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

ANITA KAMONDI AND PETER B. REINER
Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

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, I_h, were studied by use of the single-electrode voltage-clamp technique.

2. The activation curve of I_h was well fit by a sigmoidal function, with half-maximal activation occurring at -98 ± 6 mV.

3. The estimated reversal potential of I_h (E_h) in TM neurons was -35 ± 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, I_h activated with a time constant of 823 ± 35 ms, whereas at -130 mV, I_h activated with a time constant of 280 ± 65 ms. The time constant of deactivation of I_h at -60 mV was 302 ± 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 I_h and its underlying conductance g_h, while reducing $[Na^+]_o$ caused a small reduction in the amplitude of I_h with no measurable effect on g_h.

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

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

9. We conclude that I_h, carried by sodium and potassium ions, accounts for inward rectification of TM neurons. By increasing the whole-cell conductance during periods of prolonged hyperpolarization, I_h 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 I_h, this current activates over hundreds of milliseconds, in contrast to the rapid activation of the anomalous rectifier. The voltage dependence of I_h varies with cell types, but activation always begins positive to the potassium equilibrium potential. I_h, also variously known as I_Q 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; Hestrin 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 I_h to the behavioral neurophysiology of TM neurons, we have used the single-electrode voltage-clamp technique to carry out a detailed analysis of I_h 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-

brospinal 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) ASCF 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 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 26 $NaHCO_3$, and 11 glucose, saturated with 95% O_2-5% CO_2, 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 ($GABA_A$)-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 ($[K^+]_o$) 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

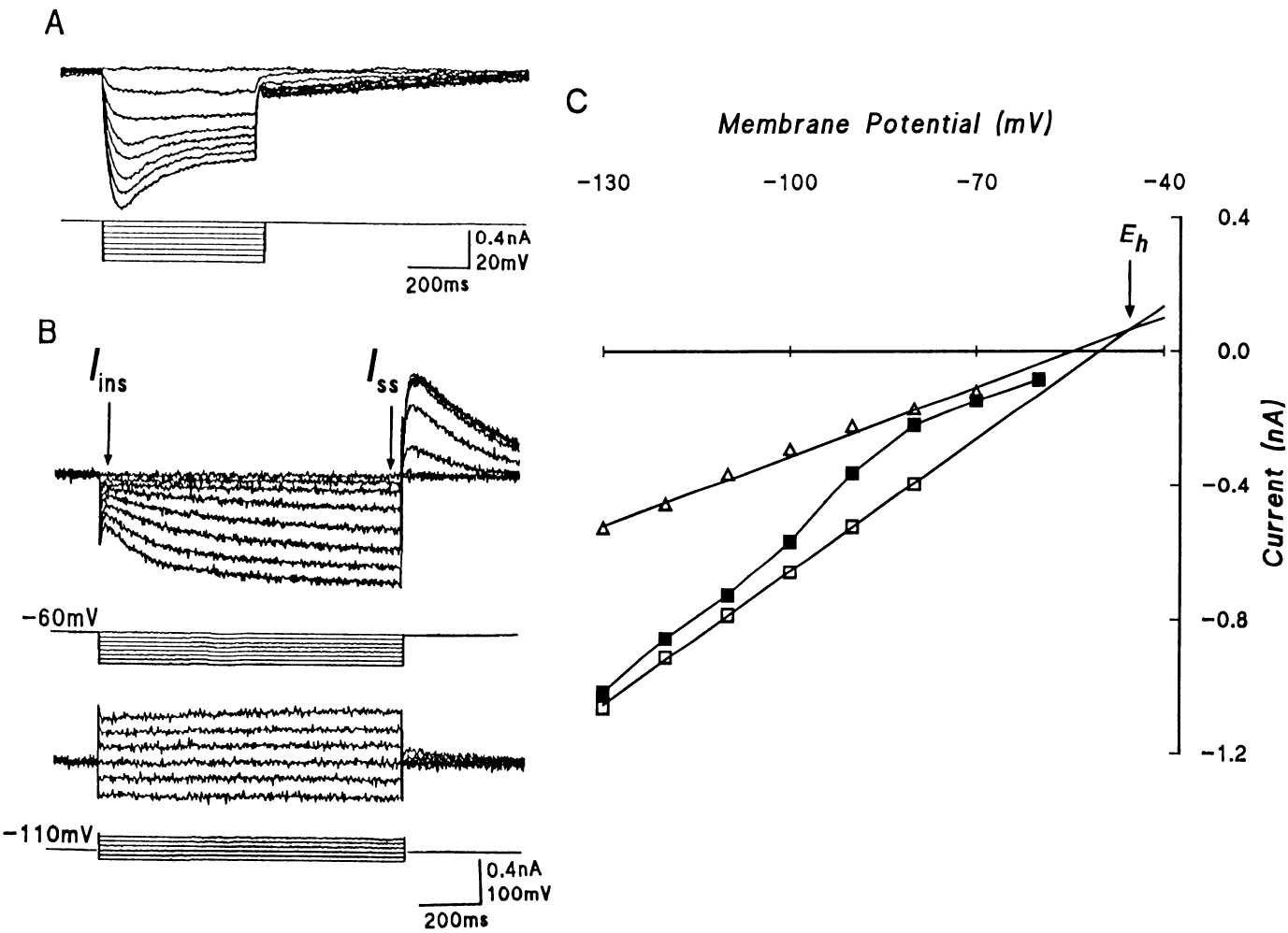

FIG. 1. Basic properties of I_h. *A*: in bridge mode, hyperpolarizing current pulses from a membrane potential of −60 mV result in a depolarizing sag of electrotonic potential. *B*: current traces recorded in single-electrode voltage-clamp mode evoked by voltage steps from holding potentials of −60 (*top*) 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* curve (■ —— ■) shows inward rectification negative to −80 mV. "Instantaneous" *I-V* curves, generated from holding potentials of −60 (△ —— △) and −110 mV (□ —— □), are linear throughout voltage range examined. Extrapolated intersection (↓) of "instantaneous" *I-V* curves gives an estimate of E_h, the reversal potential of I_h.

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. 1*A*). 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. 1*B*). I_h 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. 1*C*). 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. 1*C*) due to the invariate presence of I_h in TM neurons.

Reversal potential of I_h

Tail-current analysis of the reversal potential of I_h 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. 1*B*. "Instantaneous" *I-V* curves were generated from holding potentials of −60 mV, at which I_h is not active, and −110 mV, at which I_h is fully activated (vide infra). The intersection of the extrapolated "instantaneous" *I-V* curves gives an estimate of the reversal potential of I_h (E_h; Fig. 1*C*). In 15 TM neurons analyzed in this fashion, E_h was −35 ± 9 mV.

Voltage dependence of I_h

To maximize the sensitivity of our measurements of the voltage dependence of I_h, we designed a two-step voltage-clamp protocol (Fig. 2*A*) that allowed us to measure I_h 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 to potentials between −70 and −130 mV for 1 s. This conditioning step allowed for partial activation of I_h. After this prepulse, a test pulse was applied by stepping the membrane potential to −130 mV, where I_h appeared to be fully activated. The family of currents observed during the test pulse represents the proportion of I_h that was not activated during the conditioning pulse. The magnitude of I_h at a

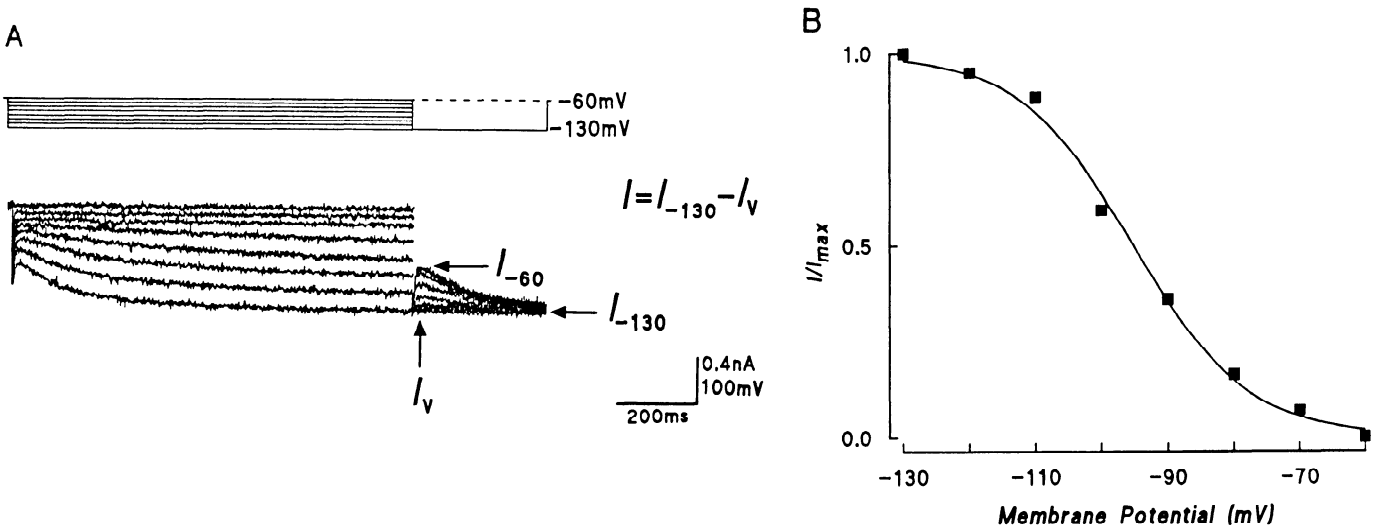

FIG. 2. Voltage dependence of I_h. *A*: 2-step voltage-clamp protocol was used to determine the voltage-dependent activation of I_h. The 1-s-duration prepulse to various holding potentials partially activates I_h. Family of currents observed during the subsequent test pulse to −130 mV was used to calculate the amount of I_h (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_V 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.

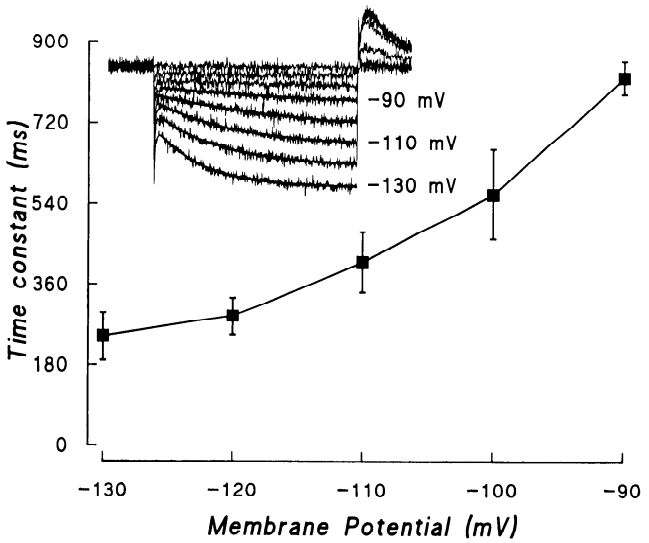

FIG. 3. Voltage sensitivity of activation kinetics of I_h. Current evoked by voltage steps to various potentials, as shown in the *inset*, was fitted to *Eq. 2*. Means ± SD time constant at various voltages are plotted against membrane potential ($n = 5$).

given membrane potential (I) was calculated by subtracting the current measured during the test pulse from the maximal value of I_h (I_{max}), as shown in Fig. 2*A*. The activation curve of I_h was generated by plotting the normalized current (I/I_{max}) against the conditioning pulse membrane potential (Fig. 2*B*). Data points were well fit by a sigmoidal function of the form

$$I/I_{max} = 1/\{1 + \exp[k(V - V_{½})]\} \quad (1)$$

where I_{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 $V_{1/2} = -98 \pm 6$ mV and $k = 0.10 \pm 0.05$ 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

$$I_t = I_{ss} + I_h \exp(-t/\text{tau}) \quad (2)$$

where I_t is the current at time t, I_{ss} is the steady-state current at the end of the voltage step, I_h is the difference between I_{ss} and the "instantaneous" current, and tau is the time constant. In 31 neurons, the time constant for activation at −130 mV was 280 ± 65 ms. These data were obtained at 30°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 mV were fitted to *Eq. 2*. At membrane potentials less than or equal to −90 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 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 4*A* shows an experiment in which an envelope

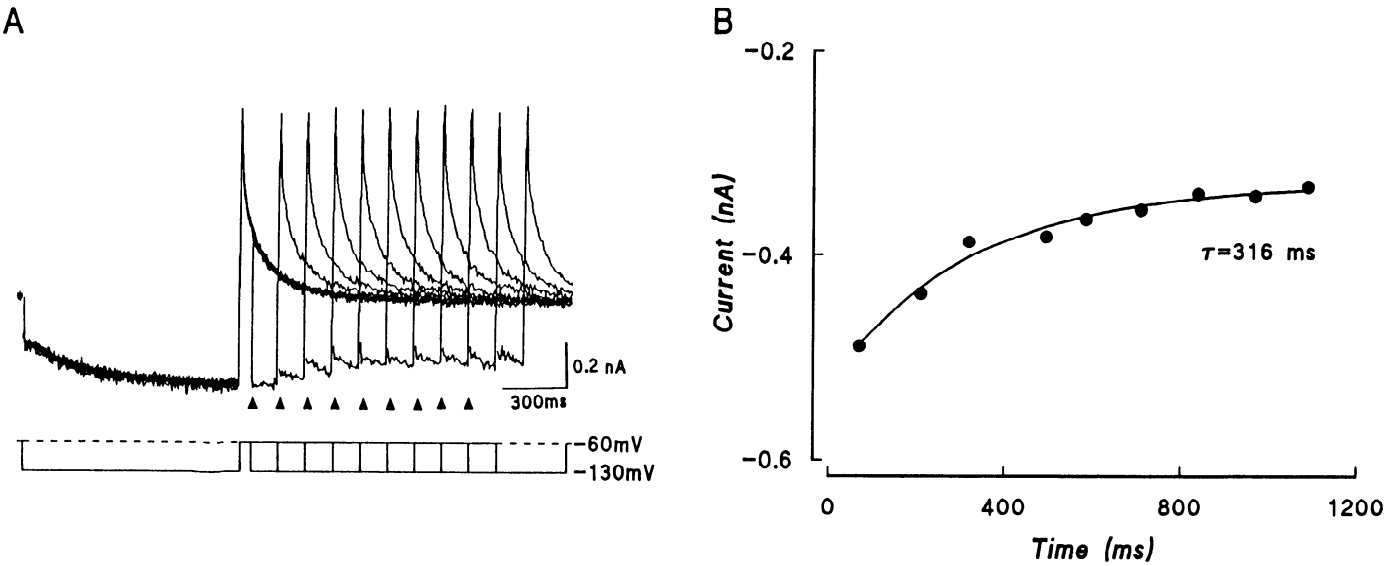

FIG. 4. Deactivation kinetics of I_h. *A*: 10 superimposed current traces show currents evoked by voltage steps from −60 to −130 mV for 1 s to fully activate I_h. After a variable-duration step to −60 mV, a 100-ms-duration step to −130 mV revealed the extent to which I_h had decayed. *B*: data points measured at arrowheads in *A* were plotted against time and fitted to *Eq. 2* to obtain the curve for deactivation.

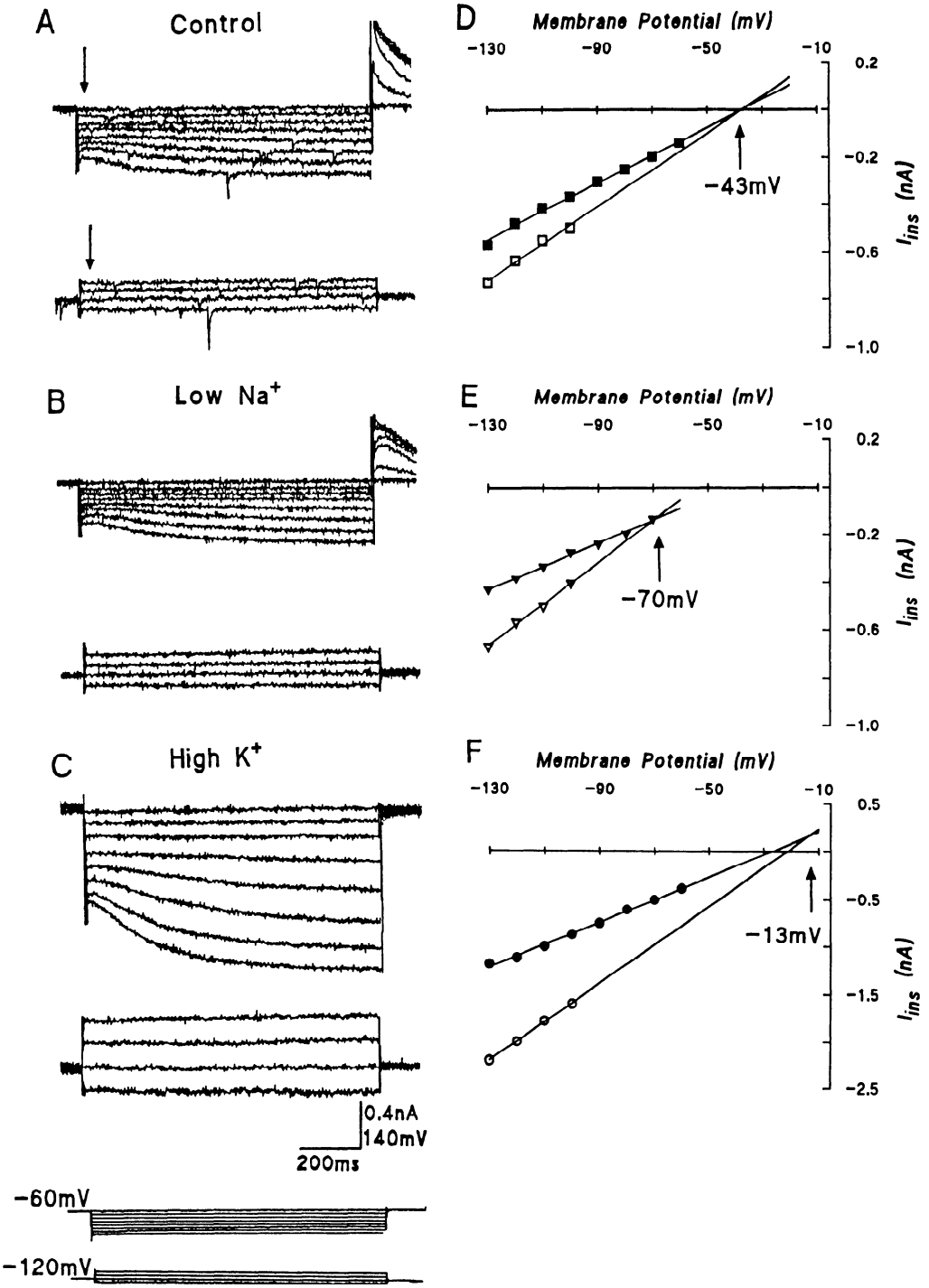

FIG. 5. Effects of low $[Na^+]_o$ and high $[K^+]_o$ on E_h. *A–C*: current traces evoked by voltage steps from holding potentials of −60 (*top*) and −120 mV (*bottom*) in control ACSF (*A*), in low-sodium (27.2 mM) solution (*B*), and high-potassium (10 mM) solution (*C*). Arrows indicate time point 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 E_h. 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.

technique was used to estimate the deactivation kinetics of I_h at −60 mV. The initial 1-s voltage step to −130 mV fully activated I_h. 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. 4*A* was plotted against time and the datapoints fitted to *Eq. 2*. The time constant for deactivation was 302 ± 35 ms (n = 5).

Effects of low sodium and high potassium

The value of E_h in TM neurons is consistent with the hypothesis that I_h 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. 7*A*). 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_h, in five cells we examined the effects of alterations of $[K^+]_o$ on the voltage dependence of g_h, calculated using

$$g_h = I/(V - E_h) \quad (3)$$

where g_h is the conductance at a membrane potential V. It was found that g_h increased in raised $[K^+]_o$ (Fig. 7*B*). In contrast to these effects of raised $[K^+]_o$, lowering $[Na^+]_o$ produced a small reduction in the amplitude of I_h (Fig. 7*A*) but did not affect g_h (Fig. 7*B*), 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

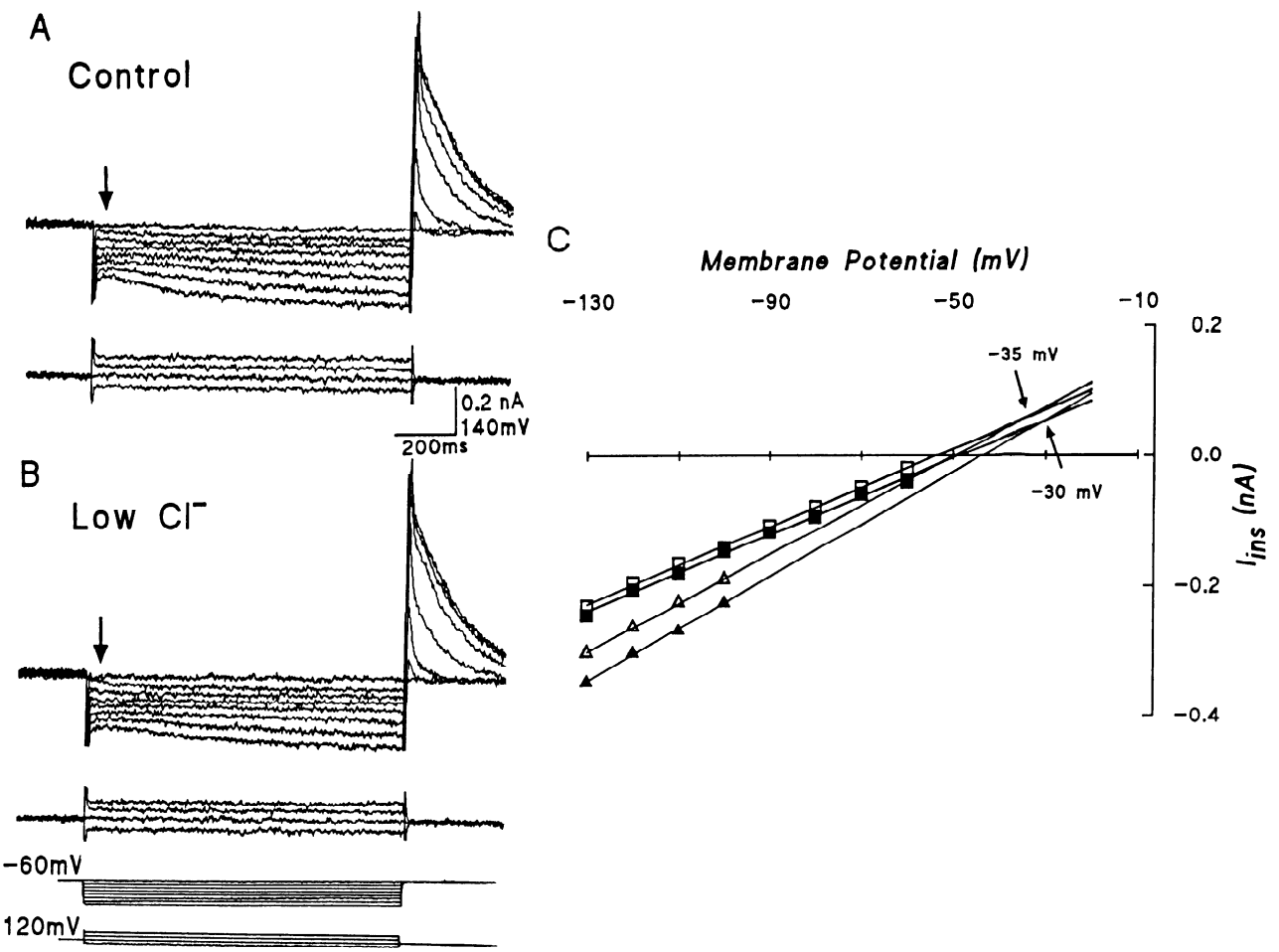

FIG. 6. Effect of low $[Cl^-]_o$ on E_h. *A* and *B*: current traces obtained in control and low-Cl^- (9.9 mM) solutions, respectively. *C*: "instantaneous" *I-V* curves obtained from holding potentials of −60 (■, □) and −120 mV (▲, △) under control conditions (■, ▲) and in low $[Cl^-]_o$ (□, △).

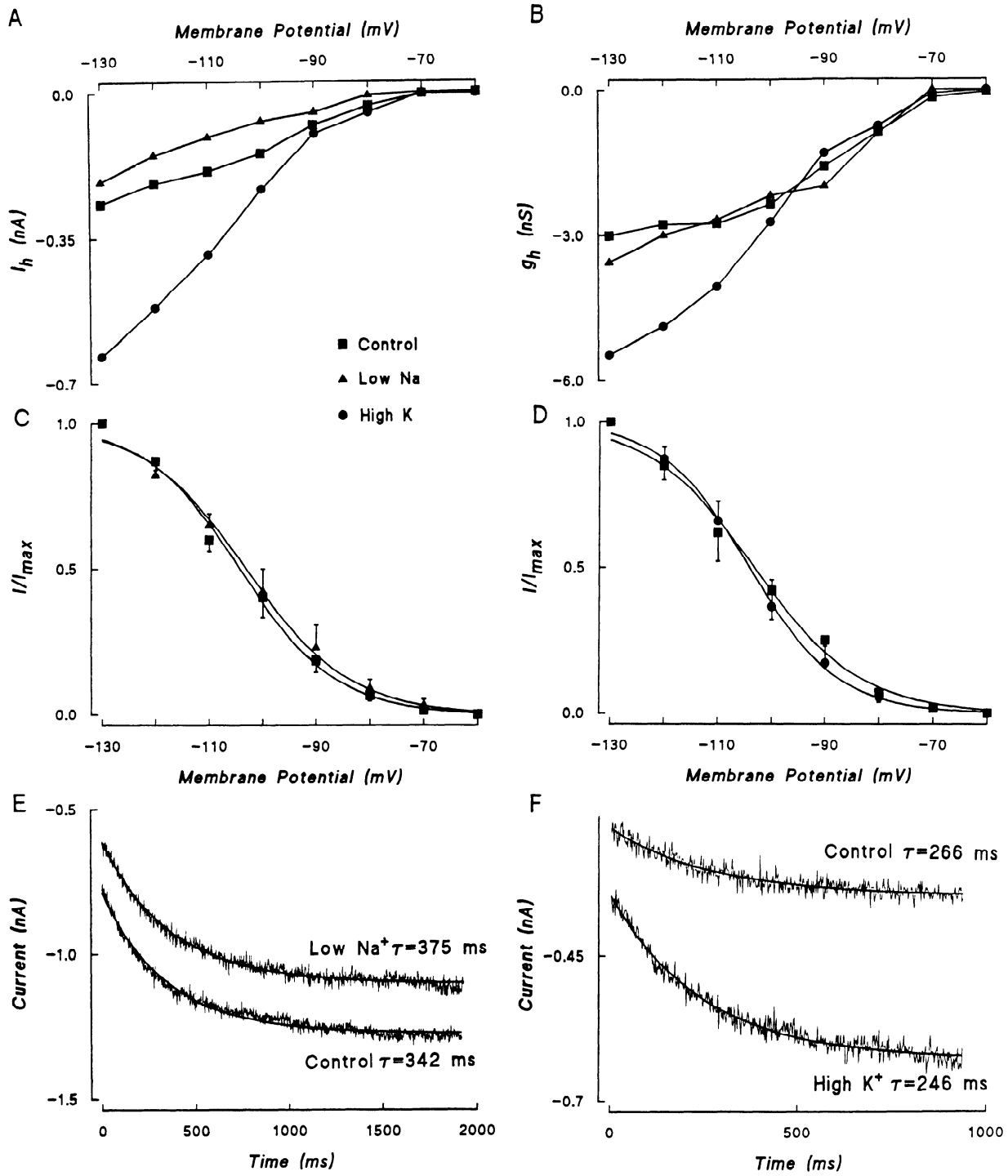

FIG. 7. Effects of low sodium and high potassium on I_h. *A*: amplitude of I_h plotted against membrane potential in control (■), low-$[Na^+]_o$ (▲), and high-$[K^+]_o$ (●) solutions. *B*: amplitude of g_h plotted against membrane potential in control, low-$[Na^+]_o$, and high-$[K^+]_o$ solutions. Symbols 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-$[Na^+]_o$ (▲) solutions (*C*) and in control (■) and high-$[K^+]_o$ (●) 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-$[Na^+]_o$ solutions (*E*) and in control and high-$[K^+]_o$ solutions (*F*) fitted to *Eq. 2*. Data in *E* and *F* are from different neurons.

7*C* shows the normalized current from three cells in control and low-sodium solution plotted against membrane potential. Figure 7*D* 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

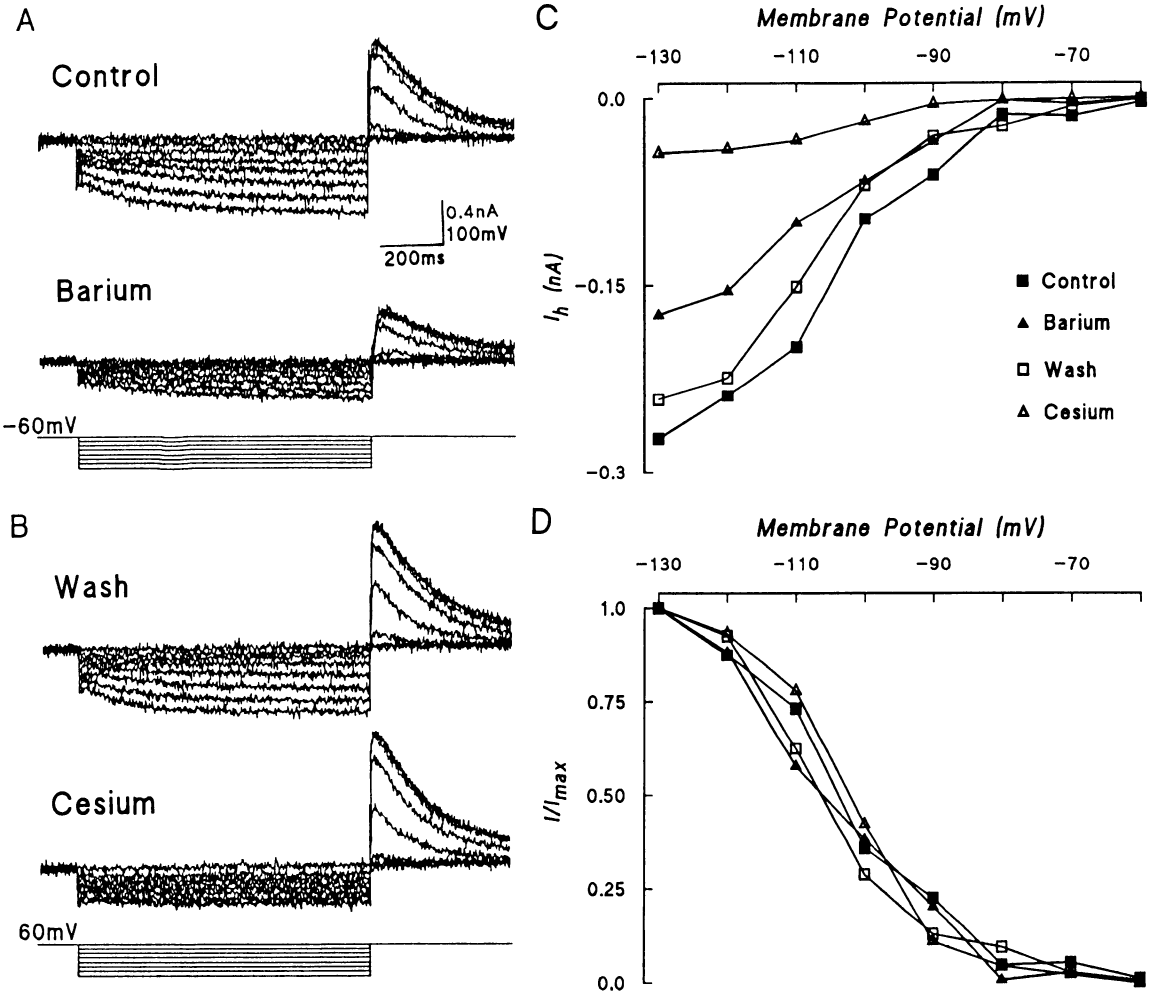

FIG. 8. Effects of barium and cesium on I_h. *A*: current traces evoked by 10-mV steps from −60 to −130 mV in control solution (*top*) and after addition of 2 mM $BaCl_2$ (*bottom*). *B*: current traces from the same cell after washout of the barium effect (*top*) and after addition of 1 mM CsCl (*bottom*). *C*: amplitude of I_h plotted against membrane potential in control (■), barium (▲), wash (□), and cesium (△) solutions. *D*: normalized I_h (I/I_{max}) plotted against the membrane potential in control, barium, wash, and cesium solutions. Symbols as in *C*.

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. 8*C*). However, neither barium nor cesium affected the voltage dependence of activation of I_h, as shown by the normalized plots in Fig. 8*D*.

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 $[K^+]_o$ and $[Na^+]_o$ on the time course of I_h activation. In hippocampus the activa-

tion kinetics of I_h slow with increased $[K^+]_o$, whereas in rabbit jejunum 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 1981; Edman and Grampp 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; Tokimasa 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 excitable cells (Bader and Bertrand 1984; DiFrancesco 1982; Edman and Grampp 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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Address for reprint requests: P. B. Reiner, Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, 2255 Westbrook Mall, Vancouver, BC V6T 1Z3, Canada.

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