In Vivo Dynamic Clamp Study of $I_h$ in the Mouse Inferior Colliculus

A. P. Nagtegaal1,2 and J.G.G. Borst1
1Departments of Neuroscience and 2Otorhinolaryngology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Submitted 15 March 2010; accepted in final form 2 June 2010

Nagtegaal AP, Borst JG. In vivo dynamic clamp study of $I_h$ in the mouse inferior colliculus. J Neurophysiol 104: 940–948, 2010. First published June 10, 2010; doi:10.1152/jn.00264.2010. Approximately half of the cells in the mouse inferior colliculus have the hyperpolarization-activated mixed cation current $I_h$, yet little is known about its functional relevance in vivo. We therefore studied its contribution to the processing of sound information in single cells by making in vivo whole cell recordings from the inferior colliculus (IC) of young-adult anesthetized C57Bl/6 mice. Following pharmacological block of the endogenous channels, a dynamic clamp approach allowed us to study the responses to current injections or auditory stimuli in the presence and absence of $I_h$ within the same neuron, thus avoiding network or developmental effects. The presence of $I_h$ changed basic cellular properties, including depolarizing the resting membrane potential and decreasing resting membrane resistance. Sound-evoked excitatory postsynaptic potentials were smaller but at the same time reached a more positive membrane potential when $I_h$ was present. With $I_h$, a subset of cells showed rebound spiking following hyperpolarizing current injection. Its presence also changed more complex cellular properties. It decreased temporal summation in response to both hyperpolarizing and depolarizing repetitive current stimuli, and resulted in small changes in the cycle-averaged membrane potential during sinusoidal amplitude modulated (SAM) tones. Furthermore, $I_h$ minimally decreased the response to a tone following a depolarization, an effect that may make a small contribution to forward masking. Our results thus suggest that previously observed differences in IC cells are a mixture of direct effects of $I_h$ and indirect effects due to the change in membrane potential or effects due to the co-expression with other channels.

INTRODUCTION

The hyperpolarization-activated mixed cation current $I_h$ contributes to various physiological functions related to the control of excitability, including setting the resting membrane potential and controlling rhythmicity and dendritic integration (reviewed in Robinson and Siegelbaum 2003; Sjöström et al. 2008). The HCN channels, which are responsible for the $I_h$ current, are highly expressed throughout the auditory brain stem (Koch et al. 2004; Notomi and Shigemoto 2004). The octopus cells of the cochlear nucleus have especially high levels of $I_h$; this contributes to the low membrane resistance and short time constant of these neurons (Bal and Oertel 2000; Golding et al. 1995). Both the fast-gating $I_h$ channel subunit HCN1 (time constant in the order of tens of milliseconds) and the slowly gating subunit HCN2 (time constant 100–1,000 ms) are prominent in the inferior colliculus (IC) (Koch et al. 2004; Notomi and Shigemoto 2004). Approximately half of IC neurons show evidence for the presence of $I_h$ both in vivo, in the form of a depolarizing sag during hyperpolarizing current steps (Tan et al. 2007), and in slices, where immunocytochemical, pharmacological, and biophysical evidence for its presence has been obtained (Koch and Grothe 2003; Koch et al. 2004). In the IC, on average, cells with $I_h$ had more depolarized membrane potentials, lower input resistance, increased excitability, more often showed rebound spiking, and more often showed accommodation or burst-firing on current injection than cells without evidence for the presence of $I_h$ (Koch and Grothe 2003; Tan and Borst 2007; Tan et al. 2007). $I_h$ may also be involved in reducing temporal summation of fast stimuli because Koch and Grothe (2003) found more summation of brief current injections when $I_h$ was pharmacologically blocked. Another possible function of $I_h$ in the auditory system may lie in its contribution to forward masking in which the detectability of a short probe signal is degraded when it is preceded by a masking stimulus (Frisina 2001). As the presence of $I_h$ leads to an afterhyperpolarization following a tone (Tan and Borst 2007), the detection threshold of the probe signal may be increased if it falls within this afterhyperpolarization, thus providing a possible central contribution to forward masking.

The goal of this study is to investigate the role of $I_h$ in the processing of current injections and tonal stimuli at the level of the mouse IC. Until now, it is unclear which of the preceding features are due to other ion channels that happen to be co-expressed with $I_h$ (Koch and Grothe 2003), an interaction of $I_h$ with for example low-threshold potassium channels (Desjardins et al. 2003; George et al. 2009; Rothman and Manis 2003), or due to a direct effect of $I_h$. To address these issues, we made in vivo whole cell patch-clamp recordings, and combined this with the dynamic clamp technique (Brette et al. 2008; Prinz et al. 2004) to artificially reintroduce a conductance with $I_h$ kinetics into the cell after pharmacological block of the endogenous channels. A major advantage of combining these two techniques is the possibility to test in a single cell the effect of the presence and absence of $I_h$ on its response to natural stimuli.

METHODS

Animals and procedures

All experiments were conducted in accordance with the European Communities Council Directive (86/609/EEC) and approved by the animal ethics committee of the Erasmus MC.

Patch-clamp recordings were performed under ketamine/xylazine anesthesia (60/10 mg/kg ip) in the IC of C57Bl/6 mice (age: 21–35 days), as described previously (Tan et al. 2007), with minor modifications. In brief, the skull and dura overlying the IC were removed. During experiments, Ringer solution (containing, in mM: 135 NaCl, 5.4 KCl, 1 MgCl2, 1.8 CaCl2, and 5 HEPES, pH adjustment to 7.2 with NaOH, osmolality: 290 mmol/kg) was applied to the brain surface to prevent dehydration. Agar (3% agarose in Ringer solution) was applied to reduce brain pulsations. We used glass pipettes (open...
pipette resistance: 4–6 MΩ) filled with internal solution containing (in mM) 126 K-glucuronate, 20 KCl, 10 Na2-phosphocreatine, 4 MgATP, 0.3 NaGTP, 0.5 EGTA, and 10 HEPES, adjusted with KOH to pH 7.2, osmolality and J.G.G. Borst; not shown). The versal potential and half-activation potential were obtained from the gating variables. Membrane potentials were sampled at 2 kHz. Re-type model of 8.0, National Instruments) routine on the basis of a Hodgkin-Huxley-tial. Recordings were only continued if the membrane potential mode, was 55

\[ I_{h} \text{ current was calculated by a custom-written LabVIEW (version 8.0, National Instruments) routine on the basis of a Hodgkin-Huxley-type model of } I_{h}, \text{ incorporating both voltage- and time-dependent gating variables. Membrane potentials were sampled at 2 kHz. Reversal potential and half-activation potential were obtained from the literature (Bal and Oertel 2000; Tan et al. 2007) and confirmed by preliminary voltage-clamp experiments in IC slices (H. P. Theeuws and J.G.G. Borst; not shown). The } I_{h} \text{ current consisted of a fast and a slow component (Tan et al. 2007), presumably reflecting the presence of different subunits within the IC (Koch et al. 2004; Notomi and Shigemoto 2004); the current was calculated by the following equation}

\[ I_{h} = (g_{\text{max fast}} \cdot h_{\text{fast}} + g_{\text{max slow}} \cdot h_{\text{slow}}) (V_{m} - E_{rev}) \]

(1)

where \( h_{\text{fast}} \) and \( h_{\text{slow}} \) are voltage- and time-dependent gating variables and the reversal potential \( E_{rev} = -38 \text{ mV} \). The gating variable \( h \) varies between 0 (closed) and 1 (open); \( h_{\text{fast}} \) and \( h_{\text{slow}} \) were obtained by numerical integration (4th-order Runge–Kutta method) of

\[ dh/dt = a \cdot (1 - h) - b \cdot h \]

(2)

The respective rate constants for activation (\( \alpha_{\text{fast}} \) and \( \alpha_{\text{slow}} \)) were determined by (Hodgkin and Huxley 1952; Roth and Häusser 2001)

\[ \alpha = a \cdot \exp(-b(V_{m} - V_{0.5})) \]

(3)

whereas the rate constants for deactivation (\( \beta_{\text{fast}} \) and \( \beta_{\text{slow}} \)) are given by

\[ \beta = a \cdot \exp(c(V_{m} - V_{0.5})) \]

(4)

Two different gating models were used in all experiments. For the fast gating \( I_{h} \), the values for the different parameters were as follows:

\[ a_{\text{fast}} = 0.012 \text{ ms}^{-1}, \quad a_{\text{slow}} = 0.0023 \text{ ms}^{-1}, \quad b = 0.05 \text{ mV}^{-1}, \quad c = 0.085 \text{ mV}^{-1}, \quad V_{0.5} = -65 \text{ mV} \]. For the slow gating \( I_{h} \), \( a_{\text{fast}} = 0.00045 \text{ ms}^{-1}, \quad a_{\text{slow}} = 0.003 \text{ ms}^{-1}, \quad b = 0.05 \text{ mV}^{-1}, \quad c = 0.085 \text{ mV}^{-1}, \quad V_{0.5} = -65 \text{ mV} \]. Because results for both gating models were similar, only the results of the fast gating models are presented except for Fig. 7. The total, maximal conductance with \( I_{h} \) kinetics was set to 4 nS; activation of this conductance generated a depolarizing sag of ~25% during 1 s, ~200 pA, hyperpolarizing constant current steps. Two-thirds of the total conductance was assigned to the first, fast component. The total conductance was based on previous observations within the IC (Tan et al. 2007); in this dataset, cells with \( I_{h} \) had a depolarizing sag of 28.5 ± 1.5% (n = 52) at the end of a 1 s hyperpolarizing constant current step.

The pipette time constant did not filter the injection of the current substantially, as the slowest pipette time constant was below the sampling interval and much faster than the fastest time constant of \( I_{h} \) in the range between ~100 and ~30 mV (>4 ms). With a sampling interval of 0.5 ms, the driving force will not be updated sufficiently rapidly during an action potential, but because the contribution of \( I_{h} \) to the total membrane current during an action potential is very small, we conclude that series resistance and sampling interval did not limit the quality of the dynamic clamp.

**Auditory stimulation**

Sound stimuli were generated with Tucker Davis Technologies hardware (System 3, RX6 multifunction processor, PA5 attenuator and ED1 electrostatic speaker driver) and delivered to the contralateral ear under closed-field conditions. Sound intensities of frequencies between 1 and 64 kHz were calibrated with an ACO pacific condenser microphone (type 7017). Recordings were performed in a single-wall sound attenuated chamber (Gretch-Ken Industries, attenuation microphone (type 7017). Auditory stimulation protocols were presented to the cells: 1 s depolarizing current injection, 100 pA above firing threshold; depolarizing and hyperpolarizing repetitive current injections [400 ms total duration, 80 stimuli of 5 ms, 10 – 90% rise time = 0.3 ms, decay τ = 3 ms, as described by Koch and Grothe (2003)]; a 50 ms tone with and without a preceding 500 ms depolarizing current injection; sinusoidal amplitude modulated (SAM) tones (400 ms total duration, carrier frequency at CF, modulation frequency 40 or 80 Hz, modulation depth 100%); up- and downward frequency-modulated (FM) sweeps (1–73 kHz, 120 ms duration, sweep rate 600 kHz/s, 5 ms rise and fall time). All protocols were applied with and without \( I_{h} \). This sequence was repeated 5–20 times (average = 14), depending on the amount of depolarization during the experiment (maximally allowed depolarization 10 mV). The minimal interval between stimuli was 400 ms.

**Analysis**

Data were analyzed with custom-written Igor procedures (Igor Pro 6.01, WaveMetrics) running within the NeuroMatic environment (version 1.98; kindly provided by Dr. J. Rothman, University College London).

Fifteen repetitions of a ~200 pA hyperpolarizing current were injected to characterize the \( I_{h} \) current. The amount of depolarizing sag was quantified and the sum of two exponential functions was fitted to both the depolarizing sag and the rebound depolarization

\[ V_{m} = V_{ss} + A_{\text{fast}} \cdot \exp(-t/\tau_{\text{fast}}) + A_{\text{slow}} \cdot \exp(-t/\tau_{\text{slow}}) \]

(5)

where \( V_{m} \) is membrane potential, \( V_{ss} \) is membrane potential at the end of a long current injection, \( A_{\text{fast}} \) and \( A_{\text{slow}} \) are amplitudes of the two exponential functions at the start of the step and \( \tau_{\text{fast}} \) and \( \tau_{\text{slow}} \) are the fast and slow time constants, respectively. Membrane resistance was calculated at the start and end of the current step.

Firing types were classified based on responses to 1 s constant-current injections at 100 pA above firing threshold, as described by Tan et al. (2007). We plotted the reciprocal (\( Y \)) of the interspike

\[ J \text{ Neurophysiol} \cdot \text{VOL 104} \cdot \text{AUGUST 2010} \cdot \text{www.jn.org} \]
As procedures were always repeated in the presence and absence of classified as sustained, ∆V_m(t) is the difference in the average membrane potential in the presence and absence of I_h, i_h(t) is the injected current and g_m(t) is the total membrane conductance. Although the latter consists of several components that are not easily dissected, it can be used to predict the response to current injections calculated with different gating models for I_h. Four different models with the same maximal conductance and steady-state voltage dependence as used in the experiments were tested. Two models were identical to the ones used in the experiments; as expected, the predicted membrane potentials always closely matched the measured response for these two models. In addition, we tested two extremes for I_h gating. In one model, the voltage-dependent gating was instantaneous, whereas in the other the open probability of I_h was fixed at the calculated value at the start of the trace. For these four models a dynamic clamp experiment was simulated using Eq. 6, where ∆V_m(t) is again the change in the membrane potential compared with the situation in the absence of any injected current, g_m(t) is the previously calculated total membrane conductance, and i_h(t) is the predicted current, calculated sample by sample according to the four different models.

**Statistics**

Data are presented as means ± SE. If responses were normally distributed, as tested with a Shapiro-Wilk test, a statistical difference was calculated using a t-test (two-tailed). The significance level was 0.05. If responses did not fit the normal distribution, a non-parametric test was used.

**TABLE 1. Effect of adding g_m on different cell properties and responses to current injections and sound stimuli in vivo**

<table>
<thead>
<tr>
<th>Cell Properties</th>
<th>No I_h</th>
<th>I_h</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_m, mV</td>
<td>−66.5±1.5 (13)</td>
<td>−62.7±1.2**</td>
</tr>
<tr>
<td>R_m, MΩ</td>
<td>134±11.3 (19)</td>
<td>78±5.6**</td>
</tr>
<tr>
<td>Spike threshold, mV</td>
<td>−50.4±1.5 (10)</td>
<td>−50.1±1.5</td>
</tr>
<tr>
<td>Relative spike threshold, mV</td>
<td>14.4±1.8 (10)</td>
<td>10.4±1.3**</td>
</tr>
<tr>
<td>Percentage depolarizing sag</td>
<td>2.1±0.4 (19)</td>
<td>24±1.0**</td>
</tr>
<tr>
<td>τ_fast depolarizing sag, ms</td>
<td>—</td>
<td>32.7±0.7 (19)</td>
</tr>
<tr>
<td>Amplitude τ_fast, mV</td>
<td>—</td>
<td>−4.2±0.9 (19)</td>
</tr>
<tr>
<td>τ_slow depolarizing sag, ms</td>
<td>—</td>
<td>250±5.8 (19)</td>
</tr>
<tr>
<td>Amplitude τ_slow, mV</td>
<td>—</td>
<td>−2.6±0.07 (19)</td>
</tr>
</tbody>
</table>

**Current injections**

| Depolarizing summation index, %  | 146±19 (14)  | 77±10*   |
| Peak - trough amplitude, mV     | 2.04±0.05 (14) | 2.10±0.06** |
| Hyperpolarizing summation index, %| 115±20 (14)  | 58±6*    |
| Peak - trough amplitude, mV     | 2.06±0.05 (14) | 2.18±0.06** |

**Tone response, 50 ms**

| Max EPSP depolarization, mV     | −54.1±1.6 (13) | −52.3±1.4** |
| EPSP amplitude, mV              | 12.5±1.6 (13)  | 10.4±1.5**  |
| EPSP full width at half maximum, ms | 41.0±1.9 (13) | 37.9±1.8*   |

**Tone response after current injection**

| Max depolarization, mV          | −54.0±1.4 (13) | −52.8±1.4** |
| EPSP amplitude, mV              | 12.6±1.6 (13)  | 10.0±1.5**  |
| Max difference with no injection, mV | 0.03±0.36 (13) | −0.42±0.21 |

**SAM tones**

| Modulated potential, mV         | 2.29±0.39 (13) | 2.23±0.37   |
| R (vector strength)             | 0.46±0.13 (4)  | 0.37±0.10   |

Values represent means ± SE. The number of cells is between parentheses. One asterisk indicates a significant difference of P < 0.05; **, P < 0.01. Max difference with no injection refers to the difference in the maximal depolarization in response to a tone with or without a preceding depolarization. EPSP, excitatory postsynaptic potential; SAM, sinusoidal amplitude modulated.

**Simulations**

In all recordings, the injected I_h current calculated by the dynamic clamp was recorded. This current was used in simulations to test the influence of different I_h gating models on the response to FM sweeps. As procedures were always repeated in the presence and absence of I_h in the experiments in which the variability in individual responses was sufficiently low, the difference in membrane potential that resulted from the current injection could be used to calculate the membrane conductance with Ohm’s law.
in the mean response between \( I_h \) and no \( I_h \) was assessed with a paired \( t \)-test, otherwise a paired Wilcoxon test was used. Significance level was set at \( P < 0.05 \).

**RESULTS**

**In vivo dynamic clamp**

To study the contribution of \( I_h \) to sound processing in the mouse inferior colliculus, we combined the dynamic clamp technique with in vivo whole cell recordings in a total of 19 cells. In none of these cells did the size of the depolarizing sag during hyperpolarizing current injection exceed 7\% under control conditions (Fig. 1, **top**), indicating that endogenous \( I_h \) channels, when present, were effectively blocked by the \( I_h \) blocker ZD7288 (100 \( \mu \)M) in the pipette solution. On addition of the conductance with \( I_h \) kinetics (\( g_h \)) to the cell using the dynamic clamp, a depolarizing sag appeared (Fig. 1, **bottom**). Similar results, but with slower kinetics, were obtained with a different gating model for \( I_h \) (Supplementary Fig. S1). ¹ The time course of the depolarizing sag was fitted by a double-exponential function; the time constants were close to what was predicted based on the H-H model and fell within the range of gating kinetics observed for native \( I_h \) channels within the IC (Table 1) (Tan et al. 2007). In the presence of \( I_h \), the membrane resistance calculated at the end of the step was clearly lower (Table 1). Following the hyperpolarizing current injection, the cells showed a rebound depolarization, which had similar kinetics as the depolarizing sag. In 6 of 19 cells, this rebound depolarization was sufficiently large to trigger spiking (Fig. 1), whereas in the absence of \( I_h \) only one cell showed rebound spiking.

**Firing pattern**

Fifteen cells were available for analysis of firing pattern during constant-current injections. Their firing pattern was classified on the basis of a fit to the ISIs, as described in METHODS. Ten cells were classified as sustained, five as accommodating. The firing patterns did not change in the presence of \( I_h \) as judged from a lack of a significant change in the fitting parameters (results not shown).

**Repetitive current injections**

We tested the effect of repetitive current injections in the presence and absence of \( I_h \) (Fig. 2). The interval between the stimuli was sufficiently short to allow summation. As a result of summation, the peak depolarization or hyperpolarization reached following the last current injection was larger than following the first. The percentage increase in this level, the summation index, was smaller in the presence of \( I_h \) for both depolarizing and hyperpolarizing current injections. Furthermore, the difference between peak and trough membrane potential of the individual responses was smaller in the absence of \( I_h \) (Table 1).

**Response to tones**

All neurons responded to tones. Their mean characteristic frequency (CF) was 20.9 ± 1.4 kHz (range: 12.1–27.9 kHz) with an average minimum threshold (MT) for evoking an EPSP of 21 ± 4 dB SPL.

The addition of \( I_h \) led to a depolarization of ~4 mV (Table 1). As a result, in the presence of \( I_h \), a more positive potential was reached in response to a tone 30 dB above MT, despite a smaller EPSP amplitude (Fig. 3; Table 1; \( n = 13 \); excluding 3 cells with an inhibitory response to tones). EPSPs were also less broad in the presence of \( I_h \), as judged from the smaller full width at half-maximum (Table 1). In the presence of \( I_h \), the amplitude of the afterhyperpolarization following the tones was slightly larger (~2.10 vs. ~1.96 mV; \( P > 0.05 \)). Ten cells showed spiking in response to tones, at an average threshold of 31 dB SPL; in the other 9 cells all sound-evoked responses were subthreshold. The firing threshold during tonal stimuli

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¹ The online version of this article contains supplemental data.

**FIG. 2.** \( I_h \) decreases temporal summation. **A:** response of a cell to repetitive current injections. Bottom traces show stimuli. For comparison purposes, resting membrane potentials (~64.9 mV for no \( I_h \), ~61.8 mV for \( I_h \)) were equalized. **B:** the 1st 7 stimuli are shown at higher time resolution. **C:** summation index [percentage increase of last postsynaptic potential (PSP) vs. 1st PSP] of all cells (\( n = 14 \)) during depolarizing repetitive current injections, with the average indicated with a thick black line. Adding \( I_h \) resulted in less summation. **D:** as C, except hyperpolarizing current injections.
in the presence of $I_h$, the amount of depolarization required to reach this threshold was reduced in the presence of $I_h$ (Table 1). We did not observe a significant effect of $I_h$ on the number of spikes fired during different tonal stimuli.

**Forward masking**

The psychophysical phenomenon of forward masking is defined as the increase in the hearing threshold of a tone when it is preceded by another tone. Instead of giving two tones, we replaced the first tone by a current injection to avoid peripheral effects (e.g., Harris and Dallos 1979) on forward masking. Similar to what was observed without the preceding current, in the presence of $I_h$, the membrane potential reached a more positive value in response to a tone 30 dB above MT, while EPSP amplitudes were smaller (Fig. 4, Table 1). In the absence of $I_h$, the maximal depolarization reached in response to the tone was similar with and without the preceding current injection. In the presence of $I_h$, a less positive membrane potential was reached following the current injection (Table 1). The observed effect was very small, however, and is therefore unlikely to contribute significantly to forward masking.

**Response to SAM tones**

The results of the repetitive current injections (Fig. 2) suggested that $I_h$ channels may reduce synaptic integration. To test this more directly, we studied the response to SAM tones and to frequency-modulated (FM) sweeps. SAM tones were given at a carrier frequency which was equal to the CF of the cells. This carrier frequency was modulated at 40 Hz, resulting in periodic fluctuations of the membrane potential (Fig. 5). The modulated potential (MP), the peak-to-peak amplitude of the cycle-averaged response of the cell (Geis and Borst 2009), was lower in the presence of $I_h$ in 8 of 13 cells, although the effects of $I_h$ on the response to a pure tone.

A: averaged response of a cell to 16 kHz, 40 dB SPL, 50 ms tones. In the presence of $I_h$, the cell reached a more depolarized membrane potential in response to the tone, despite a smaller excitatory PSP (EPSP) amplitude. B: EPSP amplitude values for all individual cells (○) and average (□, connected with —).

**FIG. 3.** Influence of $I_h$ on the response to a pure tone. A: averaged response of a cell to 16 kHz, 40 dB SPL, 50 ms tones. In the presence of $I_h$, the cell reached a more depolarized membrane potential in response to the tone, despite a smaller excitatory PSP (EPSP) amplitude. B: EPSP amplitude values for all individual cells (○) and average (□, connected with —).

**FIG. 4.** Possible contribution of $I_h$ to forward masking. A: in the absence of $I_h$, injection of a depolarizing current immediately before a tone leads to summation with the EPSP (black trace). Middle: current injection; bottom: when tone is presented. B: as A, but in the presence of $I_h$. The resulting EPSP is decreased. C: tone-evoked response of A shown at higher time resolution. Response without the preceding depolarizing current (gray trace) is also shown in Fig. 3A. D: tone-evoked response of B shown at higher time resolution. E: average EPSP amplitude ($n = 13$ cells). With $I_h$ injection, the amplitude decreases in the presence of a preceding current injection, while the amplitude does not change in the absence of $I_h$. F: average peak depolarization reached in response to the tone. In the presence of $I_h$, a more negative membrane potential is reached when the tone is preceded by a current injection.
were small, and this difference did not reach statistical significance. The vector strength, which is a measure for the ability of cells to phase-lock their spikes to the envelope of the SAM tones, was not different in the presence of \( I_h \) (Table 1). Similar results were obtained at a modulation frequency of 80 Hz (not shown).

Response to FM sweeps

Cells responded to up- or downward FM sweeps with a complex response (Fig. 6, A and B). In the presence of \( I_h \), 7 of 14 cells showed increased IPSPs (Fig. 6A), probably due to the increased driving force for inhibitory conductances at the more positive membrane potential in the presence of \( I_h \). In these cells, pure tones evoked IPSPs at the frequencies predicted by the timing of the inhibitory components within the FM sweep (not shown).

Two types of experiments suggested that \( I_h \) gating during the tones did not make a great contribution to the observed changes in inhibition during the FM sweep. First dynamic clamp experiments with an \( I_h \) current that showed slower gating were similar (Fig. 7A). Second, we used the relation between injected currents (Fig. 7B) and membrane potential changes to get an estimate of membrane conductance (Fig. 7C), as detailed in METHODS. This estimate was used in simulations to predict the change in membrane potential with other gating models. Two extreme models were compared. In one the gating was instantaneous, in the other \( I_h \) showed no gating. The membrane potential predictions for these two models were similar (Fig. 7A) despite clear differences in the injected current (B), suggesting that changes in the open probability during the FM sweep (D) do not contribute appreciably. A comparison of \( g_h \) with the total conductance shows that \( g_h \) at all times is only a small fraction of the total conductance (Fig. 7C). This was also the case in the other 13 cells in which FM tones were tested.

DISCUSSION

We made use of the dynamic clamp technique to study the contribution of \( I_h \) to sound processing in single neurons of the mouse IC. The presence of \( I_h \) changed basic membrane properties, causing a more depolarized membrane potential and a decreased resting membrane resistance. Sound-evoked EPSPs were smaller, but at the same time reached a more positive membrane potential in the presence of \( I_h \). The addition of \( I_h \) also changed more complex properties of the cells. Its presence decreased temporal summation in response to repetitive current stimuli and also induced small changes in the responses to SAM tones, FM sweeps, and a tone following a depolarization, an effect that may contribute to forward masking.

Using dynamic clamp to study \( I_h \) in vivo

The effects of \( I_h \) have been well characterized in slice recordings (Robinson and Siegelbaum 2003; Sjöström et al. 2008). Much less is known, however, about its contribution in vivo. Knock-out studies have revealed the involvement of \( I_h \) in global functions such as memory or gamma oscillations (Ludwig et al. 2003; Nolan et al. 2003, 2004), but little is known about the contribution of \( I_h \) to the processing of natural inputs in a single cell. Our approach was to block endogenous \( I_h \) and subsequently insert \( g_h \) using a dynamic clamp approach in whole cell recordings from the mouse IC. This technique has previously been used to inject synaptic conductances during intracellular in vivo recordings (Brette et al. 2008). Its use for studying voltage-dependent ion channels has as an advantage that both time-dependent gating effects and changes in the driving force can be taken into account. Changes in driving...
force are considerable for the mixed cation channel $I_{h}$, which has a reversal potential $\sim 20$ mV from the resting potential. Moreover, with this approach, it is possible to study the acute effects in a single cell, thus avoiding possible compensatory developmental or network effects that can accompany transgenic studies (Chen et al. 2010). By interspersing the presentations with and without the dynamic clamp, very small effects (<1 mV) could be picked up, even when variability between cells was much larger.

Our Hodgkin-Huxley model of the $I_{h}$ channel adequately matched the kinetic properties found within the IC. The time constants of the depolarizing sag in the presence of $I_{h}$ were in the same range as reported previously, although somewhat smaller than the fast time constant of the depolarizing sag reported in Tan et al. (2007). Its conductance was similar to previous observations in the mouse IC (Tan et al. 2007). Although the gating model used was not based on a detailed biophysical analysis of the properties of $I_{h}$ in the inferior colliculus, two lines of evidence suggest that the exact properties of the gating model were not critical. First results obtained with a fast and a slow gating model were generally similar. Second, a simulation in which extreme values for the gating of $I_{h}$ were tested did not yield clear differences either.

$I_{h}$ channels are often located at higher density in distal dendrites (Lörincz et al. 2002; Magee 1998), whereas we used somatic $I_{h}$ injections. It has been shown that many of the effects of $I_{h}$, including its effect on the somatic time course of distant synaptic inputs, do not depend on its exact subcellular distribution (Angelo et al. 2007; Bullis et al. 2007; Magee 1999). However, its effect on local temporal summation in the dendrites will not be adequately mimicked by the somatic dynamic clamp.

$I_{h}$ can trigger rebound spiking

Although other channels may also contribute to rebound depolarizations (Sivaramakrishnan and Oliver 2001; Smith 1992; Sun and Wu 2008; Tan et al. 2007), the emergence of rebound spiking in the presence of $I_{h}$ is in agreement with earlier evidence obtained both in vivo and in slices (Koch and Grothe 2003; Tan et al. 2007). This effect may contribute to off-responses in the mouse IC, which may play a role in duration tuning (Casseday et al. 2000; Tan and Borst 2007).

Lack of evidence for a role of $I_{h}$ in controlling firing pattern

Although the presence of $I_{h}$ is associated with certain firing types in the IC (Koch and Grothe 2003; Tan et al. 2007), the firing pattern during constant-current injections was not altered in the presence of $I_{h}$. This result is in agreement with an earlier study in slices (Koch and Grothe 2003), suggesting that $I_{h}$ by itself does not control firing and that the larger accommodation observed for cells with evidence for $I_{h}$ is most likely due to co-expression with a low-threshold potassium channel. A major role for $I_{h}$ on firing patterns is not expected based on its relatively low conductance compared with both synaptic and voltage-dependent currents. A limitation of our study is that we observed only cells with sustained or accommodating firing types in response to current injections. We therefore cannot exclude that the effect of $I_{h}$ on firing types such as build-up, burst-onset, burst-sustained, and accelerating (Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001; Tan et al. 2007) would have been larger, for example through an interaction with low-threshold K-channels.

$I_{h}$ and excitability

The effects of $I_{h}$ on excitability are complex. Both excitatory (Shaikh and Finlayson 2003; Tan and Borst 2007) and inhibitory effects (Huang et al. 2009; Poolos et al. 2002) have been described. Its effect on excitability depends in part on its interaction with other voltage-dependent ion channels (Desjardins et al. 2003; George et al. 2009; Rothman and Manis 2003).
and on the levels and timing of synaptic activity (Komendantov and Ascoli 2009; Migliore et al. 2004; Poolos et al. 2002; Santoro and Baram 2003). The amplitude of responses to tones decreased in the presence of $I_h$. However, the increase in resting $V_m$ compensated for the smaller EPSP size, and the net effect was that a more positive potential was reached in response to tones. Assuming that the most positive voltage reached determines the effect on excitability (George et al. 2009), this would indicate that $I_h$ increases excitability in agreement with our previous findings (Tan et al. 2007), although the effect is small and will be counteracted by the slightly more positive action potential threshold observed in the presence of $I_h$.

Possible role of $I_h$ in forward masking

Areas more central to the auditory nerve contribute to forward masking (Boettcher et al. 1990; Kaltenbach et al. 1993; Relkin and Turner 1988). We tested the hypothesis that the deactivation of $I_h$ during a depolarization induced by the masker tone contributes to a decreased response to the probe tone. We replaced the masker tone by a depolarizing current injection to prevent any peripheral effects. In the presence of $I_h$, the EPSP was smaller, and a less depolarized membrane potential was reached when the probe tone was preceded by a depolarization, whereas this was not observed in the absence of $I_h$. Due to a nonlinear relation between membrane potential and spike rate, small effects on the membrane potential can already change excitability in the inferior colliculus (Geis and Borst 2009). Furthermore, the effects of $I_h$ may be cumulative, as it is abundantly present throughout the auditory brain stem. Nevertheless, the small size of the observed effects precludes a large contribution to forward masking.

Effect of $I_h$ on integration of sensory inputs

A well studied effect of $I_h$ is that it reduces temporal summation of inputs (reviewed in Robinson and Siegelbaum 2003; Sjöström et al. 2008). Here we confirmed previous experiments performed in slices of the IC, which showed that $I_h$ decreases summation of the response to hyper- or depolarizing current injections (Koch and Grothe 2003). The effects for depolarizing and hyperpolarizing current injections appeared to be similar. The deactivation of $I_h$ during depolarizing current injections (or EPSPs) effectively creates an outward current, whereas the opposite will happen during hyperpolarizing current injections (Magee 1998; Schwindt and Crill 1997). It was not yet known whether these effects of $I_h$ on temporal summation are also relevant for physiological synaptic inputs in vivo. We observed only small effects on the response to SAM tones. $I_h$ may be relatively ineffective during synchronized inputs, as evoked by SAM tones, whereas it is expected to have a stronger effect on desynchronized inputs, such as FM sweeps (Migliore et al. 2004). Furthermore, $I_h$ is expected to attenuate responses to low-frequency inputs most effectively, whereas the 40 Hz SAM tones have a modulation frequency that is already too high to result in very large attenuation of fluctuating inputs (Nolan et al. 2004).

In response to FM sweeps, IPSPs became more conspicuous in the presence of $I_h$, probably due to a more depolarized membrane potential and a subsequent increase in driving force for inhibitory conductances (Gittelman et al. 2009). This effect may be more pronounced at a higher driving force for chloride ions, which was relatively small in our experiments (Vale and Sanes 2000). Experimental results with a slower gating model and simulated results with an $I_h$ channel that lacked gating altogether gave similar results, indicating that changes in open probability of $I_h$ during the tone did not contribute directly to the responses.

It was previously observed that the presence or absence of $I_h$ is an important predictor for the electrophysiological properties and responses to sound of IC neurons (Koch and Grothe 2003; Tan and Borst 2007; Tan et al. 2007). Here we find that the more depolarized membrane potentials, more frequent rebound spiking, lower input resistance and decreased summation during current injections that have previously been observed for IC cells with $I_h$ (Koch and Grothe 2003; Tan et al. 2007) are likely due to a direct effect of $I_h$. The different firing patterns associated with the presence of $I_h$ or the association with pause or chopper responses to tones (Tan and Borst 2007; Tan et al. 2007) are most likely due to co-expression of $I_h$ with other channels. More subtle effects on tone responses that were observed in the present study may be due to the activation of potassium currents or an increased driving force for inhibitory inputs as a result of the depolarization caused by the presence of $I_h$.

Acknowledgments

We thank C. Donkersloot for LabVIEW programming, A. Rodriguez-Contreras for helpful discussions, and M. Häusser and M. London (UCL) for comments on an earlier version of the manuscript.

Grants

This work was supported by a Neuro-Besluit subsidies investeringen kennisinfrastructuur grant (BSIK 03053; SenterNovem, The Netherlands) and the Heinsius-Houbolt fund.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

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J Neurophysiol • VOL 104 • AUGUST 2010 • www.jn.org


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