Hyperpolarization-Activated Current ($I_h$) in the Inferior Colliculus: Distribution and Contribution to Temporal Processing

Ursula Koch and Benedikt Grothe

Max-Planck Institute of Neurobiology, 82152 Martinsried, Germany

Submitted 15 April 2003; accepted in final form 2 September 2003

Koch, Ursula and Benedikt Grothe. Hyperpolarization-activated current ($I_h$) in the inferior colliculus: distribution and contribution to temporal processing. J Neurophysiol 90: 3679–3687, 2003. First published September 10, 2003; 10.1152/jn.00375.2003. Neurons in the inferior colliculus (IC) process acoustic information converging from inputs from almost all nuclei of the auditory brain stem. Despite its importance in auditory processing, little is known about the distribution of ion currents in IC neurons, namely the hyperpolarization-activated current $I_h$. This current, as shown in neurons of the auditory brain stem, contributes to the precise analysis of temporal information. Distribution and properties of the $I_h$ current and its contribution to membrane properties and synaptic integration were examined by current- and voltage-clamp recordings obtained from IC neurons in acute slices of rats (P17–P19). Based on firing patterns to positive current injection, three basic response types were distinguished: onset, adapting, and sustained firing neurons. Onset and adapting cells showed an $I_h$-dependent depolarization sag and had a more depolarized resting membrane potential and lower input resistance than sustained neurons. $I_h$ amplitudes were largest in onset, medium in adapting, and small in sustained neurons. $I_h$ activation kinetics was voltage dependent in all neurons and faster in onset and adapting compared with sustained neurons. Injecting trains of simulated synaptic currents into the neurons or evoking inhibitory postsynaptic potentials (IPSPs) by stimulating the lemniscal tract showed that $I_h$ reduced temporal summation of excitatory and inhibitory potentials in onset but not in sustained neurons. Blocking $I_h$ also abolished afterhyperpolarization and rebound spiking. These results suggest that, in a large proportion of IC cells, namely the onset and adapting neurons, $I_h$ improves precise temporal processing and contributes to the temporal analysis of input patterns.

INTRODUCTION

Postsynaptic voltage-gated ion channels strongly influence the integration of synaptic inputs. Different neuronal types can be defined and related to specific functions based on their composition of ion channels. This particularly holds for the auditory brain stem where several types of neurons are involved in the precise analysis of temporal acoustic information (Oertel 1999). A thorough analysis of their membrane properties revealed that most of these highly specialized neurons express a hyperpolarization-activated cation current ($I_h$) and a depolarization-activated low threshold potassium current (Adam et al. 2001; Bal and Oertel 2000; Banks et al. 1993; Fu et al. 1997; Oertel 1999; Smith 1995). In these neurons, $I_h$ improves the temporal precision of input processing by lowering input resistance, shortening time constants, and reducing temporal summation (Oertel 1999).

A striking feature of the auditory system is the existence of parallel pathways in the auditory brain stem that diverge at the first auditory synapse in the cochlear nucleus and converge in the inferior colliculus (IC) forming the most prominent subcortical integration center in the ascending auditory system (Oertel et al. 2002). Despite its importance in auditory processing, little is known about the basic membrane properties and the distribution of ion channels in IC neurons. Few studies exist in which neurons were classified according to their firing patterns during positive current injection (Bal et al. 2002; Li et al. 1998; Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001). In all studies, three main firing patterns could be distinguished: onset, adapting, and sustained firing neurons. These different firing patterns could each be related to a certain set of potassium channel subtypes (Sivaramakrishnan and Oliver 2001). However, nothing is known about the distribution of hyperpolarization activated currents such as $I_h$, which has been shown to be particularly large in neurons that require short time constants for precise temporal processing of inputs. Recently, it has also been proposed that $I_h$ might be involved in creating selectivity for the duration of sounds in IC neurons (Hooper et al. 2002), a characteristic that originates at the level of the IC (Casseday et al. 1994).

In this study, we address the question of whether $I_h$ is differentially distributed among various types of IC neurons and how $I_h$ contributes to the processing of synaptic inputs in IC neurons. To answer this question, we investigated $I_h$ properties in different types of IC neurons and analyzed how $I_h$ contributes to synaptic integration and to the analysis of the temporal pattern of inputs.

METHODS

Experimental procedures

Recordings were made from neurons in coronal slices of the IC from 17- to 19-day-old outbred Wistar rats. Rats were decapitated, and the brain was quickly removed and immersed in ice-cold oxygenated (95% O₂-5% CO₂) artificial cerebrospinal fluid (ACSF) solution containing (in mM) 125 NaCl, 26 NaHCO₃, 25 glucose, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, and 4 MgCl₂ (pH 7.4). A cut was made rostral to the superior colliculus and caudal to the cochlear nucleus. The block of tissue was glued with cyanoacrylate glue onto a bath chamber that was filled with ice-cold ACSF. Slices (250 μm thick) from the IC were cut on a vibrating microtome (Leica). Slices were

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subsequently stored in ACSF at 32°C for ≥45 min before being transferred to the recording chamber and superfused continuously with oxygenated ACSF (in mM: 125 NaCl, 26 NaHCO3, 25 glucose, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, and 1 MgCl2; pH 7.4) at a rate of 3–5 ml/min. All recordings were made at 32 ± 2°C. Slices were viewed with an upright microscope (Zeiss Axioscope, Oberkochen, Germany) using infrared-differential interference optics.

Electrophysiological recordings

Whole cell current- and voltage-clamp recordings were made from IC neurons using a Multiclamp700A amplifier (Axon Instruments, Foster City, CA). For all voltage- and current-clamp recordings, electrodes were filled with a solution containing (in mM) 130 K-glucuronate, 5 KCl, 10 HEPES, 1 EGTA, 2 Na2-ATP, 2 Mg2-ATP, 0.3 Na2-GTP, and 10 Na-phosphocreatine, adjusted to pH 7.3 with KOH. Glass electrodes were pulled on a vertical electrode puller (Narishige), yielding a final tip resistance of 5–10 MΩ. For current-clamp experiments, bridge balance was applied. For voltage-clamp recordings, series resistance (10–20 MΩ) was compensated by 70–80% and monitored throughout the experiment. Data were only included in the analysis when series resistance had not changed more than 10% or exceeded 20 MΩ. All signals were low-pass filtered at 5 kHz with a four-pole Bessel filter and sampled at 20–40 kHz. Voltage readings were corrected for liquid junction potentials. For blockade of Ih currents, ZD7288 (Tocris) and BaCl2 (Fluka) were dissolved in dH2O (10 mM), stored at –20°C, and diluted before the experiment.

Data acquisition and analysis

Whole cell voltage- and current-clamp signals were digitized and recorded with pCLAMP (version 8.1, Axon Instruments). Inward rectifying currents were induced with 10 mV steps from a potential of –62 mV to a final potential of –122 mV, with a duration of 1 s. More negative potentials were not measured since recordings became unstable for potentials below –122 mV. Measurements of currents were obtained after steady state conditions had been reached (800 ms after the voltage step induction). In current-clamp mode, input resistance was measured using a 100 pA current injection. Since ionic currents are activated during the current injections, input resistance was calculated for the peak (at the maximal negative voltage deflection) and for the steady state (400 ms after current step induction). Activation kinetics of Ih were measured by fitting a double exponential function \( y = K_0 + K_1 \times \exp(-K2 \times t) + K_3 \times \exp(-K4 \times t) \) to the current using fitting procedure provided by IGOR (Wavemetrics, Lake Oswego, OR).

Synaptic inputs were simulated by injecting currents with a time course of synaptic current previously obtained in voltage-clamp recordings (10–90% rise time: 0.3 ms; decay \( \tau = 3 \) ms). Repetitive synaptic waveforms (repetition rate = 200 Hz) were generated in IGOR and imported into pCLAMP. The amplitude of the artificial synaptic current was adjusted to obtain initial voltage changes of approximately 5 mV.

Data were analyzed off-line using custom-made routines in IGOR. Results are presented as means ± SE, and statistical significance was determined with the Student’s t-test.

RESULTS

Whole cell patch-clamp recordings were made under visual control from 92 neurons located in the central nucleus of the IC. In P17–P19 rats, the central nucleus of the IC can be distinguished from the surrounding dorsal cortex and the external nucleus by the large number of myelinated fibers passing through it. Neurons were selected independent of their size or location within the central nucleus of the IC. No spontaneous activity was observed in the neurons in cell-attached or in whole cell recordings. The resting potential of the cells was measured within 1 min after the whole cell configuration was obtained. Only cells with resting potential below –55 mV were included in the analysis.

Neurons in the central nucleus of the IC show three different firing patterns on positive current injection

Neurons were analyzed according to their response to 500-ms-long negative and positive current step injections of various amplitudes (–600 to +600 pA). Three neuron types could be distinguished dependent on their firing pattern evoked by depolarizing current injections (Fig. 1). One group of cells (24%), referred to as “onset cells,” responded with a single spike at the beginning of a current step injection independent of the amplitude of the injected current (Fig. 1A, left). At the end of positive current injections all onset neurons showed a prominent afterhyperpolarization (AHP) with a fast time course. Additionally, onset neurons developed a fast depolarizing sag during injection of negative current pulses and a rebound depolarization on termination of the negative current injection that in some cases (4 of 23 neurons) elicited rebound spiking. A second group of cells (29%), referred to as “adapting cells,” spiked at the beginning of the current step for low current amplitudes; for higher current amplitudes, spiking was observed throughout the current injection, however, with marked adaptation (Fig. 1A, middle). Hyperpolarizing current injections elicited a depolarizing sag similar to the one in onset neurons. As in onset neurons, a rebound depolarization was observed after hyperpolarization that in one-half of the neurons resulted in a rebound spike. The remaining neurons (47%) responded with a sustained firing pattern throughout the positive current injection for current amplitudes above spike threshold (Fig. 1A, right). We will refer to these cells as “sustained cells.” Sustained neurons showed mixed behavior to negative current injections. Whereas 20 of 33 neurons did not display a depolarizing sag for negative current injections reaching values around –87 mV, the remaining cells showed a depolarization that was larger than 5 mV for hyperpolarizing current steps to similar voltages as above. To quantitatively differentiate sustained neurons from adapting neurons, the coefficient of variation (CV) for the interspike interval histogram (ISI) was calculated for each neuron for current injection 100 pA above spike threshold. Neurons with CVs of ISI ≤ 0.3 were defined as sustained spiking. In fact the vast majority of sustained (27/34) neurons had CV ≤ 0.15. Neurons with CVs of ISI ≥ 0.3 were defined as adapting neurons.

As shown in Fig. 1B, the peak and the steady-state I-V relationships for the three different neuron types were analyzed. Peak I-V relationships were measured at the minimum voltage obtained during negative current injection (usually within the first 100 ms of the current step injection). Steady-state voltage was measured 400 ms after voltage step induction when the potential had stabilized. Onset neurons had very flat but linear steady-state I-V relationships for depolarizing and hyperpolarizing current injections, suggesting a low input resistance at all voltages measured. A similar steady-state I-V relationship was found for adapting neurons. In contrast, in sustained neurons, the I-V relationship for negative current injections was steep compared with positive current injections.

\[ I = \frac{dV}{dt} \]

\[ V = K_0 + K_1 \times \exp(-K2 \times t) + K_3 \times \exp(-K4 \times t) \]
This suggests that in onset and adapting neurons ionic currents are active at hyperpolarized and depolarized voltages, whereas in sustained neurons, the active currents dominate at voltages positive to the resting potential.

Comparing membrane properties among the three neuron types revealed significant differences in resting potential and steady-state input resistance. The resting potential of onset neurons was significantly more positive (−61.0 ± 0.8 mV; n = 25) compared with sustained firing neurons (−69.2 ± 1 mV; n = 34; P ≤ 0.001). Steady-state input resistance (measured for −100 pA current step injections) was substantially lower in onset cells (38.5 ± 4.5 MΩ) compared with sustained firing neurons (185.4 ± 16.7 MΩ, P ≤ 0.001). Adapting cells had a resting potential (−62.8 ± 0.9 mV; n = 28) and an input resistance (65.8 ± 5.6 MΩ) more similar to onset neurons.

To investigate the ionic basis of the hyperpolarization-activated current, we added the specific I_h-current blocker ZD7288 (5 μM) to the bath solution. Figure 1C shows the same examples of neurons as Fig. 1A with ZD7288 (5 μM) added to the bath to block I_h. This had several effects in the different neuron types. First, the depolarizing sag during negative current injections was abolished. Second, the neurons’ resting potential hyperpolarized and the peak input resistance increased. In all onset neurons tested (10/10), peak input resistance increased by 125 ± 9.5% and resting potential decreased by −8.2 ± 0.29 mV, indicating that in onset neurons a large proportion of I_h is already active around the resting potential. Blocking I_h current increased input resistance also in sustained neurons (44.5 ± 4.1%; n = 10); however, the effect was significantly smaller than in onset cells (P ≤ 0.05). In most sustained firing cells (7/10), little change of the resting potential was observed. Blocking I_h currents changed resting potential and input resistance of onset neurons to similar values of those of sustained firing neurons. However, the firing pattern to positive current injections did not change. While the I_h current is correlated with response type, I_h current alone does not determine the response type. It has been shown that other voltage gated currents, e.g., potassium channels are differentially distributed among different firing types of IC neurons and presum-
ably low and high-threshold potassium channels determine the firing pattern of onset cells (Sivaramakrishnan and Oliver 2001).

$I_h$ current amplitude is largest in onset neurons

To investigate whether $I_h$ amplitude was distributed differentially among IC neuron types, $I_h$ was measured using voltage-clamp recordings. Since neuron types were first determined in current-clamp mode, no additional K-channel blockers were included in the bath. However, since no influence of ZD7288 on K-currents has been described, differences in current amplitude evoked by hyperpolarization under control and drug conditions are most likely be attributed to $I_h$. Voltage clamping the neurons from $-62$ mV to 1-s-long steps ranging from $-72$ to $-122$ mV evoked an instantaneous current and a more slowly developing medium to large inward current. The amplitude of the current was measured when the inward current had reached steady state (approximately after 800 ms). The same measurement was obtained while $I_h$ was blocked with ZD7288, assuming that the difference between the control and the drug condition approximated the amplitude of the evoked $I_h$ current. Figure 2A shows representative examples of voltage-clamp measurements for onset, adapting, and sustained neurons under control conditions and during $I_h$ current blockade. Under control conditions, currents activated by hyperpolarization to $-122$ mV were $\leq 4$ nA for onset neurons, whereas currents evoked in sustained neurons were always below 1 nA. Adding ZD7288 to the bath blocked a large proportion of the current in onset and adapting cells, whereas only a small effect was observed in sustained neurons. Subtraction of the currents obtained under control and drug conditions showed that $I_h$ current amplitude was largest in onset ($-1,798 \pm 130$ pA, at $-122$ mV, $n = 7$), intermediate in adapting ($-673 \pm 198$ pA, $n = 7$), and small in most sustained neurons ($-180 \pm 66$ pA; $n = 9$; Fig. 2B). A heterogeneous behavior was observed for sustained neurons with 4 of 11 neurons showing $I_h$ amplitudes larger than 250 pA at $-122$ mV. Most of the remaining current was blocked by the addition of 200 $\mu$M BaCl$_2$, indicating that an inward rectifier potassium channel ($I_{KIR}$) of similar amplitudes was activated in all neuron types for potentials below $-80$ mV. However, in most onset neurons, a small and very slowly ($>2$ s) activating inward current remained that could not be blocked by increasing the concentration of ZD7288 to 100 $\mu$M (data not shown).

$I_h$ current activation kinetics differs among different IC neuron types

$I_h$ activation kinetics vary widely among brain regions mostly due to a differential expression of HCN isoforms, the channels underlying $I_h$. We measured the activation time constants of $I_h$ currents in IC neurons since different activation time constants indicate a differential expression of HCN iso-
**FIG. 3.** $I_h$ activation kinetics were faster in onset and adapting compared with sustained neurons. A: responses of an onset and a sustained neuron evoked by hyperpolarizing voltage steps ($-72$ to $-122$ mV). A double exponential function (dotted lines) was fitted to the currents. B: quantification of the results across the population of neurons that displayed significant $I_h$ showed that the fast and the slow activation time constant ($\tau_{fast}$ and $\tau_{slow}$) were significantly faster in onset and adapting neurons compared with sustained neurons.

This suggests that different isoforms of the HCN channel are expressed in different types of IC neurons.

Voltage dependence of $I_h$ current activation differs between onset and sustained neurons

To investigate the voltage dependence of $I_h$ activation in onset and sustained neurons, a tail current analysis was performed for potentials below $-70$ mV. This voltage range was chosen since voltage-gated K-currents that are abundant in IC neurons and determine the firing properties of neurons are mostly inactivated below $-70$ mV. Tail current amplitudes were measured for different voltage steps from $-122$ to $-72$ mV back to a potential of $-50$ mV. Typical tail currents for an onset and a sustained neuron are shown in Fig. 4A. In the onset neuron, tail current amplitude did not change considerably for voltages between $-122$ and $-102$ mV, whereas relative change of tail current amplitude was similar for all voltages in the sustained neuron. This suggests a more depolarized activation range of $I_h$ in onset compared with sustained neurons. Quantification of the results yielded a significantly more depolarized activation range for onset compared with sustained neurons ($P \leq 0.05$; Fig. 4B). This result together with $I_h$ activation kinetics in different neuron types suggests the expression of different HCN channel isoforms in sustained and onset neurons.

**FIG. 4.** $I_h$ in onset neurons activated at more depolarized voltages than in sustained neurons. A: representative tail currents on termination of hyperpolarized voltage steps to $-62$ mV of an onset and a sustained neuron. In the onset neuron, maximal activation of $I_h$ was obtained at more depolarized potentials than in sustained neurons. B: quantification of the results yielded a significantly more depolarized activation range of $I_h$ of onset neurons compared with sustained neurons.
Fig. 5. Blocking of $I_h$ increased temporal summation of simulated excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs). $A$: train (10 repetitions at 200 Hz) of positive or negative current, simulated with the time constants of a previously recorded EPSC, was injected into an onset cell (bottom). Top: voltage response to the positive and negative currents. Blocking $I_h$ with ZD7288 (5 μM) increased temporal summation significantly for both EPSPs and IPSPs. $B$: calculating a summation factor (increase of last PSP vs. 1st PSP; %) demonstrates that summation of EPSPs and IPSPs was significantly smaller in onset neurons compared with sustained firing cells. Blocking $I_h$ with ZD7288 increases summation of EPSPs and IPSPs in onset cells to a similar value as that measured in sustained neurons. $C$: synaptically evoked train of IPSPs by stimulation of the of the lemniscal tract (excitation blocked with 100 μM DNQX and 50 μM APV). No summation of IPSPs was observed during control conditions. Blocking $I_h$ with ZD7288 increased summation of IPSPs significantly.

IPSPs was measured before and during blocking $I_h$ with ZD7288. To compensate for errors due to the reduction of resting potential by blocking $I_h$, a positive holding current was injected into the cells until the potential of the control condition was achieved. Blocking $I_h$ greatly enhanced summation of simulated EPSPs and IPSPs in onset cells ($n = 6$; Fig. 5, $B$ and $A$, solid line). As expected, blocking $I_h$ did not change temporal summation of EPSPs and IPSPs in sustained firing neurons significantly. Interestingly, ZD7288 increased summation of PSPs for both depolarizing and hyperpolarizing current injections in onset neurons (Fig. 5B).

To test whether $I_h$ also affects summation of real synaptic currents, similar experiments were carried in five neurons that showed large $I_h$ amplitudes. The fibers of the lemniscal tract approximately 100 μm ventral of the IC were stimulated with five consecutive stimuli (40–80 V at 100 Hz). IPSPs were isolated by blocking AMPA and N-methyl-d-aspartate (NMDA) receptors with DNQX (10 μM) and APV (50 μM), respectively. IPSPs were measured under control conditions and during the application of ZD7288. For the neuron in Fig. 5C, the voltage measured in response to the last stimulus was 18% smaller than the response the first stimulus of the 100-Hz train. During application of ZD7288, temporal summation increased to an extent that the last IPSP was 22% larger than the first IPSP. Changes in the resting potential were adjusted with positive current injection to obtain similar driving forces for control and drug condition. During control conditions the response to the last IPSP ($5.9 \pm 0.3$ mV) was on average 10% smaller than to the first IPSP ($5.4 \pm 1.1$ mV). Blocking $I_h$ profoundly increased summation of IPSPs. Under drug conditions, the last IPSP ($8.68 \pm 1.5$ mV; $n = 4$) was on average 30% larger than the first IPSP ($6.5 \pm 0.6$ mV). However, in none of the neurons tested could a rebound spike be elicited solely by stimulation of synaptic inputs since, despite maximal stimulus intensity and low resistance stimulation electrodes, IPSPs never exceeded 7 mV.

Additionally, differences in the results between current in-
A change in driving force near the Cl⁻ reversal potential resulting in a decrease of IPSCs during stimulation (data not shown). Interestingly, in all neurons, a large proportion (66 ± 3.4%) of the IPSP amplitude was blocked by the glycine receptor antagonist strychnine (1 μM), suggesting a predominantly glycnergic input to those neurons.

**Iₜₘ current induces afterhyperpolarization and rebound spiking in IC neurons**

Most IC neurons developed a pronounced afterhyperpolarization on injecting a depolarizing current step (Fig. 6A, arrow in top trace). The decay time of this afterhyperpolarization was related to the activation time of the depolarizing sag on hyperpolarizing current injections. This relationship suggests a similar ionic base for both effects. In fact, blocking Iₜₘ current with ZD7288 abolished most of the afterhyperpolarization in the cells (Fig. 6A).

Similarly, in many cells, injecting hyperpolarizing current resulted in a rebound depolarization reaching spike threshold (Fig. 6B, arrow in top trace). One-half of the adapting cells (13/26) and four (4/23) of the onset cells exhibited rebound spiking after hyperpolarizing current injection that resulted in a hyperpolarization around −87 mV. In the remaining onset and adapting cells, a large depolarization was observed after the termination of the hyperpolarizing current step. However, the depolarization was not large enough to reach spike threshold. In all cases, ZD7288 completely abolished the depolarization and the rebound spike, suggesting that rebound spiking in the inferior colliculus is created by the activation of Iₜₘ current.

To test whether a combination of Iₜₘ and K-currents could create a selective response to the duration of the input as proposed by Hooper et al. 2002, we injected hyperpolarizing currents of different durations (2 ms–1 s) into onset and adapting neurons that showed rebound spiking (n = 5). The example in Fig. 6C shows traces from an onset neuron where the hyperpolarizing current had to be ≥6 ms long to evoke rebound spiking. However, no upper duration for rebound spiking was found, even for current step durations as long as 1 s. Similar high pass filter characteristics for the duration of the hyperpolarizing current were seen in all cells tested (n = 5). However, the minimum duration required to evoke a spike differed from cell to cell and was also dependent on the amplitude of the current injection.

**DISCUSSION**

The major finding of this study was a correlation between temporal firing pattern and Iₜₘ properties in IC neurons. Onset cells had prominent Iₜₘ with rapid activation, whereas in sustained firing neurons, Iₜₘ current was smaller and activation was significantly slower. Further analysis showed that Iₜₘ current strongly reduced temporal summation of EPSPs and IPSPs and induced afterhyperpolarization and rebound firing.

**Three types of IC neurons can be classified according to their firing pattern**

We observed three basic firing patterns in IC neurons: onset, adapting, and sustained firing neurons, with sustained neurons being the majority. This is in agreement with previous in vitro studies of IC neurons (Bal et al. 2002; Basta and Vater 2003; Li et al. 1998; Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001). In some of these studies, adapting and sustained firing neurons were divided into further subtypes (e.g., pauserbuild-up) (Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001). Although adapting and sustained firing neurons in our study were not a hommeric group, no further correlation between Iₜₘ properties and firing type were obtained.

Among previous studies, input resistance of different neuronal types has been contradictory. Input resistance measured in IC neurons of rats before or just at hearing onset (P8–P13) was generally higher than in our study and was significantly higher for onset than for sustained firing neurons (Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001), whereas sharp electrode recordings of IC neurons in adult animals (11–20 wk) showed an lower input resistance for onset compared with sustained neurons (Li et al. 1998). Further support comes from the fact that input resistance decreases with age in neurons of the cochlear nucleus (Cuttle et al. 2001).

Similar firing patterns of IC neurons observed in vitro have also been shown during in vivo recordings in response to pure tone or amplitude modulated sounds (Zhang and Kelly 2001). However, it is still unclear as to what degree these firing patterns are determined by intrinsic properties of the neurons or by the characteristics of their synaptic inputs.

**Iₜₘ current amplitude is correlated to neuron type**

We found that similar to neurons in the cochlear nucleus, two types of IC neurons, the onset cells and the adapting cells, showed large amplitudes of Iₜₘ. Iₜₘ is widely distributed among neurons in the auditory brain stem. This current has been shown to be particularly large in octopus cells of the ventral cochlear nucleus (Bal and Oertel 2000). It also found in bushy cells of the ventral cochlear nucleus (Cuttle et al. 2001), principal cells of the medial and lateral superior olive (Kandler and Friauf 1995; Smith 1995), cells of the ventral nucleus or the lateral lemniscus (Zhao and Wu 2001), and with a smaller amplitude in neurons of the dorsal nucleus of the lateral lemniscus (Fu et al. 1997). Inward rectification on hyperpolarization has previously been observed in IC neurons (Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001); however, this inward rectification could not be correlated to properties usual associated with large Iₜₘ such as a low input resistance around the resting membrane potential. This suggests that Iₜₘ might increase or change its voltage dependence shortly after hearing onset or that it is easily modulated in neurons of mature animals.

**Time course of Iₜₘ current activation**

Activation time of Iₜₘ currents varies widely among different populations of neurons (Bal and Oertel 2000; Franz et al. 2000; Santoro and Tibbs 1999). For example, the Iₜₘ current in octopus cells is activated with a time constant that is about 10 times faster than the one measured in hippocampus neurons (Bal and Oertel 2000; Franz et al. 2000). We observed that Iₜₘ current in onset and adapting cells activates significantly faster than in sustained cells, which suggests different compositions of Iₜₘ channels in different populations of cells. The four different genes of the Iₜₘ channel family have been cloned (HCN1–4).
Functionally, we found that the increase in summation induced by mediodal neurons (Lupica et al. 2001; Magee 1998, 1999). Inter-reduced temporal summation of EPSPs in hippocampal pyramidal neurons was shown to be involved in functions that require less temporal precision. Sustained cells appear to be involved in tasks requiring high temporal precision whereas sustained cells appear to be involved in tasks requiring less temporal precision.

Although an afterdepolpolarization was present in all onset cells, it was not large enough for most measured current amplitudes to reach spike threshold, most likely because larger currents are necessary to reach spike threshold in neurons with low input resistance. In contrast to our findings, Sivaramakrishnan and colleagues suggested that rebound spiking in IC neurons was only present in sustained and adapting cells and could be blocked by lowering the calcium concentration, thereby attributing rebound spiking to calcium influx (Sivaramakrishnan and Oliver 2001). This discrepancy could be explained either by the different ages of the experimental animals or the fact that I_h can be modulated by changes in the external calcium concentration (Luthi and McCormick 1999; Wang et al. 2002).

Since I_h is modulated by various substances including cAMP, calcium, and neuromodulators like serotonin and noradrenalin (McCormick and Pape 1990), it could serve as tool to alter sound processing in the IC depending on the behavioral state of the animal (Hurley and Pollak 1999, 2001; Klepper and Herbert 1991).

Similar to our results, previous studies in the cochlear nucleus and other nuclei of the auditory brain stem described cells with an onset and a sustained firing pattern. These two firing types are each correlated with a unique set of ion channels. Throughout the lower auditory brain stem, onset cells have been shown to be involved in auditory analysis that requires precise temporal processing whereas sustained cells appear to be involved in functions that require less temporal precision. We suggest that a segregation of these different processing modes is preserved in the auditory midbrain.

We thank T. Park for discussions and S. Baudoux, L. Kunz, V. Nagerl, and P. Monsivais for critical comments on the manuscript.

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