Hyperpolarization-Activated Inward Current in Neurons of the Rat’s Dorsal Nucleus of the Lateral Lemniscus In Vitro

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Fu, Xiao Wen, Borys L. Brezden, and Shu Hui Wu. Hyperpolarization-activated inward current in neurons of the rat’s dorsal nucleus of the lateral lemniscus in vitro. J. Neurophysiol. 78: 2235–2245, 1997. The hyperpolarization-activated current (\( I_h \)) underlying inward rectification in neurons of the rat’s dorsal nucleus of the lateral lemniscus (DNLL) was investigated using whole cell patch-clamp techniques. Patch recordings were made from DNLL neurons of young rats (21–30 days old) in 400 µm tissue slices. Under current clamp, injection of negative current produced a graded hyperpolarization of the cell membrane, often with a gradual sag in the membrane potential toward the resting value. The rate and magnitude of the sag depended on the amount of hyperpolarizing current. Larger current resulted in a larger and faster decay of the voltage. Under voltage clamp, hyperpolarizing voltage steps elicited a slowly activating inward current that was presumably responsible for the sag observed in the voltage response to a steady hyperpolarizing current recorded under current clamp. Activation of the inward current (\( I_h \)) was voltage and time dependent. The current just was seen at a membrane potential of about \(-70 \text{ mV} \) and was activated fully at \(-140 \text{ mV} \). The voltage value of half-maximal activation of \( I_h \) was \(-78.0 \pm 6.0 \text{ (SE) mV} \). The rate of \( I_h \) activation was best approximated by a single exponential function with a time constant that was voltage dependent, ranging from 276 ± 27 ms at \(-100 \text{ mV} \) to 186 ± 11 ms at \(-140 \text{ mV} \). Reversal potential (\( E_h \)) of \( I_h \) current was more positive than the resting potential. Raising the extracellular potassium concentration shifted \( E_h \) to a more depolarized value, whereas lowering the extracellular sodium concentration shifted \( E_h \) in a more negative direction. \( I_h \) was sensitive to extracellular cesium but relatively insensitive to extracellular barium. The current amplitude near maximal-activation (about \(-140 \text{ mV} \)) was reduced to 40% of control by 1 mM cesium but was reduced to only 71% of control by 2 mM barium. When the membrane potential was near the resting potential (about \(-60 \text{ mV} \)), cesium had no effect on the membrane potential, current-evoked firing rate and input resistance but reduced the spontaneous firing. When the membrane potential was more negative than \(-70 \text{ mV} \), cesium hyperpolarized the cell, decreased current-evoked firing and increased the input resistance. \( I_h \) in DNLL neurons does not contribute to the normal resting potential but may enhance the extent of excitation, thereby making the DNLL a consistently powerful inhibitory source to upper levels of the auditory system.

INTRODUCTION

The dorsal nucleus of the lateral lemniscus (DNLL) is a collection of neurons located within the auditory lemniscal pathway just ventral to the inferior colliculus. The DNLL is believed to play an important role in binaural processing and sound localization. DNLL neurons receive excitatory as well as inhibitory connections from multiple sources of the lower auditory brain stem bilaterally and the DNLL contralaterally. Neurons in the DNLL send inhibitory projections to both ipsilateral and contralateral inferior colliculus and to the contralateral DNLL (Glendenning et al. 1981; Hutson et al. 1991; Kudo 1981; Oliver and Shneiderman 1989; Shneiderman et al. 1988; Zook and Casseday 1987). The majority of neurons in the DNLL are sensitive to binaural stimulation (Aitkin et al. 1970; Brugge et al. 1970; Covey 1993; Markovitz and Pollak 1993). Lesion of the DNLL or suppression of activity of DNLL neurons greatly reduces the slopes of interaural intensity or time difference functions of neurons in the contralateral inferior colliculus and auditory cortex (Faingold et al. 1993; Glenn and Kelly 1992; Kelly and Li 1997; Kidd and Kelly 1996; Li and Kelly 1992), and elevates minimum audible angles for sound localization (Kelly et al. 1996). Because almost all DNLL neurons are GABAergic (Adams and Mognaini 1984; Glendenning and Baker 1988; Moore and Moore 1987; Roberts and Ribak 1987; Thompson et al. 1985; Vater et al. 1992; Winter et al. 1995), the effects of suppression or lesion of the DNLL on binaural function of the inferior colliculus and auditory cortex and on sound localization probably are manifested through the inhibitory influence of the DNLL on responses in the upper levels of the auditory system (Kelly and Li 1997; Kidd and Kelly 1996; Li and Kelly 1992).

The membrane properties of DNLL neurons already have been examined in our previous studies (Wu and Kelly 1995a,b). DNLL neurons responded to depolarizing currents with a sustained train of action potentials and a long-duration afterhyperpolarization. Injection of negative current resulted in a graded hyperpolarization that exhibited a sag back toward the resting potential. Strong hyperpolarizing currents often were followed by a large rebound depolarization that could elicit a burst of action potentials.

The sag back toward resting potential after intracellular injection of hyperpolarizing current has been described in many central neurons as an inward rectification, i.e., an increased conductance on hyperpolarization (see review, Pape 1996). There are two general types of inward rectifying currents. One type is the classical inward rectifier discovered in muscle cells by Katz, which is a pure \( K^- \) conductance that is activated at values negative to the \( K^- \) equilibrium potential. It has faster kinetics and much greater sensitivity to barium than cesium. Another type, called \( I_n \), has been identified in rod photoreceptors, sino-atrial node cells, Purkinje fibers of the mammalian heart, and many types of neurons. \( I_n \) current is a slowly developing inward current activated on hyperpolarization of the membrane beyond the resting potential. In many central neurons, the hyperpolarization sag, i.e., inward rectification by hyperpolarization from the resting membrane potential, has been postulated to be...
due to $I_h$. $I_h$ current is a mixed potassium and sodium current and behaves in a way expected of a time- and voltage-dependent membrane conductance. The voltage dependence of the activation of $I_h$ can be regulated by neurotransmitters and metabolic stimuli via the intracellular adenosine 3′,5′-cyclic monophosphate (cAMP) pathway. A general pharmacological feature of $I_h$ is its sensitivity to low concentration of extracellular cesium and its relative insensitivity to barium.

Our previous whole cell patch-clamp studies described several types of potassium currents, including A-current, Ca$^{2+}$-dependent, and Ca$^{2+}$-independent-sustained current, and dextrorotatory-sensitive current in DNLL neurons. The membrane excitability and spike repolarization of DNLL neurons are dependent on these currents (Fu et al. 1996a). In the present study, we used whole cell patch-clamp techniques to characterize the hyperpolarization-activated, mixed potassium and sodium $I_h$ current that underlies inward rectification in DNLL neurons. Our purpose was to investigate the basic properties of this current and to assess its possible role in modulation of DNLL activity. Some of these data have been published in abstract form (Fu et al. 1996b).

**METHODS**

The rat’s DNLL brain slice preparation has been described in detail in a previous report (Fu et al. 1996a). In the present study, transverse DNLL slices (400 μM) were cut from freshly dissected brains of 21- to 30-day-old Wistar rats with a Vibratome. Sectioning was performed with tissue immersed in 30°C oxygenated artificial cerebrospinal fluid (ACSF) that had the following composition (in mM): 129 NaCl, 3 KCl, 1.2 KH$_2$PO$_4$, 2.4 CaCl$_2$, 1.3 MgSO$_4$, 20 NaHCO$_3$, and 10 dextrose. The ACSF was infused continuously with 95%O$_2$-5%CO$_2$ to ensure adequate oxygenation of slices and to maintain a pH of 7.4.

For recordings, a slice was transferred to a circular recording chamber mounted on the stage of a Diastar microscope (Reichert) equipped with Zeiss water-immersion objectives. The chamber, which had a volume of ~200 μl, was continuously perfused with ACSF at a rate of 6–8 ml/min. In voltage-clamp experiments tetrodotoxin (0.3 μM) was added to block voltage-dependent sodium currents. Cesium- and barium-containing solutions were made by adding of 1 mM CsCl and 2 mM BaCl$_2$, respectively, to the ACSF solution. The low-sodium (27.2 mM) solution was made by equimolar substitution of chloro and sodium for NaCl. The high-potassium solution (27.5 mM) was made by substitution of 7.5 mM KCl for NaCl (Kamondi and Reiner 1991). Experimental drugs and test solutions were added to the chamber by redirecting the flow of liquid through a system of tubing and stopcocks. All drugs were prepared with ACSF as the vehicle. Tetrodotoxin and 4-aminopyridine (4-AP) were obtained from Sigma.

Whole cell current- and voltage-clamp recordings from DNLL neurons were obtained with patch electrodes, which were made from 1.1-mm OD and 0.8-mm ID thin-walled glass tubing (Kimax-51, Kimble) with a vertical puller (Kopf Instruments). The recording electrodes had a tip diameter of ~1 μm and a resistance of 3–5 MΩ. The electrodes were filled with a solution containing the following (in mM): 100 K-glucosone, 30 KCl, 1 MgCl$_2$, 5 ethylene glycol-bis(β-aminooethyl ether)-N,N,N′,N′-tetraacetic acid, and 10 N-2-hydroxyethylpipperazine-N′-2-ethanesulfonic acid. The pH of the internal solution was adjusted to 7.25 with KOH.

A List EPC-7 (Medical Systems) or Axopatch 200A (Axon Instruments) amplifier was used for recording in the whole cell voltage- and current-clamp experiments. In current-clamp experiments, currents were injected to hold the cell membrane potential at an appropriate level, and additional current pulses were superimposed on the holding current. In voltage-clamp experiments, there was no compensation for series resistance because the currents rarely exceeded 300 pA, and the resulting voltage errors were <3 mV. The data were digitized on-line, stored, and analyzed on a PC computer with pClamp 6.0 (Axon Instruments). Averaged data are presented as means ± SE.

**RESULTS**

The data represent recordings from 42 DNLL neurons. The mean resting potential was −65.0 ± 3.8 mV ($n = 30$). The mean input resistance was 408.0 ± 10.4 MΩ ($n = 20$).

With current clamp, injections of hyperpolarizing current into DNLL neurons led to an initial hyperpolarization followed by a slowly developing sag of the membrane potential, i.e., inward rectification. Figure 1A shows an example. Hyperpolarizing current pulses of sufficient magnitude resulted in a slowly developing depolarization sag of the electrotonic potential. The rate and magnitude of the sag depended on the amount of hyperpolarizing current injected, i.e., larger currents lead to larger and faster decays. Current-voltage ($I$–$V$) curves (Fig. 1B) showed an essentially linear current-voltage relationship for the initial hyperpolarization (Fig. 1A, ●), but a nonlinear current-voltage relationship for the final hyperpolarization (Fig. 1A, ○) due to a decrease in the input resistance of the membrane. Thirty-six of 42 DNLL neurons (86%) exhibited an observable sag after the injection of hyperpolarizing current. Thus the slowly developing inward rectification that constitutes the relaxation phase of hyperpolarization is a common electrical feature of the DNLL cell membrane. During voltage clamp in this cell (Fig. 1C), hyperpolarizing voltage steps of 10-mV increments from a holding potential of −50 mV elicited an instantaneous inward leak current. At membrane potential more negative than −70 mV, there was an additional, slowly activating inward current that did not inactivate during voltage steps of 900-ms duration (Fig. 1C). The instantaneous current jump ($I_m$; ●) measured immediately after the capacitive transient, the steady-state current ($I_s$; ∆) measured at the end of the 900-mV voltage step, and the difference between $I_m$ and $I_s$ are plotted as a function of the membrane potential in Fig. 1D. The difference in the current measured at the beginning and the end of the voltage step represents the hyperpolarization-activated inward current, $I_h$ (○) (Fig. 1D, $I_h = I_s - I_m$). A marked inward current appeared at membrane potentials more negative than −70 mV. This slowly activating inward current presumably underlies the sag observed during hyperpolarization in current-clamp records. The voltage- and time-dependent properties and pharmacological characteristics of $I_h$ in DNLL neurons will be shown below.

**Reversal potential of $I_h$**

In many other types of neurons, $I_h$ is carried jointly by sodium and potassium ions and has a reversal potential ($E_h$) more positive than the resting membrane potential under normal physiological conditions (Banks et al. 1993; Bayliss et al. 1994; Dekin 1993; Kamondi and Reiner 1991; Mayer and Westbrook 1983; McCormick and Pape 1990). We used the method described by Mayer and Westbrook (1983), Ka-
FIG. 1. General characteristics of $I_h$. A: in current clamp, the voltage responses (top) to hyperpolarizing and depolarizing current pulses (bottom) were recorded in a dorsal nucleus of the lateral lumniscus (DNLL) neuron. Depolarizing sag reflects activation of $I_h$. Resting potential was $-73$ mV. B: peak (●) and steady-state (○) voltages were plotted as a function of injected currents. C: currents (top) were evoked from a holding potential of $-50$ to $-140$ mV by hyperpolarizing voltage steps (bottom). An inward current slowly developed during hyperpolarization. D: instantaneous current ($I_{ins}$; ●) measured at the end of the capacitive transient and steady-state current ($I_{ss}$; Δ) measured at the end of voltage steps were plotted against the membrane potential measured at the same time. Difference between $I_{ins}$ and $I_{ss}$ represents the time- and voltage-dependent inward current $I_h$ (○). E: currents (top) were evoked from a holding potential of $-130$ mV by voltage steps (bottom). F: $I$-$V$ curves for instantaneous currents from C (●) and E (▲) are linear throughout the voltage range steps. Extrapolated intersection (↑) of instantaneous $I$-$V$ curves gives an estimate of the reversal potential ($E_h$) of $I_h$.

mondi and Reiner (1991), and Bayliss et al. (1994) to estimate the reversal potential of $I_h$ in a DNLL cell. The voltage-clamp experiment was conducted in the same DNLL neuron as shown in Fig. 1A. $I_{ins}$ was elicited by voltage steps from a holding potential of $-50$ mV at which $I_h$ was not activated (Fig. 1C, ●), and then from a holding potential of $-130$ mV where $I_h$ was strongly activated (Fig. 1E, ▲). Plots of $I_{ins}$ as a function of the membrane potential are presented in
The voltage dependence of \( I_h \), a 2-step protocol was used to determine the voltage-dependent activation of \( I_h \). The 900-ms-duration prepulse to various holding potentials between \(-50\) and \(-140\) mV partially activated \( I_h \). Current observed during the subsequent test pulse to \(-140\) mV was used to calculate the amount of \( I_h \) at different membrane potentials. \( I_{160} \) is the maximal current, indicated by the horizontal arrow, evoked by stepping the membrane potential to \(-140\) mV, and \( I_h \) is the current evoked during the \(-140\) mV test pulse after a prepulse of membrane potential \( V \), measured at the time indicated by the vertical arrow. \( I_h \) was calculated for each test pulse by subtracting \( I_{140} \) from \( I_{160} \). A: plot of relative \( I_h \) current \(( \frac{I}{I_{\text{max}}} \) against the membrane potential from the cell shown in A. The data were fitted to Eq. 1 (see text), with a calculated \( V_{1/2} = -90\) mV and \( K = 0.1\) mV.

**Voltage dependence of \( I_h \)**

We used the method described by Kamondi and Reiner (1991) to measure the voltage dependence of \( I_h \) in DNLL neurons. A two-step voltage-clamp protocol was used to examine the activation of \( I_h \). The membrane potential was held at \(-50\) mV and then stepped in \(-10\)-mV increments to potentials between \(-60\) and \(-140\) mV for 900 ms (Fig. 2A). This conditioning step led to a partial activation of \( I_h \). After this prepulse, a test pulse was applied by stepping the membrane potential to \(-140\) mV, where \( I_h \) appeared to be fully activated (\( I_{-140} \)). The currents observed during the test pulse represents the proportion of \( I_h \) that was not fully activated during the conditioning pulse. The magnitude of \( I_h \) at a given holding membrane potential was calculated by subtracting the current \( (I_t) \) measured during the test pulse from the maximal value of \( I_h \) (\( I_{-140} \)). The activation curve of \( I_h \) was obtained by plotting the normalized current \((\frac{I}{I_{\text{max}}} \) against the membrane potential (Fig. 2B). The data were fitted by a Boltzman function

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp[k(V - V_{1/2})]} \tag{1}
\]

where \( I_{\text{max}} \) is the maximum current recorded at \(-140\) mV after the conditioning pulse, \( I \) is the amplitude of the current recorded at a membrane potential \((V) \) after the prepulse, \( V_{1/2} \) is the membrane potential at which the current is half-maximally activated, and \( k \) is a slope factor. The experimental data were fitted to the form giving \( V_{1/2} = -90\) mV for this neuron. An average of \( V_{1/2} \) was \(-78.0 \pm 6.0\) mV, and of \( k \) was \(0.10 \pm 0.06 \) (\( n = 6 \)).

The voltage dependence of the time constant of \( I_h \) activation was examined with the voltage protocol shown in Fig. 3A. The currents were evoked by hyperpolarizing voltage steps from a holding potential of \(-50\) mV to various levels between \(-60\) and \(-140\) mV. The data (full traces) were best fitted by a single exponential function (\( F \) test, \( P < 0.05 \)) of the form

\[
I = I_s + I_h \exp(-t/\tau) \tag{2}
\]

where \( I \) is the current at the time \((t) \), \( I_s \) is the steady-state current at the end of the voltage step, \( I_h \) is the difference between \( I_s \) and \( I_{\text{max}} \), and \( \tau \) is the time constant. In six DNLL neurons, the voltage sensitivity of activation kinetics of \( I_h \). A: current evoked by voltage steps to various membrane potentials was fitted to Eq. 2 (see text). B: mean \( \pm \) SE time constants at various voltage steps are plotted against the membrane potential (\( n = 6 \)).
neurons, the time constant for activation of \( I_h \) was 186 ± 11 ms at -140 mV, 216 ± 17 ms at -120 mV, and 276 ± 27 ms at -100 mV. Figure 3B shows the mean activation time constants plotted against the membrane potential for these six neurons. The activation kinetics of \( I_h \) in DNNL neurons showed voltage sensitivity in the membrane potential range from -100 to -140 mV, with the activation time constant decreasing as the membrane potential was further hyperpolarized.

Effects of low sodium and high potassium on \( I_h \)

To investigate which cation species contributing to \( I_h \) in DNNL neurons, we examined the effects of varying extracellular concentration of sodium (\([Na^+]_o\)) and potassium (\([K^+]_o\)) on \( I_h \). The results of a typical experiment with a DNNL neuron are illustrated in Fig. 4. After perfusion of a modified ACSF containing high potassium (10 mM), the instantaneous currents increased (Fig. 4B). In contrast, when \([Na^+]_o\) was reduced to 27.2 mM, the instantaneous currents decreased (Fig. 4C). These changes were accompanied by a marked alteration in \( E_h \) measured with the same voltage protocol shown in Fig. 1. C and E. \( E_h \) was shifted from -48 to -15 mV when \([K^+]_o\) was increased to 10 mM (Fig. 4D) and from -48 to -70 mV when \([Na^+]_o\) was decreased to 27.2 mM (Fig. 4E). Similar shifts of \( E_h \) toward depolarization by high \([K^+]_o\) and toward hyperpolarization by low \([Na^+]_o\) were observed from five and three other cells, respectively. These results suggest that sodium and potassium are the charge carriers for \( I_h \) in DNNL neurons (see DISCUSSION).

Pharmacology of \( I_h \)

We examined the relative sensitivity of \( I_h \) of DNNL neurons to extracellular cesium (Cs\(^+\)) and barium (Ba\(^{2+}\)). The time- and voltage-dependent hyperpolarization-activated \( I_h \) current was blocked almost completely by 1 mM Cs\(^+\) in the DNNL neuron shown in Fig. 5A. Using the voltage protocol shown in Fig. 1C, plots of the amplitude of \( I_h \) (\( I_h = I_h - I_{\text{inj}} \)) against the membrane potential in normal and in Cs\(^+\)-containing ACSF in this cell are shown in Fig. 5B. \( I_h \) was diminished greatly by Cs\(^+\) at all membrane potentials more negative than -60 mV.

Similarly, barium also reduced \( I_h \) but had less effect than cesium. The protocol shown in Fig. 1C was used for measuring \( I_h \) in normal and in Ba\(^{2+}\)-containing ACSF. The reduction of \( I_h \) by 2 mM barium is shown in Fig. 6B. Ba\(^{2+}\) reduced \( I_h \) at all membrane potentials more negative than -60 mV, but to a lesser degree than Cs\(^+\).

The suppression of \( I_h \) by Cs\(^+\) and Ba\(^{2+}\) was more prominent when the cell membrane was more hyperpolarized than when the cell membrane was less hyperpolarized (Figs. 5B and 6B), indicating the effects of Cs\(^+\) and Ba\(^{2+}\) on \( I_h \) were voltage dependent. Both Cs\(^+\) and Ba\(^{2+}\) also reduced the whole cell resting conductance of DNNL neurons. Similar results were obtained with 2 mM 4-AP (not shown), which is a blocker for potassium channels. Thus Cs\(^+\) and Ba\(^{2+}\) also may block the leakage conductance of DNNL neurons nonspecifically.

The combined data for the effects of cesium and barium on \( I_h \) are shown in Fig. 7. The amplitude of \( I_h \) was measured at the level of maximal activation and was plotted as a percentage of the control amplitude. \( I_h \) was reduced to 40% (35 and 45%, \( n = 2 \)) of the control in 1 mM cesium and to 71% (59–84%, \( n = 7 \)) of the control in 2 mM barium. The reduction in \( I_h \) was significantly different for the cesium and barium groups (Mann-Whitney \( U = 0, P < 0.05 \)). Thus the \( I_h \) observed in DNNL neurons was more sensitive to cesium than to barium.

Functional properties of \( I_h \)

To further investigate what role \( I_h \) may play in physiologicalfunctions of DNNL neurons, we examined the effects of cesium on spontaneous and current-evoked firing because cesium substantially suppresses or blocks \( I_h \) in many types of neurons (Banks et al. 1993; Bayliss et al. 1994; Dekin 1993; Kamondi and Reiner 1991; Mayer and Westbrook 1983; McCormick and Pape 1990; Mercuri et al. 1995; Strohmann et al. 1994), as well as in DNNL neurons (Fig. 5). Bath application of CsCl (2 mM) was used to determine the contribution of \( I_h \) to firing rate. Figure 8 shows that 2 mM cesium reduced the frequency of spontaneous activity when the cell membrane potential was held at -59 mV. The spontaneous firing rate of DNNL neurons was 5.6 ± 0.7 Hz in normal ACSF and was decreased to 1.3 ± 0.3 Hz (\( n = 3 \)) with the brain slice of the DNNL in 2 mM CsCl, i.e., cesium perfusion reduced the spontaneous rate by 23% (t-test, \( P < 0.05 \)). The alteration of firing rate of DNNL neurons by cesium at least in part may be attributed to blockade of \( I_h \) (see DISCUSSION).

The contribution of \( I_h \) to evoked discharge rate also was tested with CsCl in three DNNL neurons. Figure 9 shows an example of the experiments. The cell was held at four different membrane potentials (-60, -70, -80, and -90 mV) by injecting the appropriate amount of DC current. Two depolarizing current pulses (20 and 40 pA) of 200-ms duration were applied with the brain slice in normal and Cs\(^+\)-containing ACSF (Fig. 9A). Two millimolar CsCl had no effect on the membrane potential, input resistance or firing rate of this cell at a holding membrane potential of -60 and -70 mV. As the holding potential was hyperpolarized to -80 or -90 mV, 2 mM cesium hyperpolarized the cell, increased the input resistance, and decreased the firing rate, i.e., the lower the holding membrane potential, the greater the effects of cesium. The number of spikes evoked by 40 pA in this DNNL neuron is plotted as a function of the holding potential under normal conditions and with 2 mM CsCl in Fig. 9B. Cesium reduced spike frequency to 50 and 20% of the control at holding potentials of -80 and -90 mV, respectively, but did not affect the firing rate at holding potentials of -60 and -70 mV.

DISCUSSION

The major finding of the present study is the presence of a mixed cation current (\( I_h \)) that underlies the inward rectification caused by hyperpolarization in rat DNNL neurons. The data add to our previous results showing a variety of potassium channels in DNNL neurons and represent a further step in understanding the mechanisms that govern the membrane excitability of DNNL neurons.
In the present study, we have characterized the hyperpolarization-activated inward current ($I_h$) in DNLL neurons using whole cell patch-clamp recordings. Our data show a close temporal correspondence between the depolarizing sag of the hyperpolarizing electrotonic potential and the development of $I_h$, indicating that $I_h$ underlies the inward rectification on hyperpolarization. Activation of $I_h$ in DNLL neurons is voltage dependent. This voltage dependency is well de-
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1991) and neurons of the medial nucleus of the trapezoid body (Forsythe and Barnes-Davies 1993). The value of the reversal potential of \( I_h \) current in DNLL neurons is also consistent with that in other types of neurons. Measurements of the reversal potential for \( I_h \), estimated by extrapolation methods similar to our own, range from \(-17\) mV in rat hippocampal neurons (Maccaferri et al. 1993) to \(-50\) mV in cells from the sensorimotor cortex of the cat (Spain et al. 1987). The reversal potential of \( I_h \) in DNLL neurons was positive to the normal resting potential and was affected by extracellular potassium or sodium concentrations. These results indicate that \( I_h \) in DNLL neurons is a mixed potassium and sodium current. Although the possibility of contribution of the chloride current to \( I_h \) was not investigated in this study, it is not likely that the \( I_h \) has a significant chloride

scribed by a Boltzmann function and is similar to that of the corresponding membrane conductance in many other neurons (Banks et al. 1993; Bayliss et al. 1994; Dekin 1993; Kamondi and Reiner 1991; Maccaferri et al. 1993; Mayer and Westbrook 1983; McCormick and Pape 1990; Mercuri et al. 1995; Travagli and Gillis 1994; Womble and Moises 1993). \( I_h \) in DNLL neurons is activated at a voltage range negative to the resting potential, i.e., \(-70\) to \(-140\) mV. The threshold for activation of \( I_h \) in DNLL neurons is comparable with reported values for threshold of \( I_h \) activation in other types of neurons, including \(-69\) mV for rat midbrain dopaminergic neurons (Mercuri et al. 1995), \(-70\) mV for guinea pig bulbospinal neurons (Dekin 1993), and \(-80\) mV for rat hypothalamus histaminergic neurons (Kamondi and Reiner

FIG. 5. Effect of cesium on \( I_h \). A: current evoked by hyperpolarizing voltage steps (bottom) from \(-50\) to \(-140\) mV in control ACSF (top) and after addition of 1 mM CsCl (middle). B: the amplitude of \( I_h \) is plotted against the membrane potential in control (●) and in Cs⁺ containing ACSF (○).

FIG. 6. Effect of barium on \( I_h \). A: current evoked by hyperpolarizing voltage steps (bottom) from \(-50\) to \(-140\) mV in control ACSF (top) and after an addition of 2 mM BaCl₂ (bottom). B: amplitude of \( I_h \) is plotted against the membrane potential in control (●) and in Ba²⁺-containing ACSF (○).
conclusion was drawn from studies of $I_h$ in rat hypothalamus histaminergic neurons and midbrain dopaminergic neurons, as the activation threshold of $I_h$ in those neurons was also more negative than their resting potentials (Kamondi and Reiner 1991; Mercuri et al. 1995).

$I_h$ has been shown to be a “pacemaker” current and to provide pacemaker depolarization during generation of rhythmic oscillatory activity in sino-atrial node cells, Purkinje fibers of heart, and thalamic neurons, suggesting that $I_h$ plays a significant role in controlling autorhythmic activity (DiFrancesco 1981, 1986; McCormick and Pape 1990). DNNL neurons usually do not exhibit an autorhythmic oscillation, but some fire spontaneously both in vivo (Aitkin et al. 1970) and in vitro (Wu and Kelly 1995a,b). We hypothesize that the spontaneous activity of DNNL neurons could be regulated in part by $I_h$. The action potential of DNNL neurons usually is followed by an afterhyperpolarization (AHP) that exhibits either a single component or double components (an initial rapid hyperpolarization followed by a 2nd slower phase of hyperpolarization) (Wu and Kelly 1995a,b). The AHP of DNNL neurons could take the membrane potential to a value between $-70$ and $-80$ mV (Wu and Kelly 1995a,b) (Fig. 8), causing activation of $I_h$, acceleration of spike repolarization and an increase in firing. Pharmacological suppression of $I_h$ by application of cesium reduced the spontaneous firing rate of DNNL neurons (Fig. 8) probably because of a prolonged AHP. Thus $I_h$ in DNNL neurons increases the rate of spike AHP. As a con-

![FIG. 7. Mean data for the effects of cesium and barium on $I_h$. Amplitude of $I_h$ was measured at its maximal activation (−140 mV). $I_h$ was decreased to 40% of the control in 1 mM cesium ($n = 2$) and to 71% of the control in 2 mM barium ($n = 7$) (see text for details).](image)

In some types of neurons, $I_h$ appears to assist in determining the resting potential. This is due to the fact that the resting potential of these neurons lies within the voltage range of $I_h$ activation between $-50$ and $-100$ mV (Bayliss et al. 1994; Kamondi and Reiner 1991; McCormick and Pape 1990; Mercuri et al. 1995; Travagli and Gillis 1994). $I_h$ in DNNL neurons was suppressed greatly by extracellular cesium but was less sensitive to extracellular barium. Thus all the properties of $I_h$ in DNNL neurons, including the voltage range of activation, the kinetics of activation, the reversal potential and its alteration with extracellular potassium and sodium concentrations, and the differential sensitivity to cesium and barium are comparable with those previously described for $I_h$ in many other neurons (Bank et al. 1993; Bayliss et al. 1994; Dekin 1993; Kamondi and Reiner 1991; McCormick and Pape 1990; Strohmann et al. 1994). These observations suggest that the inward rectification in DNNL neurons results from the activation of $I_h$ current rather than the classical inward rectifier.

FIG. 8. Effect of cesium on spontaneous firing rate. Firing rate was 4 Hz in normal ACSF and was reduced to 2 Hz in 2 mM CsCl in this cell. Frequency of cell firing was determined by the number of the spikes during 2–4 s. Cell membrane potential was $-59$ mV.

![FIG. 8. Effect of cesium on spontaneous firing rate.](image)
sequence, the level of DNLL spontaneous activity is enhanced. Travagli and Gillis (1994) have made a similar suggestion that the spontaneous activity is modulated in part by $I_h$ current in neurons of the rat dorsal motor nucleus of the vagus (DMV neurons), as the spontaneous firing rate of DMV neurons expressing $I_h$ was reduced with cesium.
Although cesium also may block leak conductance or other types of voltage-dependent conductances, such as delayed rectifier and K⁺ inward rectifier channels (Pape 1996), our evidence of the voltage range of the AHP at which the Iₛ could be activated in DNLL neurons supports the idea that the Iₛ at least in part may regulate the spontaneous activity in DNLL neurons. A further study using various pharmacological blocking agents differentially affecting these conductances and Iₛ may disclose the exact fraction of contribution of Iₛ current to electrical activity of DNLL neurons.

Another possible role of Iₛ current in DNLL neurons might be to modulate synaptic excitation. DNLL neurons receive excitatory projections probably from neurons in the contralateral cochlear nucleus, the contralateral lateral superior olive, and the ipsilateral medial superior olive (Glendenning et al. 1981). Electrical stimulation of the lateral lemniscus, which is a major afferent pathway from the lower auditory brain stem, often elicits excitatory postsynaptic potentials (EPSPs) in DNLL neurons in a brain slice preparation (Wu and Kelly 1995a,b). Both short- and longer-latency EPSPs can be evoked in the same neuron. A single spike with relatively constant latency can be elicited from the short-latency EPSP, whereas a single or multiple spikes with highly variable latencies can be evoked from the long-latency EPSP. Studies of the synaptic pharmacology of DNLL neurons suggest that the short-latency EPSP is mediated by glutamate via non-N-methyl-D-aspartate (NMDA) receptors, whereas the longer-latency EPSP is mediated through NMDA receptors (Fu et al. 1997; Wu and Kelly 1996). During synaptic excitation, especially the NMDA-receptor–mediated excitation with a long time course and multiple action potentials (Fu et al. 1997), Iₛ may increase the rate of spike repolarization and thus increase the firing rate. These longer excitatory events would be enhanced by Iₛ. Therefore, Iₛ in DNLL neurons would augment the influence of excitatory inputs. We speculate that an important role of Iₛ in DNLL neurons is to increase the efficacy of glutamatergic-mediated excitatory postsynaptic potentials.

Neurons in the DNLL are largely GABAergic and provide a major source of inhibitory projection to the auditory midbrain (Adams and Mugnaini 1984; Hutson et al. 1991; Oliver and Shneiderman 1989, 1991; Shneiderman et al. 1993). The inhibition from the DNLL exerts an important influence on binaural processing in upper levels of the central auditory system (Fangold et al. 1993; Glenn and Kelly 1992; Kelly and Li 1997; Kidd and Kelly 1996; Li and Kelly 1992) and plays an active role in sound localization (Ito et al. 1996; Kelly et al. 1996). By limiting prolonged hyperpolarization, activation of Iₛ in DNLL neurons would augment spontaneous and excitatory synaptic activity. As a result, a higher level of excitation in the DNLL would be translated into an enhanced inhibition of the postsynaptic neurons with which the DNLL neurons make contact. Thus Iₛ current may serve as a modulator to regulate excitation to an extent that makes the DNLL a consistently powerful inhibitory source to upper levels of the auditory system.

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