periods (Fig. 2D). The mean diameters of the VGCs were 22.8 ± 0.3 μm at PD5–7 (n = 250), 22.3 ± 0.6 μm (n = 35) at PD12–16, and 22.2 ± 0.5 μm at PD20–22 (n = 32). The cross-sectional areas of the VGCs were 398.6 ± 8.3 μm² at PD5–7 (n = 250), 396.2 ± 23.2 μm² at PD12–16 (n = 35), and 394.3 ± 19.3 μm² at PD20–22 (n = 32). There were no significant differences in the diameter or cross-sectional areas among the three postnatal periods (P > 0.5:...
The firing type of VGCs on the current-clamp mode in PD5–7 rats (Fig. 3, A) was different from those in the phasic VGCs (Fig. 3, B). Potassium currents in the phasic VGCs were activated at around –30 mV, whereas those in the tonic VGCs were activated at around –50 mV, suggesting the presence of low-voltage-activated K⁺ channels in phasic VGCs.

In phasic VGCs, the low-voltage-activated component was mostly blocked by α-DTX, a high-affinity blocker of Kv1 channels (Fig. 4A). The α-DTX-sensitive component showed little inactivation during 500 ms (Fig. 4A). The remaining α-DTX-insensitive currents activated at more positive potentials were attenuated by a low concentration of TEA (1 mM; Fig. 4B). The voltage dependence of the α-DTX- and TEA-sensitive conductances were fitted using a Boltzmann equation (Fig. 4C; see METHODS). The half-activation potential (V1/2) for the α-DTX-sensitive conductance was –44.8 mV with a slope factor (k) of 8.4 mV, whereas those for the TEA-sensitive conductance were –4.5 and 15.3 mV, respectively.

**Different voltage dependence of potassium currents between phasic and tonic VGCs**

To explore the mechanism underlying the differences in the firing properties of VGCs, we investigated the properties of K⁺ currents on the voltage-clamp mode after determining the firing type of VGCs on the current-clamp mode in PD5–7 rats (Fig. 3, A and B). Potassium currents were evoked by stepping from a holding potential of –90 to +50 mV in 10-mV increments in the presence of TTX (1 μM) and Cd²⁺ (100 μM). The current-voltage relationships in phasic (n = 9) and tonic (n = 8) VGCs showed different thresholds for activation of the K⁺ currents in the two types of cells (Fig. 3C). Potassium currents in the phasic VGCs were activated at around –30 mV, whereas those in the tonic VGCs were activated at around –50 mV, suggesting the presence of low-voltage-activated K⁺ channels in phasic VGCs.

All values are means ± SE n values are in parentheses. Cₘ, membrane capacitance; Vₑ, resting membrane potential; Rᵢₑ, input resistance.

**TABLE 1. Electrophysiological properties of vestibular ganglion cells at different postnatal periods**

<table>
<thead>
<tr>
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<th>PD5–7</th>
<th>PD12–16</th>
<th>PD20–22</th>
<th>Difference</th>
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<tbody>
<tr>
<td>Cₘ, pF</td>
<td>11.6 ± 0.3 (247)</td>
<td>12.2 ± 1.1 (35)</td>
<td>11.2 ± 0.6 (32)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Vₑ, mV</td>
<td>–58.0 ± 0.7 (247)</td>
<td>–59.1 ± 1.5 (35)</td>
<td>–59.2 ± 1.6 (32)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Rᵢₑ, GΩ</td>
<td>0.27 ± 0.1 (211)</td>
<td>0.32 ± 0.04 (35)</td>
<td>0.28 ± 0.02 (32)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Threshold for firing spikes, pA</td>
<td>30.0 ± 2.0 (261)</td>
<td>28.2 ± 2.6 (35)</td>
<td>27.1 ± 1.9 (32)</td>
<td>&gt;0.5</td>
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There were no significant differences in these properties among the VGCs at the three postnatal periods.

**α-DTX-sensitive current underlies the intrinsic firing characteristic of phasic VGCs**

We next examined the effects of α-DTX and TEA on the firing properties of the phasic VGCs obtained from PD5–7 rats in the current-clamp mode. Application of α-DTX markedly increased the maximal number of APs in response to current steps by 622.3 ± 19.4% (n = 9; P < 0.01) with little effect on the size or shape of APs (Fig. 5A). In the presence of α-DTX, the phasic VGCs showed sustained AP trains that lasted throughout the stimulation period. The number of APs gradually decreased with increased injected currents (Fig. 5, A and B). This firing property was different from those of the typical tonic VGCs, which showed monotonic increase in the number of APs, suggesting the contribution of other factors to heterogeneous firing properties among VGCs. The addition of TEA

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**FIG. 3. Different outward currents between phasic and tonic VGCs obtained from PD5–7 rats. A: voltage (left) and current (right) responses in a phasic VGC. The outward currents were evoked by command pulses from –90 to +40 mV by 10-mV steps in the presence of TTX (1 μM) and Cd²⁺ (100 μM). B: voltage (left) and current (right) responses in a tonic VGC. C: the current-voltage (I-V) relationships of phasic (n = 9) and tonic (n = 8) VGCs. Current amplitudes were measured at 10 ms from the onset of a command pulse.**
in the presence of α-DTX reduced the number of AP firing during the current steps by 69.9 ± 5.4% (n = 4) as well as increasing the width of each AP by 140 ± 25% (n = 4; Fig. 5B). Neither α-DTX nor TEA significantly altered the resting membrane potentials (0.63 ± 1.4 mV by α-DTX, n = 9; 2.25 ± 2.4 mV by TEA, n = 4; P > 0.5 per each drug). Similar effects of α-DTX and TEA on the firing properties were also observed in the phasic VGCs obtained from both PD12–16 (n = 3) and PD20–22 rats (n = 3).

We tested drugs that have high affinity for subunits of the Kv1 subfamily on the firing properties of the phasic VGCs obtained from PD5–7 rats: 4-AP (1 mM) and three toxins, MgTx (10 or 20 nM), DTX-k (100 or 200 nM), and TsTx (100 or 200 nM). MgTx primarily blocks channels containing Kv1.3 subunits (Garcia-Calvo et al. 1993), whereas DTX-k and TsTx specifically block channels containing Kv 1.1 and Kv1.2 subunits, respectively (Hopkins 1998). The broad-spectrum voltage-gated potassium channel blocker 4-AP increased AP firing in phasic VGCs, whereas MgTx (n = 5 at 10 nM and n = 2 at 20 nM), DTX-k (n = 5 at 100 nM and n = 2 at 200 nM), or TsTx (n = 4 at 100 nM and n = 2 at 200 nM) did not have any effect on the AP firing in those VGCs (Fig. 6A). We also tested the effects of these drugs on K⁺ currents evoked in the phasic VGCs (Fig. 6B). The low-voltage-activated components of the K⁺ currents up to ~30 mV were mostly blocked by 4-AP (n = 4), whereas these components were hardly affected by MgTx, TsTx, or DTX-k (n = 3 per each drug). The voltage dependence of the 4-AP-, MgTx-, and DTX-k-sensitive conductances could be fitted using a Boltzmann equation, whereas that of the TsTx-sensitive conductance could not be fitted by the equation. The half-activation potential (V1/2) was −18.8 mV for the 4-AP-sensitive conductance, −5.9 mV for the MgTx-sensitive conductance, and −12 mV for the DTX-k-sensitive conductance. The slope factor (k) was 17.1 mV for the 4-AP-sensitive conductance, 7.2 mV for the MgTx-sensitive conductance, and 8.5 mV for the DTX-k-sensitive conductance. Because α-DTX blocks the three potassium channel subunits, Kv1.1, Kv1.2, and Kv1.6, these data suggest that Kv1.6 or heteromeric channels containing Kv1.6 may contribute to the control of AP firing of phasic VGCs through the low-voltage activated component of K⁺ currents.

Effects of α-DTX and TEA on AP firing and potassium currents in tonic VGCs

We tested the effects of α-DTX and TEA on the firing properties of tonic VGCs obtained from PD5–7 rats (Fig. 7A). Application of α-DTX did not alter the mean number of APs during prolonged current injection in tonic VGCs (n = 5). The addition of TEA in the presence of α-DTX reduced the number of AP firing by 41.1 ± 5.5% (P < 0.01) during the current steps with
increasing the width of each AP by 82.9 ± 12% \((P < 0.05, n = 5)\). We also tested the effects of \(\alpha\)-DTX and TEA on \(K^+\) currents evoked in the tonic VGCCs (Fig. 7, B and C). \(\alpha\)-DTX did not affect the \(K^+\) currents \((n = 4)\), whereas TEA blocked the \(K^+\) currents activated at more positive potentials than \(-20 mV\) by 75.9 ± 6.3% \((n = 4)\). These results suggest that tonic VGCCs have little \(\alpha\)-DTX-sensitive conductance, while TEA-sensitive conductance has some role in the frequency and duration of AP firing.

**Effects of \(\alpha\)-DTX on AP firing in intermediate VGCCs**

We tested the effects of 4-AP, \(\alpha\)-DTX, TEA, MgTx, TsTx, and DTX-k on the firing properties of the intermediate VGCCs (Fig. 8). 4-AP, \(\alpha\)-DTX, MgTx, TsTx, and DTX-k increased the number of AP firing in intermediate VGCCs and changed the pattern to sustained firing during the depolarizing stimuli \((n = 4–5 per each blocker)\), suggesting contribution of multiple \(K^+\) channels in the control of the later component of AP firing in the intermediate VGCCs. On the other hand, TEA decreased the number of APs by 49.1 ± 11.6% in the intermediate VGCCs accompanied by increase in the width of each AP by 28.4 ± 7.9% \((n = 5)\).

**Contribution of calcium-activated potassium current to firing property of VGCCs**

\(Ca^{2+}\)-activated potassium channels modulate the AP waveform and repetitive firing properties of neurons (Dutia and Johnson 1998; Sah and Faber 2002). Two types of \(Ca^{2+}\)-activated potassium channels, named BK and SK, are specifically blocked by iberiotoxin (IbTx) and apamin, respectively. We examined the effects of IbTx (100 nM) and apamin (100 nM) on the firing properties of VGCCs obtained from PDS-7 rats (Fig. 9, A and B). Neither IbTx nor apamin had any effect.
on the firing properties of phasic VGCs (n = 5 each). On the other hand, IbTx decreased the number of APs by 62.5 ± 8.3% in intermediate VGCs (n = 3) and by 48.6 ± 3.6% in tonic VGCs (n = 4), suggesting that BK channels contribute to maintaining AP firing in the tonic and intermediate VGCs. Apamin did not have any effect on the firing properties in the tonic VGCs (5.1 ± 8.8%, n = 4) but increased the number of APs by 120.5 ± 36% in intermediate VGCs (n = 4).

Neither IbTx nor apamin altered the resting membrane potentials of VGCs significantly (for IbTx: 0 ± 1.3 mV in phasic VGCs, 1.7 ± 1.8 mV in intermediate VGCs, and 1.8 ± 1.6 mV in tonic VGCs; for apamin: 0.3 ± 1.9 mV in phasic VGCs, 0 ± 1.1 mV in intermediate VGCs, and 0.25 ± 1.7 mV in tonic VGCs; P > 0.1).

**DISCUSSION**

By using whole cell current-clamp recording in acutely dissociated rat VGCs, we have shown that VGCs have heterogeneous firing properties during sustained membrane depolarization where a low-voltage-activated, noninactivating K⁺ current acts as a principal ionic mechanism underlying firing adaptation. The majority (66%) of VGCs exhibited a strong adaptation generating just a single or a few spikes at PD5–7, but these turned into a minority (23%) during the 2–3 wk of postnatal period and the regular discharging VGCs (tonic type) increased up to ~60%. It has been shown that primary vestibular afferents have immature physiological properties for the first few postnatal weeks in rats and mice (Curthoys 1979; Desmadryl 1991; Desmadryl et al. 1986). Frequency of the spontaneous activities, which are already present at birth, showed an increase through postnatal periods, and the regular discharging vestibular afferents are rare at birth, but start to increase reaching up to ~40% during the postnatal period (Curthoys 1979). Sensitivities of the responses to angular acceleration stimulations and galvanic stimulations also showed an increase during the postnatal periods (Curthoys 1979; Desmadryl 1991). Low-voltage-activated K⁺ channels that underlie the firing adaptation of isolated VGCs might contribute to the maturation of discharge properties of primary vestibular afferents.

**Heterogeneous intrinsic firing properties of rat VGCs**

The relationships between afferent morphology and physiology have been studied in mammals (Baird et al. 1988; Curthoys 1979; Goldberg 2000; Goldberg et al. 1990a,b), chicks (Yamasita and Ohmori 1990), and bullfrogs (Honrubia et al. 1989). In the adult chinchilla, the irregularly discharging afferents have phasic response dynamics and high sensitivity to angular and linear
accelerations acting on the head and have thick axons ending as calyces or dimorphic terminals, whereas the regularly discharging afferents have tonic response dynamics and low sensitivity to acceleration acting on the head and have thin axons ending as boutons and dimorphic terminals (Goldberg 2000). On the other hand, Yamashita and Ohmori (1990) found that in the newborn chicken, calyx neurons were regularly discharging and showed tonic increase of firing frequency in response to mechanical stimuli, whereas bouton neurons were irregularly discharging and showed phasic increase of firing frequency in response to the same stimuli. Honrubia et al. (1989) found that vestibular neurons of bullfrogs, which lack type I hair cells and calyceal synapses, can still be grouped into irregular and regular discharging types and showed a broad range of response dynamics to rotational stimuli. Thus the diversities in firing regularity and dynamic responses of vestibular afferents cannot be solely explained by types of hair cells they innervate or types of synaptic input.

We did not find any spontaneous firing of APs in the isolated VGCs at any developmental stages between PD5 and PD22. Spontaneous firing of vestibular afferents has been observed on extracellular recordings from in vivo preparations (Curthyos 1979; Goldberg 2000) and in vitro explants containing the vestibular afferents and the labyrinths (Desmadryl et al. 1986). One possible reason that can account for the lack of spontaneous firing in the present study is that our preparation lack synaptic contacts between hair cells and vestibular afferents while others preserve these contacts. This explanation is consistent with Goldberg’s suggestion that spontaneous activities of vestibular afferents arise from activities of hair cells or afferent synapses (Goldberg 2000). The lack of spontaneous firing in our preparations prevented us from classifying our neurons as irregular or regular and comparing our results with those obtained from in vivo preparations directly.

We showed that rat VGCs have diverse intrinsic firing properties even in the absence of peripheral synaptic input and were able to classify them into phasic, intermediate, and tonic types based on the maximal duration of the spike trains in response to sustained depolarizing stimuli. Risner and Holt (2006) reported diverse firing properties of postnatal mouse VGCs and categorized them into low- and high-threshold neurons according to the thresholds required to evoke APs with current injections. Their high- and low-threshold types may partly correspond to the phasic and tonic types of our classification, respectively, because most low-threshold neurons fired multiple APs in response to depolarizing stimuli. We hypothesize that the heterogeneous intrinsic firing properties can contribute to the diversity of the afferent firing characteristics as well as the response properties at the hair cells and at the afferent terminals. It has been reported that irregular neurons tend to show phasic response dynamics to rotational stimulus acting on the head or mechanical stimulus to hair cells in the semicircular canals, whereas regular neurons tend to show tonic response dynamics to the same stimuli in in vivo preparations (Baird et al. 1988; Fernandez and Goldberg 1976; Goldberg and Fernandez 1971) and in vitro preparations con-

**FIG. 7.** Effects of α-DTX and TEA on the firing properties of tonic VGCs obtained from PD5–7 rats. A: the response of a tonic VGC to a current injection of 60 pA before (left) and after application of α-DTX (middle) and co-application of α-DTX and TEA (right). Dependence of the mean number of APs during 480-ms step current injection in tonic VGCs on the control external solution, α-DTX, and co-application of α-DTX and TEA (n = 5 each). B: the K+ current of a tonic VGC before and after application of α-DTX in response to command pulses from –90 to +30 mV (left). The average current-voltage relationships before and after application of α-DTX (n = 3; right). C: the K+ current of a tonic VGC before and after application of TEA (left). The average current-voltage relationships before and after application of TEA (n = 3; right).
taining semicircular canals and vestibular afferents (Yamashita and Ohmori 1990). To examine whether the irregular and regular neurons correspond to the phasic and tonic firing types, respectively, in the present study, preparations containing VGCs and labyrinths suitable for whole cell recording will need to be developed.

We found a change in the distribution of the firing patterns of rat VGCs during early postnatal period. About two-thirds of VGCs belonged to the phasic type at PD5–7, but these turned into less than one-fourth at PD12–16. On the other hand, the tonic type increased from 14 to 60% during the same period. Similar developmental changes in the firing pattern have been reported in chick vestibular nucleus neurons (Shao et al. 2006). We also showed that the average gain of the responses to depolarizing stimuli increases between PD5–7 and PD12–16 in rat VGCs. Desmadryl (1991) recorded neural activities of the primary vestibular afferents in response to externally applied galvanic currents in developing mice and showed that the gain of the responses is very low at birth but increased during postnatal periods. It is possible that this developmental increase in gain to galvanic stimuli may be partly attributed to the developmental changes of intrinsic firing patterns of VGCs that we observed.

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properties remains unclear. It is known that speeds of maturation are different among each vestibular endorgan (Sher 1971), but several studies showed that VGCs are not exclusively organized in an end-organ specific fashion and that the neurons from different endorgans show an overlapping distribution (Kevetter and Perachio 1985; Maklad and Fritzch 1999). It is probable that there is not much difference between the superior and inferior VGCs in the timing of developmental changes of the firing properties.

Neurotrophic factors, a family of polypeptide growth factors, play important roles in regulating neuronal survival and morphology during development (Henderson 1996; Markus et al. 2002). They have also been shown to affect electrophysiological properties such as voltage-gated ion channels (Lesser et al. 1997), mechanotransduction (Carroll et al. 1998), and synaptic transmissions (Schuman 1999). Adamson et al. (2002) examined the effects of neurotrophic factors on firing properties of mouse spiral ganglion neurons, where neurons from the base of the cochlea fire APs with more rapid accommodation than apical neurons. They showed that exposure to brain-derived neurotrophic factor and neurotrophin-3 alters these firing properties of the spiral ganglion neurons, suggesting that the electrophysiological diversity of those neurons is based on extrinsic regulation. It is possible that the heterogeneous firing properties of VGCs and their developmental changes are also regulated by certain neurotrophic factors.

**Contribution of potassium channels to intrinsic firing properties**

The physiological relevance of the diversity of K⁺ channels on the cell body has been thought to be representative of channels that could reflect their requirement of rapid firing and secure conduction (Brew and Forsythe 1995) as well as functionally important in their distribution on the axon for the regulation of frequency response properties (Stancefeld et al. 1986). Low-voltage-activated K⁺ channels start to activate on modest depolarization below the thresholds for generating APs and prevent repetitive firing on prolonged depolarization. Indeed, low-voltage-activated K⁺ channels have been shown to regulate presynaptic spike discharge to minimize the risk of repetitive discharge and to influence transmitter release at nuclei of the central auditory synapses (Dodson and Forsythe 2004; Dodson et al. 2002; Ishikawa et al. 2003). In hippocampal neurons, a slowly inactivating α-DTX-sensitive current was responsible for a delayed spike discharge on depolarization (Halliwell et al. 1986). Rat neostriatum neurons possess DTX-sensitive currents responsible for a long latency to spike discharge (Nisenbaum et al. 1994). On the other hand, McKay et al. (2005) found that Kv1 potassium channels in Purkinje cells maintain low frequencies of Na⁺ spike discharges but played a limited role in setting the delay to spike discharge. These Kv1 channels also maintained low frequencies of Ca²⁺ spikes (McKay et al. 2005). These different roles of low-voltage-activated Kv1 potassium channels may be related to the relative density of low-threshold current or the presence of additional depolarizing inward currents such as regurist Na⁺ current and slow Na⁺ and Ca²⁺ currents underlying plateau potentials (Raman and Bean 1997; Rothman and Manis 2003). Thus it seems that the firing pattern is determined by the balance of Kv1, Kv3, and other channels including Na⁺ channels.

Heterogeneous potassium currents have been reported in VGCs (Chabbert et al. 2001; Limón et al. 2005; Risner and

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**FIG. 9.** Effects of Ca²⁺-activated K⁺ channel blockers on the firing properties of VGCs obtained from PD5–7 rats. A: application of iberiotoxin (IbTX; 100 nM) had no effect on the number of APs in phasic VGCs but decreased the number in intermediate and tonic VGCs. B: application of apamin (apamin, 100 nM) had no effect on the number of APs in phasic and tonic VGCs but increased the number in intermediate VGCs. Insets: magnification of beginning parts of the firing. Calibration: (inset) 40 mV, 10 ms.
Holt 2006), Chabbert et al. (2001) characterized three distinct types of potassium currents in mouse VGCs, which were blocked, respectively, by TEA, α-DTX, and blood depressing substance. Consistent with the previous study, we showed that rat VGCs have at least three types of potassium currents: α-DTX-sensitive currents, TEA-sensitive currents, and α-DTX and TEA-insensitive currents. We correlated differential expression of low-voltage-activated K\(^{+}\) currents with the intrinsic firing properties of VGCs and found that the α-dendrotoxin-sensitive K\(^{+}\) channel is critical in determining the patterns of spike discharges of rat VGCs.

Sensitivity to α-DTX is confined to potassium channels including Shaker-related subunits, and α-DTX has been shown to specifically block Kv1.1, Kv1.2, and Kv1.6 (Harvey 1997). The firing properties of phasic VGCs were not affected by MgTx, TsTx, or DTX-k, which block Kv 1.3, 1.2, and 1.1, respectively. These results suggest that the low-voltage-activated K\(^{+}\) currents that regulate the firing properties of VGCs are homomeric Kv 1.6 channels or heteromeric channels containing Kv 1.6 subunits. This is consistent with our results indicating little overlap between TEA- and α-DTX-sensitive conductances. Recombinant homomeric Kv 1.1 has a moderate TEA sensitivity (IC\(_{50}\), 0.3 mM), whereas Kv 1.2 or Kv 1.6 are less sensitive to TEA (Coetzee et al. 1999). Because multiple Kv 1 subunits are expressed in VGCs, the exact composition of the Kv 1 channels regulating AP firing remains to be determined. On the other hand, in neurons from the ciliary ganglion (Wisgrida and Dryer 1993) and the lateral lemniscus (Fu et al. 1996), high-voltage-activated, α-DTX-sensitive currents were identified, which may play some role in the repolarization of APs.

Ca\(^{2+}\)-activated K\(^{+}\) channels also contribute to the shaping of AP waveform and firing discharge patterns of neurons (Dutia and Johnson 1998; Faber and Sah 2002). Smith and Goldberg (1986) showed that the relationship between discharge regularity and sensitivity to synaptic input in vestibular afferents can be accounted for by interactions between the synaptic noise and the slope of the afterhyperpolarization (Smith and Goldberg 1986), which is partly dependent on the Ca\(^{2+}\)-activated K\(^{+}\) current. Limón et al. (2005) reported rat VGCs possess four distinct types of Ca\(^{2+}\)-activated K\(^{+}\) currents: BK, SK, IK, and resistant current. We found that BK channels play a regulatory role in maintaining AP firings of the intermediate and tonic VGCs using specific blockers for each Ca\(^{2+}\)-activated K\(^{+}\) current. Limón et al. (2005) reported that the effect of IbTx, a specific blocker of BK, varied among VGCs and that IbTx increased the number of APs during depolarizing stimuli in some VGCs. We did not encounter such VGCs in the present study. The reason for this discrepancy is unknown at present. One possible reason is the different methods of cell isolation. Limón et al. used collagenase as an enzyme to isolate rat VGCs, but we used papain to isolate them. Armstrong and Roberts (1998) showed that papain incubation alters Ca\(^{2+}\)-activated K\(^{+}\) current and some kinds of voltage-activated K\(^{+}\) current in frog saccular hair cells. But papain had minimal effects on voltage-activated K\(^{+}\) currents evoked in inner hair cells of guinea pigs (Kimitsuki et al. 2005). Other possible reasons such as developmental stage of animals or recording condition could partly account for the differences.

Immunocytochemical investigations of vestibular receptors have demonstrated that calbindin-D 28k and calretinin were co-localized in the largest VGCs, which exclusively innervate type I vestibular hair cells via calyx synapses, although ~2/3 of the total population was nonimmunoreactive for CaBP antibodies (Dememes et al. 1992; Desmadryl and Dechesne 1992). The most-frequently suggested role of the calcium-binding proteins in neurons is short-term cytosolic calcium buffering, which might modulate the excitability and firing patterns of neurons. Neither BK nor SK channels have clear calcium binding domains on their intracellular face. Instead a series of negatively charged amino acids referred to as calcium sensor (Sah and Faber 2002; Schumacher et al. 2001). Thus how far Ca\(^{2+}\)-activated K\(^{+}\) channels contribute in firing regularity of VGCs remains to be studied.

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REFERENCES


Dememes D, Raymond J, Atger P, Grill C, Winsky L, Dechesne CJ. Identification of neuron subpopulations in the rat vestibular ganglion by antibodies (Dememes et al. 1992; Desmadryl and Dechesne 1992). This work was supported by grants from the Japanese Ministry of Education, Science, Sports, and Culture (C-17591778 and C-20791181).


Harvey AL. Recent studies on dendrotoxins and potassium ion channels. Gen Pharmacol 28: 7–12, 1997.


