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

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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 cyanoacrylic 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
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 Mg-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 and subsequently stored in ACSF at 32°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 \( y = K_0 + K_1 \times \exp(-K_2 \times x) + K_3 \times \exp(-K_4 \times x) \) 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. Initially, the combined synaptic current was fit with a double exponential function, and subsequently simulated with a single exponential function to match the fit for the initial part.

Results were 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. Neurons were identified as IC neurons using infrared-differential interference optics. 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- to 1000-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) of the interspike interval histogram (ISI) of each neuron 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.
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 Ih-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 Ih. 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 Ih is already active around the resting potential. Blocking Ih 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 Ih 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 Ih current is correlated with response type, Ih 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 ≤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 ± 130 pA, at −122 mV, n = 7), intermediate in adapting (−673 ± 198 pA, n = 7), and small in most sustained neurons (−180 ± 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 μM 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 μM (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-

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**FIG. 2.** Effect of ZD7288 (5 μM) on hyperpolarization-evoked currents in different neuron types. A: example of inward currents evoked by 1-s hyperpolarizing voltage step ranging from −72 to −122 mV in onset, adapting, and sustained neurons. In onset and adapting neurons, ZD7288 blocked a large component of this current, whereas only a small effect of ZD7288 was seen in sustained neurons. A smaller current with immediate onset was left in all neuron types after blocking I_h. **B:** effect of blocking I_h on I-V relationship for the 3 different neuron types. I_h was significantly larger in onset compared with adapting neurons. In the majority of sustained neurons, little change was observed when I_h was blocked.
forms. Figure 3A displays currents 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 ($\tau_{\text{fast}}$ and $\tau_{\text{slow}}$) were significantly faster in onset and adapting neurons compared with sustained 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.

$I_h$ current decreases temporal summation of inputs similarly for excitatory postsynaptic potentials and inhibitory postsynaptic potentials

To investigate how $I_h$ influences temporal summation of excitatory and inhibitory inputs, excitatory postsynaptic current (EPSC)- and inhibitory postsynaptic current (IPSC)-like currents (see methods) were injected into the cells (Fig. 5A). The rise and decay time of the artificial synaptic waveforms were modeled after voltage-clamp recordings of evoked EPSCs and IPSCs from IC neurons that had been preformed previously. Excitatory postsynaptic potential (EPSP)- and inhibitory postsynaptic potential (IPSP)-like responses were compared in different types of cells by measuring the amount of summation (summation factor (%) = increase of amplitude between first PSP and last PSP in train). For example, the EPSP summation factor for the onset neuron in Fig. 5A was 25%, very similar to that of the IPSPs (28%). Comparing the summation factor of EPSPs and IPSPs in different populations of IC neurons showed that summation of EPSPs was significantly more pronounced in sustained neurons (223 ± 89%; n = 4) compared with onset neurons (47 ± 16%; $P \leq 0.05$; n = 6; Fig. 5B). A significant difference was also obtained for summation of IPSPs between onset and sustained cells ($P \leq 0.01$; Fig. 6B).

To investigate the effect of $I_h$ on temporal summation in different neuron types, summation of simulated EPSPs and IPSPs was measured for different membrane potentials (Fig. 6). The results for EPSPs were similar to those for IPSPs, and the depolarized activation range of $I_h$ in onset neurons was in agreement with the depolarized activation range of $I_h$ in onset neurons compared with sustained neurons.
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 (5.4 ± 1.1 mV) was on average 10% smaller than to the first IPSP (5.9 ± 0.3 mV). Blocking $I_h$ profoundly increased summation of IPSPs. Under drug conditions, the last IPSP (8.68 ± 1.5 mV; $n = 4$) was on average 30% larger than the first IPSP (6.5 ± 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 injections.
nection and synaptic summation can be explained by 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 glycineergic input to those neurons.

\(I_h\) 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 \(I_h\) 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 \(I_h\) current.

To test whether a combination of \(I_h\) 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 \(I_h\) properties in IC neurons. Onset cells had prominent \(I_h\) with rapid activation, whereas in sustained firing neurons, \(I_h\) current was smaller and activation was significantly slower. Further analysis showed that \(I_h\) 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., pauser-build-up) (Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001). Although adapting and sustained firing neurons in our study were not a homomorphic group, no further correlation between \(I_h\) 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 extent these firing patterns are determined by intrinsic properties of the neurons or by the characteristics of their synaptic inputs.

\(I_h\) 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 \(I_h\). \(I_h\) 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 Friau 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 usually associated with large \(I_h\) such as a low input resistance around the resting membrane potential. This suggests that \(I_h\) 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 \(I_h\) current activation**

Activation time of \(I_h\) currents varies widely among different populations of neurons (Bal and Oertel 2000; Franz et al. 2000; Santoro and Tibbs 1999). For example, the \(I_h\) 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 \(I_h\) current in onset and adapting cells activates significantly faster than in sustained cells, which suggests different compositions of \(I_h\) channels in different populations of cells. The four different genes of the \(I_h\) channel family have been cloned (HCN1–4)
(Gauss et al. 1998; Santoro et al. 1998). Functional expression of HCN1 and HCN2 shows that different isoforms have different activation times with HCN1 being faster than HCN2 isoform (Moosmang et al. 2001; Santoro and Tibbs 1999). This has also been confirmed in a study that links fast activation kinetics of $I_h$ in hippocampal and cortical neurons with high expression of HCN1 subunits, whereas thalamic neurons that completely lack the HCN1 subunit, have a significantly slower activation time of $I_h$ (Franz et al. 2000). An in situ hybridization study in the rat provides evidence that IC neurons express medium levels of HCN1, HCN2, and HCN4 mRNA (Monteggia et al. 2000). Their existence in IC neurons matches our findings of various activation time constants in the IC, suggesting different compositions of HCN isoforms in different types of IC neurons. Since addition of cAMP accelerates HCN2 activation kinetics much stronger than HCN1 activation kinetics (Wainger et al. 2001), neuromodulators, such as noradrenalin or serotonin, would differentially influence neuronal processing in onset and adapting neurons depending on their HCN channel types.

Functional relevance of $I_h$ currents in auditory processing

In the auditory system, the largest $I_h$ currents were measured in octopus cells of the cochlear nucleus (Golding et al. 1995). In these cells, $I_h$ current dramatically lowers the input resistance that accelerates voltage changes in response to synaptic currents, thereby improving the temporal precision of the synaptic response and increasing the ability to faithfully follow inputs at high repetition rates. Moreover, low input resistance attenuates voltage changes for single inputs and therefore renders the cells only responsive for multiple coincident inputs (Bal and Oertel 2000; Ferragamo and Oertel 2002; Golding et al. 1995). Thus the large $I_h$ current in octopus cells should improve the temporal precision of signal processing. Consistent with this idea our present results show that blocking $I_h$ considerably increases temporal summation of simulated EPSPs and IPSPs in onset cells. Similarly, dendritic $I_h$ current reduced temporal summation of EPSPs in hippocampal pyramidal neurons (Lupica et al. 2001; Magee 1998, 1999). Interestingly, we found that the increase in summation induced by blocking $I_h$ was similar for hyperpolarizing and depolarizing PSPs. This is contradictory to the idea that $I_h$ has a larger effect on hyperpolarizing than on depolarizing inputs (Luthi and McCormick 1998).

Another response feature that we found to be associated with rebound $I_h$ current was rebound spiking. In many adapting and onset neurons, rebound spiking was induced by $I_h$ current activation. Previous studies have shown that neurons in many parts of the brain exhibit rebound depolarization or spiking after the termination of hyperpolarizing current injections or an offset response after sound stimulation. This has also been observed in IC neurons, where a response to the offset of a sound was in all cases blocked by the glycine receptor antagonist strychnine but never by the GABA$_A$ receptor antagonist bicuculline (Kiran and Wenstrup 2003). A functional role for rebound spiking in the auditory system has been proposed to explain response properties of neurons that exhibit a band-pass filter for the duration of a sound (Casseday et al. 1994). To create this duration sensitivity a model has been proposed in which a delayed excitatory input with constant latency coincides with a rebound depolarization to the offset of a sound. In this case, $I_h$ could induce the rebound depolarization in this neuron. Another recently published model suggests that in inferior colliculus neurons band-pass duration sensitivity is created by an interaction of a slowly activating $I_h$ current and a slowly inactivating transient potassium current (Hooper et al. 2002). However, in the present study, only a lower limit but not an upper limit in the duration of the current injection to initiate rebound spiking was observed. This suggests that slowly inactivating transient potassium channels are not involved in duration tuning of single neurons but synaptic inputs must be involved in band-pass duration selectivity.

Although an afterdepolarization 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.

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