Hyperpolarization-Activated Inward Current in Histaminergic Tuberomammillary Neurons of the Rat Hypothalamus

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SUMMARY AND CONCLUSIONS

1. Intracellular recordings were obtained from histaminergic tuberomammillary (TM) neurons of rat hypothalamus in an in vitro slice preparation. The properties of a time- and voltage-dependent inward current activated on hyperpolarization, Ih, were studied by use of the single-electrode voltage-clamp technique.

2. The activation curve of Ih was well fit by a sigmoidal function, with half-maximal activation occurring at $-98 \pm 6$ mV.

3. The estimated reversal potential of Ih ($E_h$) in TM neurons was $-35 \pm 9$ (SD) mV.

4. The time constant of activation was well fit by a single exponential function and exhibited marked voltage dependence: at $-90$ mV, Ih activated with a time constant of $823 \pm 35$ ms, whereas at $-130$ mV, Ih activated with a time constant of $280 \pm 65$ ms. The time constant of deactivation of Ih at $-60$ mV was $302 \pm 35$ ms.

5. Raising the extracellular potassium concentration ($[K^+]_o$) to 10 mM shifted $E_h$ to a more depolarized value, while lowering the extracellular sodium concentration ($[Na^+]_o$) shifted $E_h$ in the negative direction. Altering the extracellular chloride concentration ($[Cl^-]_o$) had little effect on $E_h$.

6. Increasing $[K^+]_o$ to 10 mM increased the amplitude of both Ih and its underlying conductance $g_h$, while reducing $[Na^+]_o$, caused a small reduction in the amplitude of Ih with no measurable effect on $g_h$.

7. The time constant of activation of Ih became shorter in raised $[K^+]_o$ and longer in lowered $[Na^+]_o$.

8. Extracellularly applied cesium blocked Ih in a voltage-dependent manner. Extracellular barium reduced Ih but was less effective than cesium.

9. We conclude that Ih, carried by sodium and potassium ions, accounts for inward rectification of TM neurons. By increasing the whole-cell conductance during periods of prolonged hyperpolarization, Ih may act as an ionic shunt, decreasing the efficacy of synaptic inputs. This effect would be most apparent during rapid-eye-movement sleep, when TM neurons fall silent.

INTRODUCTION

The existence of an inward current activated on hyperpolarization, termed inward rectification, has been described in many neuronal and nonneuronal systems. Despite the ubiquitous nature of inward rectification, much confusion exists regarding this phenomenon. At least two different types of inward rectification have been described. One is the classic anomalous rectifier, first described by Katz (1949) in muscle and since then shown to be present in a variety of cell types (Constanti and Galvan 1983; Hagiwara and Takahashi 1974; Leech and Stanfield 1981; Uchimura et al. 1989). The anomalous rectifier is a pure potassium conductance that, in most cells, is active only negative to the potassium equilibrium potential ($E_K$). Activation occurs within a few milliseconds of an appropriate change in membrane potential (Sakmann and Trube 1984). Recordings of single anomalous rectifying potassium channels in guinea pig heart have revealed the channel to be ohmic in nature, with its voltage dependence arising secondary to voltage-dependent blockade by internal magnesium ions (Matsuda et al. 1987; Vandenberg 1987).

A second form of inward rectification can be distinguished from the anomalous rectifier by its kinetics, voltage dependence, and ionic charge carrier. Termed the hyperpolarization-activated inward current, or Ih, this current activates over hundreds of milliseconds, in contrast to the rapid activation of the anomalous rectifier. The voltage dependence of Ih varies with cell types, but activation always begins positive to the potassium equilibrium potential. Ih, also variously known as $I_o$ and $I_f$, is a mixed cationic current carried by both sodium and potassium ions (Angstadt and Calabrese 1989; Bader and Bertrand 1984; Benham et al. 1987; Crepel and Penit-Soria 1986; DiFrancesco 1981; Edman et al. 1987; Halliwell and Adams 1982; Hestin 1987; Mayer and Westbrook 1983; McCormick and Pape 1990; Spain et al. 1987; Tokimasa and Akasu 1990; Yanagihara and Irisawa 1980).

Previous studies have shown that the histaminergic neurons of the tuberomammillary (TM) nucleus of the rat hypothalamus exhibit inward rectification (Haas and Reiner 1988). These neurons are of particular interest insofar as they exhibit marked changes in spontaneous activity across behavioral states, falling silent during rapid-eye-movement (REM) sleep (Vanni-Mercier et al. 1984). To gain some insight into the possible contribution of Ih to the behavioral neurophysiology of TM neurons, we have used the single-electrode voltage clamp technique to carry out a detailed analysis of Ih in TM neurons in slices maintained in vitro. Preliminary versions have been published in abstract form (Kamondi and Reiner 1990a,b).

METHODS

The methods for preparing slices and recording from histaminergic TM neurons were similar to those employed in previous studies (Greene et al. 1990; Haas and Reiner 1988). Young male rats were anesthetized with halothane and decapitated. Their brains were quickly removed and immersed in cold artificial cere-
brosplinal fluid (ACSF). The hypothalamus was dissected free, and 300-μm-thick coronal slices containing the TM nucleus were cut on a vibratome. Before recording, slices were stored in a holding chamber in ACSF at room temperature for ≥1 h. For electrophysiological recordings, individual slices were transferred to a recording chamber, where they were superfused with warmed (30°C) ACSF at a flow rate of 1.8-2.0 ml/min. Intracellular recordings were obtained with 2 M KCl-filled microelectrodes, the resistances of which ranged from 30 to 60 MΩ. Single-electrode voltage-clamp measurements were obtained with an Axoclamp 2A amplifier with a switching frequency of 3-5 kHz and were filtered at 1 kHz. Headstage output was continuously monitored on a separate oscilloscope and capacitance was fully compensated.

For voltage-command generation, data acquisition, and current-voltage (I-V) curve analysis, we used PCLAMP software (Axon Instruments), whereas, for time constant measurement and activation curve analysis, we used FIGP software (Biosoft). Data were independently digitized and stored on videotape for off-line analysis. Results are expressed as means ± SD. For data treated statistically, significance was defined as P < 0.05.

The standard ACSF had the following composition (in mM): 2.5 KCl, 126 NaCl, 2.5 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 26 NaHCO₃, and 11 glucose, saturated with 95% O₂-5% CO₂, pH 7.4. In most experiments, tetrodotoxin (0.3 μM) was added to block voltage-dependent sodium currents, and bicuculline (10 μM) was added to block spontaneous γ-aminobutyric acid-A (GABAₐ)-mediated synaptic currents (Reiner and Haas 1990). The low-sodium (27.2 mM) solution was made by equimolar substitution of choline chloride for NaCl. When the extracellular potassium concentration ([K⁺]ₑ) was raised to 10 mM (high-potassium solution), 7.5 mM KCl was substituted for NaCl. Low-chloride solution contained 126 mM Na-isothionate instead of NaCl, resulting in a final chloride concentration of 9.9 mM. In experiments employing cesium and barium, the relevant cation was added directly to the ACSF. All drugs were obtained from Sigma.

**RESULTS**

The data are based on recordings from 102 TM neurons that exhibited electrophysiological properties identical to

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**FIG. 1.** Basic properties of $I_h$. A: in bridge mode, hyperpolarizing current pulses from a membrane potential of −60 mV result in a depolarizing sag of electrotonic potential. B: current traces recorded in single-electrode voltage-clamp mode evoked by voltage steps from holding potentials of −60 (top) and −110 mV (bottom). Hyperpolarizing voltage steps from −60 mV result in a slowly developing inward current, at offset of steps, a transient outward current is observed. Arrows show the cursor position for “instantaneous” (I_{ins}) and steady-state (I_{ss}) current measurements for I-V curves. C: steady-state I-V curves, generated from holding potentials of −60 ($\Delta$) and −110 mV ($\triangle$), are linear throughout the voltage range examined. Extrapolated intersection ($\times$) of “instantaneous” I-V curves gives an estimate of $E_h$, the reversal potential of $I_h$. 

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**RESULTS**

The data are based on recordings from 102 TM neurons that exhibited electrophysiological properties identical to
those previously identified as histaminergic neurons by the use of immunohistochemical techniques (Haas and Reiner 1988). Input resistance was calculated by applying sufficiently small hyperpolarizing current pulses to produce a 10- to 15-mV deflection from a holding potential of -60 mV. Mean input resistance was 280 ± 112 MΩ (n = 17), and action potential threshold in these spontaneously active neurons was -54 ± 3 mV (n = 11). On application of 0.3 μM tetrodotoxin, the resting potential was usually between -55 and -60 mV.

During bridge-mode recordings, hyperpolarizing current pulses of sufficient magnitude to bring the membrane potential negative to -80 mV resulted in a slowly developing depolarizing sag of the electrotonic potential (Fig. 1A). A similar phenomenon has been described in a variety of preparations (Benham et al. 1987; Crepel and Penit-Soria 1986; Mayer and Westbrook 1983; Silva et al. 1990; Spain et al. 1987; Sugihara and Furukawa 1989). In single-electrode voltage-clamp mode, hyperpolarizing voltage steps of 10-mV increments from a holding potential of -60 mV evoked a small instantaneous inward current and, negative to -80 mV, a slowly activating inward current that did not inactivate during the course of voltage steps of 1- to 5-s duration (Fig. 1B). Ih thus appears to underlie the depolarizing sag noted in current-clamp recordings.

As one would expect when recording from a cell with extended geometry, the first 5-10 ms after a voltage step were dominated by an artifact resulting from charging of the membrane capacitance. This precluded measurement of true instantaneous currents. However, the current measured after the capacitative artifact had settled (which we term “instantaneous” current to differentiate it from the true instantaneous current) was essentially linear over a voltage range that straddled the potassium equilibrium potential (approximately -110 mV; Fig. 1C). Thus, under our recording conditions, anomalous rectification was not observed. In contrast to the ohmic behavior of the “instantaneous” current, the steady-state I-V curve shows strong inward rectification negative to -80 mV (Fig. 1C) due to the invariable presence of Ih in TM neurons.

**Reversal potential of Ih**

Tail-current analysis of the reversal potential of Ih proved impractical in TM neurons because of the presence of a transient outward current insensitive to 4-aminopyridine (Greene et al. 1990). Therefore we utilized the method described by Mayer and Westbrook (1983) in dorsal root ganglion neurons and shown in Fig. 1B. “Instantaneous” I-V curves were generated from holding potentials of -60 mV, at which Ih is not active, and -110 mV, at which Ih is fully activated (vide infra). The intersection of the extrapolated “instantaneous” I-V curves gives an estimate of the reversal potential of Ih (Eh; Fig. 1C). In 15 TM neurons analyzed in this fashion, Eh was -35 ± 9 mV.

**Voltage dependence of Ih**

To maximize the sensitivity of our measurements of the voltage dependence of Ih, we designed a two-step voltage-clamp protocol (Fig. 2A) that allowed us to measure Ih at a rather hyperpolarized membrane potential where the driving force on the current is large. The membrane potential was held at 60 mV and stepped in 10-mV increments from potentials between -70 and -130 mV for 1 s. This conditioning step allowed for partial activation of Ih. After this prepulse, a test pulse was applied by stepping the membrane potential to -130 mV, where Ih appeared to be fully activated. The family of currents observed during the test pulse represents the proportion of Ih that was not activated during the conditioning pulse. The magnitude of Ih at a

![FIG. 2. Voltage dependence of Ih. A: 2-step voltage-clamp protocol was used to determine the voltage-dependent activation of Ih. The 1-s-duration prepulse to various holding potentials partially activates Ih. Family of currents observed during the subsequent test pulse to -130 mV was used to calculate the amount of Ih (I) at different membrane potentials using measurements shown at right. I_{130} is maximal current evoked during the test pulse to -130 mV, I_{-60} is current evoked by a voltage step from -60 to -130 mV, and I_h is current evoked during the step to -130 mV after a prepulse of membrane potential V, measured at the time indicated by the arrow. B: plot of relative current (I/I_{max}) vs. membrane potential from the cell shown in A. Data points were fitted to Eq. 1, with a calculated $V_{1/2} = -97$ mV and $k = 0.11$ mV.](A)
given membrane potential \( I \) was calculated by subtracting the current measured during the test pulse from the maximal value of \( I_h \) \( (I_{\text{max}}) \), as shown in Fig. 2A. The activation curve of \( I_h \) was generated by plotting the normalized current \( I/I_{\text{max}} \) against the conditioning pulse membrane potential (Fig. 2B). Data points were well fit by a sigmoidal function of the form

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

where \( I_{\text{max}} \) is the maximum current, \( I \) is the calculated current at a membrane potential of \( V \), \( V_{1/2} \) is the potential at which the current is half-maximally activated, and \( k \) is a slope factor. The experimental data were fitted to Eq. 1, giving an average \( V_{1/2} = -98 \pm 6 \text{ mV} \) and \( k = 0.10 \pm 0.05 \text{ mV} \) \( (n = 20) \).

**Kinetics of \( I_h \)**

The activation kinetics of \( I_h \) in TM neurons were best fit by a single exponential function of the form

\[
I_t = I_{\text{ss}} + I_h \exp(-t/\tau)
\]

where \( I_t \) is the current at time \( t \), \( I_{\text{ss}} \) is the steady-state current at the end of the voltage step, \( I_h \) is the difference between \( I_{\text{ss}} \) and the "instantaneous" current, and \( \tau \) is the time constant. In 31 neurons, the time constant for activation at \(-130 \text{ mV} \) was \( 280 \pm 65 \text{ ms} \). These data were obtained at \( 30^\circ \text{C} \); one would expect the kinetics of \( I_h \) to be somewhat faster at physiological temperatures.

The voltage dependence of the time constant of \( I_h \) activation was studied with the voltage-clamp protocol shown in Fig. 3 (inset). The currents evoked by hyperpolarizing voltage steps in 10-mV increments from a holding potential of \(-60 \text{ mV} \) were fitted to Eq. 2. At membrane potentials less than or equal to \(-90 \text{ mV} \), single exponential curve fitting gave satisfactory results. At less hyperpolarized voltages, the goodness of fit was poor and we excluded these data from the analysis. Figure 3 shows the mean activation time constants for five neurons plotted against membrane potential. The activation kinetics of \( I_h \) in TM neurons showed strong voltage sensitivity in the membrane potential range from \(-90 \) to \(-130 \text{ mV} \), with the activation time constant becoming shorter as the membrane was further hyperpolarized, as has been described in several other systems (Angstadt and Calabrese 1989; Halliwell and Adams 1982; Mayer and Westbrook 1983; Yanagihara and Irisawa 1980).

Figure 4A shows an experiment in which an envelope
FIG. 5. Effects of low [Na⁺] and high [K⁺] on *Eₜ*. A–C: current traces evoked by voltage steps from holding potentials of −60 (top) and −120 mV (bottom) in control ACSF (A), in low-sodium (27.2 mM) solution (B), and high-potassium (10 mM) solution (C). Arrows indicate time point used for measurement of “instantaneous” current. D–F: “Instantaneous” *I–V* curves obtained at holding potentials of −60 and −120 mV. Intersections of extrapolated best fit lines represent the estimated *Eₜ*. Filled symbols represent steps from a holding potential of −60 mV; open symbols represent steps from a holding potential of −120 mV. All data obtained from the same cell.

The effect of low sodium and high potassium

The technique was used to estimate the deactivation kinetics of *Iₜ* at −60 mV. The initial 1-s voltage step to −130 mV fully activated *Iₜ*. Subsequently, the membrane potential was stepped back to −60 mV for various periods of time, followed by a 100-ms hyperpolarizing step to −130 mV. The current measured at the arrowheads in Fig. 4A was plotted against time and the datapoints fitted to Eq. 2. The time constant for deactivation was 302 ± 35 ms (n = 5).

The value of *Eₜ* in TM neurons is consistent with the hypothesis that *Iₜ* is a mixed cationic current. To determine
the ionic species contributing to \( I_h \) in TM neurons, we examined the effects of varying \([Na^+]_o\) and \([K^+]_o\). The results of a typical experiment are illustrated in Fig. 5. When we switched to a modified ACSF containing 27.2 mM sodium, the "instantaneous" current was slightly reduced, as was \( I_h \). In contrast, when \([K^+]_o\) was increased to 10 mM, both the "instantaneous" current and \( I_h \) increased dramatically. These changes were accompanied by marked alterations in \( E_h \), as measured with the protocol shown in Fig. 1. When \([Na^+]_o\) was reduced to 27.2 mM, the apparent value of \( E_h \) shifted to a more hyperpolarized level, in this case from \(-43\) to \(-70\) mV. This is to be expected, inasmuch as the calculated value of \( E_{Na} \) is shifted \(-45\) mV by this manipulation. When \([K^+]_o\) was increased to 10 mM, shifting \( E_K \) by \(+36\) mV, \( E_h \) shifted in the depolarized direction, in this case to \(-13\) mV. Similar results were obtained from another four cells. These data strongly suggest that sodium and potassium are the charge carriers of \( I_h \) in TM neurons.

Because a voltage-dependent chloride current with properties similar to \( I_h \) has been described in Aplysia neurons (Chesnoy-Marchais 1983) and because our recordings were made with 2 M KCl electrodes, we considered the possibility that \( I_h \) might be a chloride current. In the experiment illustrated in Fig. 6, \( E_h \) was measured both in control and low-chloride (9.9 mM) solutions. Although the chloride replacement shifts \( E_{Cl} \) by \(+70\) mV, there was only a \(-5\)-mV shift in \( E_h \) (Fig. 6), well within the range of experimental error. Thus we conclude that chloride is not a charge carrier of \( I_h \).

Raising \([K^+]_o\) to 10 mM resulted in a net inward current and increased the amplitude of \( I_h \) (Fig. 7A). These changes could not be accounted for simply by the enhanced driving force conferred by the new \( E_K \). To determine whether the change in \([K^+]_o\) might have altered the underlying conductance \( g_{hK} \), in five cells we examined the effects of alterations of \([K^+]_o\) on the voltage dependence of \( g_{hK} \), calculated using

\[
g_{hK} = \frac{I_h}{(V - E_h)}
\]

where \( g_{hK} \) is the conductance at a membrane potential \( V \). It was found that \( g_{hK} \) increased in raised \([K^+]_o\) (Fig. 7B). In contrast to these effects of raised \([K^+]_o\), lowering \([Na^+]_o\) produced a small reduction in the amplitude of \( I_h \) (Fig. 7A) but did not affect \( g_{hK} \) (Fig. 7B), reinforcing the notion that the effect observed in raised \([K^+]_o\) is a phenomenon worthy of further study.

We also examined the possibility that changes in \([K^+]_o\) and \([Na^+]_o\) might alter the voltage dependence of \( I_h \). Figure

**FIG. 6.** Effect of low \([Cl^-]_o\) on \( E_h \). A and B: current traces obtained in control and low-Cl\(^-\) (9.9 mM) solutions, respectively. C: "instantaneous" \( I-V \) curves obtained from holding potentials of \(-60\) (●, ■) and \(-120\) mV (▲, ▲) under control conditions (●, ▲) and in low \([Cl^-]_o\) (■, ▲).
FIG. 7. Effects of low sodium and high potassium on $I_h$. A: amplitude of $I_h$ plotted against membrane potential in control (■), low-[Na+] (▲), and high-[K+] (●) solutions. B: amplitude of $g_h$ plotted against membrane potential in control, low-[Na+] (■), and high-[K+] (●) solutions. Symbols as in A. Data in A and B obtained from the cell shown in Fig. 6. C and D: normalized current (mean ± SD) in control (■) and low-[Na+] (▲) solutions (C) and in control (■) and high-[K+] (●) solutions (D) plotted against membrane potential ($n = 3$ for C; $n = 5$ for D). E and F: current traces evoked by voltage steps from a holding potential of $-60$ to $-130$ mV in control and low-[Na+] solutions (E) and in control and high-[K+] solutions (F) fitted to Eq. 2. Data in E and F are from different neurons.

$7C$ shows the normalized current from three cells in control and low-sodium solution plotted against membrane potential. Figure $7D$ shows similar data from another five cells in control and high-potassium solution. Neither low sodium nor high potassium significantly changed the voltage-dependent activation of $I_h$ (paired comparisons $t$ test).

In the low-sodium ACSF, the activation time constant measured at $-130$ mV was significantly longer than under
control conditions by 70 ± 26 ms (n = 9), whereas the activation time constant was significantly shorter in high-potassium ACSF than under control conditions by 58 ± 49 ms (n = 5; paired comparisons t test; Fig. 7, E and F).

Effects of cesium and barium

Extracellular CsCl (1 mM) reduced the whole-cell conductance and markedly reduced $I_h$ in TM neurons without affecting the holding current (Fig. 8, B and C). Bath application of 2 mM BaCl$_2$ produced an inward current at the holding potential of −60 mV, blocked the fast component of the transient outward current, dramatically reduced the whole-cell conductance, and partially blocked $I_h$ (Fig. 8, A and C). Blockade of $I_h$ by both barium and cesium exhibited voltage dependence, becoming more effective at more hyperpolarized voltages (Fig. 8C). However, neither barium nor cesium affected the voltage dependence of activation of $I_h$, as shown by the normalized plots in Fig. 8D.

DISCUSSION

The major conclusion of the present study is that the inward rectification exhibited by histaminergic TM neurons is due to the presence of a mixed sodium-potassium current, $I_h$. These data add to a growing body of evidence regarding the membrane properties of TM neurons (Greene et al. 1990; Haas and Reiner 1988) and represent a further step in understanding the mechanisms that control the excitability of these neurons.

$I_h$ in TM neurons shares many properties with the similar current observed in other excitable cells. Although it is inappropriate to base an extensive analysis of the biophysical properties of $I_h$ on recordings using the single-electrode voltage-clamp technique, some of our observations are relevant to the issue. The major differences seen in $I_h$ in various excitable cells all relate to the kinetics of activation. For instance, some have found that, as in TM neurons, $I_h$ activates with single exponential kinetics (Benham et al. 1987; Crepel and Penit-Soria 1986; DiFrancesco 1984; Halliwell and Adams 1982; Yanagihara and Irisawa 1980), whereas in other cells double exponential kinetics have been described (Hestrin 1987; Spain et al. 1987). There is even less consensus on the effects of altering [K$^+$], and [Na$^+$], on the time course of $I_h$ activation. In hippocampus the activa-
ton kinetics of $I_h$ slow with increased $[K^+]_o$, whereas in rabbit jejunal smooth muscle high $[K^+]_o$ has no effect on the activation time course (Benham et al. 1987; Halliwell and Adams 1982). In low-sodium solution the activation time constant increases in heart and thalamic neurons but does not change in hippocampus and lobster stretch receptor neurons (Crepel and Penit-Soria 1986; DiFrancesco and Tortora 1981; Edman and Gramp 1989; McCormick and Pape 1990). In TM neurons, both manipulations altered the kinetics of activation. Whether these differences in kinetics are due to different subtypes of $I_h$ awaits molecular analysis of the channel itself.

$I_h$ has been shown to be a “pacemaker” current in Purkinje fibers of the heart (DiFrancesco 1981) and thalamic neurons (McCormick and Pape 1990). Because TM neurons are spontaneously active in vitro, we considered the possibility that $I_h$ might be a pacemaker current in these neurons. If so, one would predict that $I_h$ would be active in the immediate subthreshold region ($-55$ to $-60$ mV). However, the voltage dependence of $I_h$ demonstrates that the current is not active positive to $-80$ mV, even though our protocol for measuring the activation of $I_h$ was carried out in a way that would maximize detection of the current near its reversal potential. Moreover, cesium is very effective in blocking $I_h$ but has no effect on the holding current at $-60$ mV. These data would appear to negate the hypothesis that $I_h$ is the pacemaker current of TM neurons.

$I_h$ has been shown to contribute to repolarization of the membrane potential during the early portion of the afterhyperpolarization in cat neocortical pyramidal neurons (Spain et al. 1987). In TM neurons the afterhyperpolarization takes the membrane potential to the region of $-80$ mV. Consider the cell illustrated in Fig. 5. At $-80$ mV, $I_h$ produces a current of 20 pA, which would depolarize a typical 200-MΩ TM neuron by only 4 mV and only after the current had maximally activated. Inasmuch as the kinetics of activation of $I_h$ at this membrane potential are very slow, it seems unlikely that this current exerts a significant depolarizing influence during the interspike interval in TM neurons.

These conclusions should be considered in light of recent observations that the voltage dependence of $I_h$ can be altered by agonist-mediated production of cyclic AMP (Bobker and Williams 1989; DiFrancesco and Tortora 1991; DiFrancesco and Tromba 1988; Pape and McCormick 1989; Tokiama and Akasu 1990), by changes in intracellular Ca$^{2+}$ (Hagiwara and Irisawa 1989), or by direct G-protein coupling (Yatani et al. 1990). Given the ubiquity of this phenomenon, it is possible that agonists shift the activation curve of $I_h$ in TM neurons such that it plays a role in control of spontaneous activity under some conditions. Nonetheless, our data would suggest that spontaneous activity in TM neurons occurs even when $I_h$ does not contribute as a pacemaker current. Thus, without precluding a role for $I_h$ in the control of TM neuronal firing rate under some circumstances, we can conclude that in TM neurons other ionic mechanisms of pacemaking must exist.

One of the remarkable properties of histaminergic TM neurons is the change in firing rate observed during different behavioral states. As with other aminergic neurons, TM neurons fire regularly during waking, at a reduced rate during slow-wave sleep, and not at all during episodes of REM sleep that last many minutes (Vanni-Mercier et al. 1984). Although the mechanism underlying the silence of TM neurons during REM sleep is unknown, the most plausible hypothesis is that it is due to an active hyperpolarizing influence, inasmuch as TM neurons are spontaneously active in slices in which most afferent inputs have been severed as well as after application of excitatory amino acid antagonists (Reiner, unpublished observations). A chloride current is unlikely to be responsible, because the chloride equilibrium potential in TM neurons is very nearly equal to the resting membrane potential (Reiner and Haas 1990). Therefore the silence of TM neurons during REM sleep seems most likely to be due to activation of a potassium current.

Both the voltage dependence and kinetics of activation of $I_h$ make it likely that this current would be activated during a prolonged potassium-mediated hyperpolarization, such as that presumed to occur during REM sleep. Activation of $I_h$ increases the whole cell conductance of TM neurons and would therefore act as a shunt on incoming synaptic activity. Because TM neurons have a restricted extracellular space (Wouterlood et al. 1986), a prolonged increase in potassium conductance (as might occur during REM sleep) would result in an increase in $[K^+]_o$. Our data demonstrate that the underlying conductance $g_h$ increases with increased $[K^+]_o$, while voltage dependence is unaffected, as has been observed in many other excitatory cells (Bader and Bertrand 1984; DiFrancesco 1982; Edman and Gramp 1989; Hestrin 1987; Mayer and Westbrook 1983; Spain et al. 1987). Thus the ability of $I_h$ to act as an ionic shunt would be even more pronounced during REM sleep than during other behavioral states. We hypothesize that the major role of $I_h$ in TM neurons is to diminish the efficacy of excitatory postsynaptic potentials during REM sleep, thereby ensuring that these neurons remain silent during this behavioral state.

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