Voltage-Activated K⁺ Currents of Hypoglossal Motoneurons in a Brain Stem Slice Preparation From the Neonatal Rat

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Lape, Remigijus and Andrea Nistri. Voltage-activated K⁺ currents of hypoglossal motoneurons in a brain stem slice preparation from the neonatal rat. J. Neurophysiol. 81: 140–148, 1999. Whole cell, patch-clamp recordings were performed on motoneurons of the hypoglossus nucleus in a brain stem slice preparation from the neonatal rat brain. The aim was to investigate transient outward currents activated by membrane depolarization under voltage clamp conditions. In a Ca²⁺-free medium containing tetrodotoxin and Cs⁺, depolarizing voltage commands from a holding potential of −50 mV induced slow outward currents (I slow) with 34 ± 6 ms (SE) onset time constant at 0 mV and minimal decline during a 1 s pulse depolarization. When the depolarizing command was preceded by a prepulse to −110 mV, the outward current became biphasic as it comprised a faster component (I fast), which could be investigated in isolation by subtracting the two sets of records. I_fast was selectively blocked by TEA (10–30 mM) while I_slow was preferentially depressed by 2–3 mM 4-aminopyridine. Analysis of tail current reversal indicated that both I_fast and I_slow were predominantly due to K⁺ with minor permeability to Na⁺ (92/1 and 50/1, respectively). These results suggest that membrane depolarization activated distinct K⁺ conductances that, in view of their largely dissimilar kinetics, are likely to play a differential role in regulating the firing behavior of hypoglossal motoneurons.

INTRODUCTION

Hypoglossal motoneurons are responsible for tongue muscle movements and for maintaining patent airways through the appropriate tone of tongue protrusor muscles. Dysfunction of these cells results in a condition termed sleep apnea, when serious obstruction to breathing develops periodically in infants or adults (Gauda et al. 1987; Wiegand et al. 1991; Willinger 1989). To understand in detail the intrinsic voltage activated conductances of hypoglossal motoneurons subserving their electrical behavior requires, as an experimental model, a brain stem slice preparation in which the electrophysiological properties of single cells can be studied. Using rodent brain slices for this experimental model has demonstrated that these neurons contain several voltage-activated membrane conductances (Haddad et al. 1990; Mosfalt Laursen and Rekling 1989; Viana et al. 1993a,b) and that they display characteristic firing patterns following membrane depolarization (Sawczuk et al. 1995, 1997; Viana et al. 1995). The latter aspect is of special relevance because it would reveal how the integration of the electrical behavior of the cell at somatic level is translated into output signals to the tongue muscles. Thus depolarizing current pulses induce primary and secondary range firing with different degrees of accommodation of spike activity (Sawczuk et al. 1995, 1997; Viana et al. 1995). These responses presumably rely on the interplay between inward excitatory and outward inhibitory currents. Whereas inward currents, especially those carried by Ca²⁺ (Umemiya and Berger 1994, 1995), have been amply characterized, data on outward currents studied under voltage clamp are presently lacking. Our aim was, therefore, to provide a first description of some voltage-activated outward currents of rat hypoglossal motoneurons. In addition to a slow K⁺ current, reminiscent of the classical delayed rectifier, we also observed a fast transient current hitherto unreported in hypoglossal motoneurons.

METHODS

Slice preparation

Experiments were carried out using brain stem slices obtained from 0 to 9 day-old-rats. Thin slices were prepared following the procedure described by Viana et al. (1994). The brain stem was isolated from neonatal rats and placed into modified, ice-cold Krebs solution (see below). A tissue block containing the lower medulla was then fixed with insect pins onto an agar block inside a Vibratome chamber filled with ice-cold Krebs solution (bubbled with O₂-CO₂) to obtain 200 μm thick slices. Slices were first transferred to an incubation chamber for 1 h at 32°C under continuous oxygenation and subsequently maintained at room temperature for ≥1 h before use.

Recording

Brain stem slices placed in a small recording chamber were viewed with an infrared video camera to identify single hypoglossal motoneurons within the XII nucleus. Parallel experiments using choline acetyltransferase immunocytochemistry indicated that >90% of the cells of this area were positively stained (R. Donato, unpublished result), thus identifying them as motoneurons (see Viana et al. 1990). The conventional whole-cell, patch-clamp recording technique (Hamill et al. 1981) was employed with the use of an EPC-7 patch-clamp amplifier. Patch electrodes pulled from borosilicate glass had 3–5 MΩ DC resistance. Seal resistance was usually higher than 2 GΩ. After seal rupture, series resistance (5–25 MΩ) was routinely monitored and compensated for (usually by 50%, range 20–90%). For the sample of cells used for measurements of outward current kinetics and steady state properties the average voltage error found at the largest depolarized potential employed (±20 mV) was 7.2 ± 0.5 mV (range 4.3–9.6 mV). Voltage pulse generation and data acquisition were performed with...
a PC running pClamp 6.1 software. Currents elicited by voltage steps were filtered at 3–10 kHz and sampled at 5–10 kHz.

**Solutions**

The solution for slice preparation and maintenance was (in mM) 130 NaCl, 3 KCl, 26 NaHCO3, 1.5 Na2HPO4, 1 CaCl2, 5 MgCl2, 10 glucose (osmolarity ~290–310 mOsm). The extracellular solution for electrophysiological recording was (in mM) 140 NaCl, 3 KCl, 2 CaCl2, 2 MgCl2, 10 HEPES, 10 glucose (pH 7.4, 290–310 mOsm). The patch pipette solution was (in mM) 110 K-gluconate, 20 KCl, 5 NaCl, 2 MgCl2, 1 CaCl2, 10 HEPES, 10 EGTA, 2 ATP-Mg (pH 7.2, 260–270 mOsm).

Voltage-activated fast sodium currents were blocked by tetrodotoxin (TTX; 1–2 μM) in most experiments. In some experiments QX-314 (5–10 mM) was added to the pipette solution to block sodium channels and the hyperpolarization activated inward rectifying cationic current (Ih) (Perkins and Wong 1995). Alternatively, CsCl (4 mM) was added extracellularly to block Ih. In most experiments extracellular CaCl2 was replaced by the same amount of CoCl2 (Ca-free-Co solution) or CdCl2 (200 μM) was added to block Ca2+-dependent currents and Ca2+-dependent K+ currents. When tetraethylammonium (TEA; chloride salt) or 4-aminopyridine (4-AP) was added to the recording solution in concentrations larger than 5 mM, an equivalent concentration of NaCl was removed. The recording chamber was continuously superfused at 2–5 ml/min. Drugs were added by switching to an appropriate extracellular solution maintained for 5–10 min for equilibration.

**Analysis**

Cell input resistance (Rm) was calculated from 10 or 20 mV hyperpolarizing commands from a holding potential (Vh) of −70 mV or from the linear part of the I/V line (ramp test) near the cell resting potential (Vrest). Sigma Plot and Clampfit softwares were used for exponential fitting of membrane currents and for linear regression analysis of experimental data. Data are presented as means ± SE. All potential values were corrected off-line for the liquid junction potential, which was measured as 10 mV. Current leak subtraction was performed either on-line using P/8 subtraction procedure or off-line using the Clampfit module.

**RESULTS**

**Basic characteristics of hypoglossal motoneurons**

Recordings were obtained from 49 neurons with 44 ± 4 pF somatic capacitance and 400 ± 100 MΩ input resistance (Rm).

In standard solution membrane currents elicited by depolarizing voltage commands comprised multiple components. An example is shown in Fig. 1A where depolarizing steps (10 mV increments from −70 mV holding potential; Vh) evoked fast inward currents (apparent threshold = −50 mV) followed by slower outward currents (apparent threshold = −20 mV). The fast inward current was blocked completely by 1–2 μM TTX (Fig. 1B), suggesting that it was a voltage-activated Na+ current. Because this current was usually too fast, even under the most favorable voltage-clamp conditions, to be adequately recorded, no further analysis of it was attempted. Although not shown in Fig. 1, A and B, most cells also displayed a slower and smaller inward current that was blocked by Cd2+ (0.2 mM) or by replacing Ca2+ with Co2+, indicating that it was mostly carried by Ca2+. As the properties of voltage dependent, Ca2+ currents and channels of hypoglossal motoneurons have been systematically investigated by Umemiya and Berger (1994, 1995), they will not be further reported here.

The outward current, clearly detected in TTX medium (Fig. 1B), was partly diminished (for instance by 11% at 0 mV) in Ca-free-Co solution or by extracellular application of Cd2+ (not shown). This outward component was more strongly depressed (40 ± 8% at 0 mV; n = 3 cells) by the K+ channel blocker TEA (20 mM; not shown). These preliminary observations suggested to us that the outward current was presumably due to K+ efflux via several conductances, mainly voltage and partly Ca2+ activated. The present report thus focusses on voltage activated K+ currents.

**Slow transient outward current**

The delayed transient outward current (Islow) was routinely investigated in Ca-free-Co solution containing TTX (1 μM).
After measuring the peak of voltage emerged for responses generated by pulses to positive which for example at 0 mV had a time constant of 34 nA. The slow amplitude (evoked by 20 mV and 70 mV) did not decline during the 1 s pulse. Larger steps generated currents of larger amplitude and faster peaking response. Some decline in current amplitude emerged for responses generated by pulses to positive membrane potentials. Preliminary trials showed that the conditioning step (200–500 ms) to −50 mV did not influence the slow current development, but it removed the contaminating presence of a faster current (described below). For this reason, whenever the slow current was studied in isolation, a 400 ms prepulse to −50 mV was always applied before depolarizing test steps. We also checked that extracellular application of Cs⁺ (4 mM) did not influence Islow because this monovalent cation can block a variety of K+ channels in other cells (Rudy 1988). Thus, comparing the steady state Islow amplitude (evoked by +20 mV steps) in Cs⁺ free solution with the one obtained after the addition of Cs⁺ (4 mM) indicated that there was no significant change (3 ± 8%; n = 4; P = 0.7 with ANOVA test).

The average I/V relation (n = 15 cells) for Islow is shown in Fig. 2B. Islow activated at membrane potentials positive to −50 mV. Its amplitude monotonically increased with increasing depolarization within the tested potential range of −50 to +20 mV. The dependence of Islow chord conductance (g; expressed in nS) on membrane potential is shown in Fig. 2C. After measuring the peak of Islow the values for g were calculated according to the equation

\[ g = \frac{I_{\text{slow}}}{(V_m - E_{\text{K}})} \]  

where \( E_{\text{K}} \) is the K⁺ equilibrium potential (measured as described below) and \( V_m \) is the test membrane potential at which Islow was recorded. The values of g grew for potentials positive to −50 mV and showed an average increment of 3.9 ± 0.4 nS/mV. Fitting these data according to the Boltzmann equation yielded an average slope of 19 ± 3 mV; the g maximum value was estimated as 26 ± 1 nS as it occurred at a potential level (+60 mV) outside the range tested in the present experiments.

Figure 2D shows a plot for the voltage dependence of Islow activation in which the current rising phase could be fitted monoexponentially: it is clear that it had a slow onset, which for example at 0 mV had a time constant of 34 ± 6 ms. Once Islow reached its peak, it remained at a plateau or gradually declined for test depolarizations positive to −10 mV. For example, Islow decline had a time constant of 4.2 ± 0.4 s at +20 mV membrane potential (n = 3 cells). Using the time constant of tail currents at the end of depolarizing voltage commands, the deactivation properties of Islow were studied. In this case, the deactivation time constant was on average 40 ± 4 ms (n = 7 cells; range 27–60 ms within −105 and −40 mV). These data, therefore, indicate that Islow had rather slow kinetic characteristics, which made it unsuitable to control motoneuronal excitability within a narrow time frame and prompted a more systematic search for a faster outward current component in these cells that was hitherto undescribed.

Fast transient outward current

We suspected that any hypothetical fast outward current (Ifast) might have been largely masked by the use of relatively depolarized \( V_i \) values because this appears to be the case for the fast outward current of other central neurons (Rogawski 1985; Rudy 1988). To demonstrate its existence in hypoglossal cells, we used a subtraction procedure as detailed in Fig. 3, A and B. In fact, we first obtained a family of outward currents consisting of an initial, rapid peak followed by a slower component (Fig. 3A) when the 1 s depolarizing test steps were applied immediately after a hyperpolarizing prepulse (to −110 mV). An analogous protocol with only one
difference was subsequently repeated on the same cell, namely, instead of a hyperpolarizing prepulse, a depolarizing one (to −50 mV; Fig. 3B) was used. In the latter case the outward current typically consisted of \( I_{\text{low}} \) only (see also Figs. 1 and 2). After point-to-point subtraction of the current traces obtained with depolarized prepulses from those obtained with hyperpolarizing prepulses, a transient and rapid outward current (\( I_{\text{fast}} \)) could be demonstrated in isolation (Fig. 3C). \( I_{\text{fast}} \) grew to a peak and inactivated in <200 ms (Fig. 3C). We also investigated whether the presence of 4 mM Cs\(^+\) in the bathing solution might have affected \( I_{\text{fast}} \) development. Comparing the peak amplitude of \( I_{\text{fast}} \) (induced by steps to +20 mV) before and after adding Cs\(^+\) showed that there was no significant change (7 ± 9%; \( n = 3; P = 0.8 \)), suggesting that this cation did not interfere with \( I_{\text{fast}} \) recording.

The peak current-voltage (\( I/V \)) relation for \( I_{\text{fast}} \) is shown in Fig. 3D. The threshold for activation of \( I_{\text{fast}} \) was approximately −60 mV with a monotonic increase in \( I_{\text{fast}} \) amplitude as the test depolarization increased. The \( I_{\text{fast}} \) rise time (calculated as 10–90% time to peak) decreased with increasing depolarization (Fig. 3E), suggesting voltage dependence of the current activation process. The rise time was 24 ± 7 ms at −50 mV and 8 ± 3 ms at +20 mV (\( n = 5 \) cells).

The activation and inactivation properties of \( I_{\text{fast}} \) were next examined. For activation the peak conductance (\( g_{\text{peak}} \)) was simply determined by dividing the \( I_{\text{fast}} \) peak amplitude by the driving force (Eq. 1) and then normalized (\( g_{\text{norm}} \)) to the maximum conductance, \( g_{\text{max}} \). The latter value was obtained from chord conductance-voltage plots, which indicated \( g \) saturation at +20 mV. Hence

\[
g_{\text{norm}} = \frac{g_{\text{peak}}}{g_{\text{max}}} \tag{2}
\]

For nine cells the normalized conductance (\( g_{\text{norm}} \)) values were thus plotted against membrane potentials to give the activation curve (Fig. 4A; ●). These experimental points were fitted with the Boltzmann equation, which indicated half activation of \( I_{\text{fast}} \) at −27.6 ± 0.9 mV with a 16 ± 1 mV slope. The conductance was thus activated at potentials positive to −70 mV and was virtually fully activated at +20 mV.

The protocol to study steady-state inactivation of \( I_{\text{fast}} \) is depicted in Fig. 4B. The motoneuron membrane potential was conditioned (for 200 ms) to different potentials (in the range from −140 mV to −10 mV) and then depolarized to a fixed test potential of +30 mV, which corresponded to full activation of \( I_{\text{fast}} \) (see Fig. 4A). The faster timebase records of Fig. 4C1 show that, with progressively more depolarized prepulses, \( I_{\text{fast}} \) disappeared. For full isolation of \( I_{\text{fast}} \) the current trace after a prepulse to −50 mV (when \( I_{\text{fast}} \) was absent) was subtracted from all the other traces. \( I_{\text{fast}} \) could thus be measured separately (Fig. 4C2) and the underlying \( g_{\text{peak}} \) calculated and normalized to \( g_{\text{max}} \) (Eqs. 1 and 2). The inactivation curve was generated by plotting \( g_{\text{norm}} \) versus the conditioning step potential (Fig. 4A; ○) and fitted with the Boltzmann equation. Half inactivation was estimated at −92.9 ± 0.2 mV with a −10.8 ± 0.2 mV slope (\( n = 9 \) cells). The conductance inactivation was complete at −50 mV and was completely removed at −140 mV. When activated \( I_{\text{fast}} \) inactivated completely or to a small residual current (Fig. 3C).

It seemed useful to characterize how quickly \( I_{\text{fast}} \) inactivation could develop, because this property may influence the firing characteristics of the cell. This result was obtained by measuring the time constants of single exponential decay of \( I_{\text{fast}} \) (Fig. 5A). In these examples the values were 86, 75, and 61 ms at −40, −20, and 0 mV membrane potentials, respectively. These observations allowed us to plot the average time constants of inactivation versus membrane potential.

**FIG. 3.** Hypoglossal motoneurons possess a fast transient outward current (\( I_{\text{fast}} \)). A and B: two sets of outward current traces evoked by a series of depolarizing voltage steps from −60 to 20 mV preceded by either a hyperpolarizing voltage step to −110 mV (A) or a depolarizing voltage step to −50 mV (B) for 400 ms. C: \( I_{\text{fast}} \) isolated by subtracting current traces in B from those in A. D: current-voltage relation of \( I_{\text{fast}} \) (\( n = 8 \) cells). E: \( I_{\text{fast}} \) activation time (calculated as 10–90% rise time) vs. membrane potential (\( n = 5 \) cells).
initial control amplitude was plotted versus time (Fig. 5). The control value. The by a test voltage step to 30 mV after conditioning prepulses from subsequently applied. Note that after progressively longer sojourns at −110 mV, the amplitude of $I_{\text{fast}}$ gradually returned to control value. The $I_{\text{fast}}$ peak (normalized with respect to the initial control amplitude) was plotted versus time (Fig. 5D; ○). Figure 5D also shows that on 3 cells similar data were obtained when the initial $V_{h}$ and sojourns values were −110 mV or −90 mV (○, ■, respectively). In both cases the time course of recovery from inactivation was well fitted by two exponentials (Fig. 5D). The time course for recovery was well fitted by two exponentials with time constants of 4.4 ± 0.6 and 17 ± 2 ms at −110 mV and 5.1 ± 0.2 and 25.8 ± 0.9 ms at −90 mV, indicating a slight voltage dependence for this process. In conclusion, recovery from inactivation was less dependent on membrane potential than the process of inactivation itself.

Deactivation properties of $I_{\text{fast}}$ were obtained by measuring the time constant of decay of tail currents (e.g., Fig. 6) at potentials between −105 and −65 mV because at less negative level the inactivation process became predominant. In this case, deactivation was relatively independent from membrane voltage and averaged 19 ± 3 ms (range 4–38 ms; $n = 8$ cells).

**Ionic properties of outward currents**

The ionic selectivity of $I_{\text{fast}}$ and $I_{\text{slow}}$ was studied with tail current analysis. Voltage steps to 0 mV from −70 mV $V_{h}$ with or without a prepulse (for 200–500 ms) to −110 mV were applied to evoke these currents. When the current reached its peak (200 ms for $I_{\text{slow}}$ and 15 ms for $I_{\text{fast}}$), the membrane potential was stepped to different voltages and tail currents were recorded. An example of $I_{\text{fast}}$ tail currents recorded at membrane potentials from −95 mV to −60 mV is shown in Fig. 6A. In this cell the tail current reversed at −80 mV. The mean reversal potential of $I_{\text{fast}}$ tail currents was −75 ± 2 mV ($n = 11$ cells), whereas the corresponding value for $I_{\text{slow}}$ tail currents was −81 ± 1 mV ($n = 11$ cells).

Average $I_{\text{fast}}$ tail currents plotted versus membrane voltage in 3 ($n = 11$ cells), 6 ($n = 3$), or 12 ($n = 3$) mM external K⁺ concentrations are presented in Fig. 6B. A compensatory reduction in NaCl was effected whenever K⁺ was raised. The results indicate that the tail current reversal potential, $E_{\text{rev}}$, moved to more positive values when the external K⁺ was increased and that there was an approximately parallel, rightward shift of these plots. The $E_{\text{rev}}$ dependence on the external K⁺ concentration is shown in Fig. 6C for both $I_{\text{slow}}$ (□) and $I_{\text{fast}}$ (■). The theoretical $E_{\text{rev}}$ for K⁺ at three K⁺ concentrations was calculated with the Nernst equation and is shown as a dashed line in Fig. 6C. From these data, it is apparent that $E_{\text{rev}}$ for both outward currents moved together with changes in external K⁺ concentration, suggesting that $I_{\text{slow}}$ and $I_{\text{fast}}$ were predominantly K⁺ currents. Nevertheless, as the observed values differed from the calculated one, at least three factors could have accounted for the deviation: 1) K⁺ accumulation in the extracellular space, 2) a degree of membrane permeability to other ions such as Na⁺, or 3) imperfect voltage clamp-conditions. The first possibility seemed unlikely because the tail current amplitude of $I_{\text{fast}}$ or $I_{\text{slow}}$ at −50 mV (to maximize its size) did not change during 2 Hz test pulses, a condition which should have enhanced any K⁺ accumulation. The second possibility was explored by 50% replacement of external NaCl with the presumably impermeant N-methyl-glucamine HCl: in this case, the tail $E_{\text{rev}}$ of $I_{\text{fast}}$ shifted in the negative direction by 5 ± 1 mV ($n = 3$ cells). This result suggests a measurable contribution

**FIG. 4.** Voltage dependence of activation and inactivation of $I_{\text{fast}}$. A: activation (■) and inactivation (○) curves for $I_{\text{fast}}$. Experimental points were obtained by dividing $I_{\text{fast}}$ peak by its driving force ($V_{h}$–$E_{\text{rev}}$) and normalizing them by the maximal conductance $g_{\text{max}}$ taken as 1. Lines represent Boltzmann equation fits; the potentials for half inactivation and half activation were −92.9 ± 0.2 mV and −27.6 ± 0.9 mV, respectively, while the slope factors were 10.8 ± 0.2 mV and 16 ± 1 mV for inactivation and activation, respectively ($n = 9$ cells). B: a set of outward currents elicited by a test voltage step to 30 mV after conditioning prepulses from −140 mV to −50 mV for 200 ms. C1: same set of current traces on a faster time scale. C2: individual $I_{\text{fast}}$ responses were isolated by subtracting the current trace obtained after the depolarizing prepulse to −50 mV from the current traces shown in C1.
by Na\(^+\) to the transient outward currents. Using the Goldman-Hodgkin-Katz equation (Hille 1992), we calculated the permeability ratio of K\(^+\) to Na\(^+\) \((P_{K}/P_{Na})\). For the Na\(^+\) and K\(^+\) concentrations of the patch pipette and extracellular solutions the permeability ratio values of 1/0.01 (92/1) and 1/0.02 (50/1) were obtained for \(I_{slow}\) and \(I_{fast}\) respectively. These data suggest that the contribution by Na\(^+\) permeability to the transient K\(^+\) currents was relatively small and that the deviation of the outward current \(E_{rev}\) from the one calculated for a pure K\(^+\) mediated response was perhaps also due to the difficulty to obtain isopotential conditions for a large cell with dendritic arborization in a slice preparation.

**Pharmacological dissection of \(I_{fast}\) and \(I_{slow}\)**

Two well known K\(^+\) channel antagonists, TEA and 4-AP, were tested. Application of 10–30 mM TEA \((n = 6 cells)\) readily depressed \(I_{slow}\), as indicated by the example in Fig. 7A1 and 2, in which the whole set of outward current traces (elicited by the same protocol shown in Fig. 2A) recorded in control solution (Ca-free-Co, TTX, Cs\(^+\)) was attenuated by 20 mM TEA. Fig. 7A3 shows the \(I/V\) curve related to the same data, indicating that there was a consistent depression (by 70 \pm 3%) of \(I_{slow}\) at various test potentials. This effect was reversible after 15–20 min washout. \(I_{fast}\) was comparatively resistant to Na\(^+\) to the transient outward currents. Using the Goldman-Hodgkin-Katz equation (Hille 1992), we calculated the permeability ratio of K\(^+\) to Na\(^+\) \((P_{K}/P_{Na})\). For the Na\(^+\) and K\(^+\) concentrations of the patch pipette and extracellular solutions the permeability ratio values of 1/0.01 (92/1) and 1/0.02 (50/1) were obtained for \(I_{slow}\) and \(I_{fast}\) respectively. These data suggest that the contribution by Na\(^+\) permeability to the transient K\(^+\) currents was relatively small and that the deviation of the outward current \(E_{rev}\) from the one calculated for a pure K\(^+\) mediated response was perhaps also due to the difficulty to obtain isopotential conditions for a large cell with dendritic arborization in a slice preparation.

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to 10–30 mM TEA; in fact, $I_{\text{fast}}$ amplitude at +20 mV slightly increased by 1.1 ± 0.2 times ($n = 4$ cells). $I_{\text{fast}}$ was preferentially blocked by 4-AP. An example is shown in Fig. 7B in which $I_{\text{fast}}$ elicited by a depolarizing step to 20 mV after a conditioning step to −110 mV was recorded in Ca-free-Co solution containing TTX (1 μM), TEA (20 mM), and Cs⁺ (4 mM). Increasing concentrations of 4-AP (1, 2, and 3 mM) were cumulatively added to the external solution. The peak amplitude of $I_{\text{fast}}$ was not decreased by 1 mM 4-AP, although the current decay became faster (33 ms time constant in control and 21 ms time constant in presence of 1 mM 4-AP). At 2 or 3 mM concentration, 4-AP diminished $I_{\text{fast}}$ peak by 6 and 19%, respectively, together with a progressive reduction in current decay (13 and 10 ms time constants, respectively). Nevertheless, the late component of $I_{\text{fast}}$ remained relatively unaffected. Even very high concentrations of 4-AP (up to 10 mM) were unable to block $I_{\text{fast}}$ completely, whereas at concentrations higher than 6 mM 4-AP also depressed (by 28% in 7 mM 4-AP solution) $I_{\text{slow}}$. At 5 mM concentration 4-AP depressed $I_{\text{fast}}$ and $I_{\text{slow}}$ by 37 and 6%, respectively.

**DISCUSSION**

The present report provides the first quantitative description of two voltage-activated, Ca²⁺-independent K⁺ currents ($I_{\text{slow}}$ and $I_{\text{fast}}$), under voltage-clamp conditions, in neonatal hypoglossal motoneurons.

**Voltage activated currents of hypoglossal motoneurons**

Motoneurons integrate excitatory synaptic inputs to convert them into trains of the action potential that determine the characteristics of muscle activity. The pattern and frequency of action potential firing are due to the interplay of various membrane intrinsic conductances. In hypoglossal motoneurons (like in most nerve cells) the TTX-sensitive, fast Na⁺-inward current (shown in Fig. 1) is presumably responsible for the initial rising phase of the action potential. As it is notoriously difficult to study this Na⁺ current under voltage-clamp conditions (see for example, Takahashi 1990), it was not further investigated in the present report. Membrane depolarization of hypoglossal motoneurons is also known to activate four types of Ca²⁺ channels (Umemiya and Berger 1995) that underlie distinct Ca²⁺ currents systematically investigated by Umemiya and Berger (1994).

In addition to the transient inward currents generated by depolarization, multiple K⁺ conductances have been suggested to shape action potential repolarization and repetitive firing behavior (Viana et al. 1993b).

Molecular biology studies have indicated that rat brain stem nuclei contain mRNA for various K⁺ channel subunits, particularly Kv3.3 (and, to a lesser degree, Kv3.1), which in expression studies generates a sustained, slowly inactivating current (Weiser et al. 1994), and Kv4.2 and Kv4.3 both responsible for fast activating and inactivating currents (Serodio and Rudy 1998). It appears likely that hypoglossal motoneurons possess a repertoire of K⁺ currents that so far have not been systematically analyzed under voltage-clamp conditions.

In our experiments the total outward current could be separated into two broad components: one composed of voltage-activated K⁺ currents and a second of Ca²⁺-dependent K⁺ currents, which are typically responsible for the medium and slow afterhyperpolarization (AHP) (Viana et al. 1993b). In our experiments, Ca²⁺-dependent K⁺ currents were very small by comparison with Ca²⁺-independent K⁺ currents (Fig. 1B) presumably because of the presence of the strong Ca²⁺ chelator EGTA, which is known to suppress the Ca²⁺-dependent AHPs (Viana et al. 1993b).

In our study, voltage-activated K⁺ currents were investigated in the presence of TTX to block Na⁺ currents and in Ca-free-Co solution to eliminate Ca²⁺ currents and any residual Ca²⁺-dependent K⁺ currents. Cs⁺ was always present in the external solution to block the hyperpolarization activated current $I_h$ (Bayliss et al. 1994) and did not appear to interfere with the voltage activated outward currents. Under these conditions a slow outward current ($I_{\text{slow}}$) was generated by 1 s voltage steps from −70 mV $V_h$ (see Fig. 1B), peaking in tens of milliseconds and remaining at a plateau with a slow decline. Hyperpolarizing prepulses allowed us to unmask a much faster current ($I_{\text{fast}}$). $I_{\text{slow}}$ and $I_{\text{fast}}$ could therefore be distinguished on the basis of the substantial

![Image](http://jn.physiology.org/)

**FIG. 7.** Pharmacological properties of $I_{\text{slow}}$ and $I_{\text{fast}}$. A: current traces obtained by depolarizing voltage steps (same protocol as in Fig. 2) in control solution (Ctrl, containing TTX, Ca-free-Co, Cs⁺) (A1) and in the presence of TEA (20 mM) (A2). A3: I/V relation of $I_{\text{fast}}$ in control (○) solution or in the presence of 20 mM of TEA (●) for the same cell shown in A1 and 2. B: 4-AP preferentially blocks $I_{\text{slow}}$. Current traces (elicited by voltage step to 20 mV) were recorded in a solution containing TTX, TEA (20 mM), Ca-free-Co, and different concentrations of 4-AP (0, 1, 2, and 3 mM). All records are from the same cell.
differences in their voltage dependence of activation and inactivation.

$I_{\text{slow}}$ properties

Based on its activation kinetics and voltage dependence, $I_{\text{slow}}$ resembled the classical delayed rectifier first reported for the squid giant axon by Hodgkin and Huxley (1952). $I_{\text{slow}}$ activated at membrane potentials positive to $-50$ mV and showed voltage-dependent kinetics of activation. For example, the activation time constant value decreased nearly 10-fold from $-20$ mV to $+20$ mV. At membrane potentials positive to $-10$ mV, $I_{\text{slow}}$ inactivated slowly with a time course of seconds. During repolarization at the end of voltage step, $I_{\text{slow}}$ deactivated with monoexponential time constant. In addition, $I_{\text{slow}}$ was suppressed by TEA and partly depressed by very high doses of 4-AP.

$I_{\text{fast}}$ properties

$I_{\text{fast}}$ was unaffected by TEA application, a treatment which actually allowed us to observe it in isolation with its characteristically faster kinetics. $I_{\text{fast}}$ resembled the fast transient outward current $I_o$, first observed in gastropod neurons (Connor and Stevens 1971a). $I_{\text{fast}}$ was almost completely inactivated near resting potential because depolarizing voltage steps from $-70$ mV $V_h$ did not evoke $I_{\text{fast}}$. Half-maximal inactivation was at $-92.9 \pm 0.2$ mV. By membrane hyperpolarization the inactivation of $I_{\text{fast}}$ was rapidly removed with recovery to $50\%$ of control amplitude in about 10 ms (see Fig. 5D). Subsequent depolarization to values positive to $-60$ mV activated $I_{\text{fast}}$ (with a time course depending on membrane voltage), which peaked and fully inactivated within 200 ms. (At 0 mV the inactivation time constant was $70 \pm 20$ ms.) $I_{\text{fast}}$ deactivation after the end of the command pulse was comparatively faster ($19 \pm 3$ ms) and showed little voltage sensitivity. In contrast to $I_{\text{slow}}$, $I_{\text{fast}}$ was selectively attenuated after application of 4-AP (up to $5$ mM), which, in mM concentrations, is a well-established blocker of $I_{\text{fast}}$ in other nerve cells (Rudy 1988). The characterization of $I_{\text{fast}}$ in hypoglossal motoneurons is thus based on distinct electrophysiological properties and pharmacological sensitivity.

It should be noted that $E_{\text{rev}}$ for $I_{\text{fast}}$ (and also $I_{\text{slow}}$) was near the calculated value for K$^+$ and was shifted positively (as predicted by the Nernst equation) by raising extracellular K$^+$ concentration. These observations suggest that both currents were mainly mediated by an increased membrane permeability to K$^+$. Nevertheless, there was a small but systematic difference between the current $E_{\text{rev}}$ and $E_K$. This was partly due to a slight, yet consistently measurable permeability to Na$^+$ inherent in both currents and perhaps partly to the difficulty in obtaining complete isopotential conditions in neurons with dendritic arborization.

Functional implications of $I_{\text{fast}}$ and $I_{\text{slow}}$ for motoneuronal firing properties

Analogous to spinal (Barrett et al. 1980) and facial (Nishimura et al. 1989) motoneurons, hypoglossal motoneurons were shown to possess at least two transient K$^+$ currents, the functional role of which should be considered. It has been previously demonstrated that TEA- and 4-AP-sensitive K$^+$ conductances shape the action potential of hypoglossal motoneurons (Viana et al. 1993b) because TEA (1–10 mM) or 4-AP (0.1–0.5 mM) prolong action potential duration. When hypoglossal motoneurons are depolarized by long current pulses, their firing frequency decreases in a multimodal fashion with linearly developing initial adaptation (Sawczuk et al. 1995, 1997). In the study by Viana et al. (1993b), firing accommodation could not be tested in 4-AP solution owing to the development of intense synaptic activity while it seemed to be enhanced by TEA, although a quantitative analysis was not provided.

Our experiments suggest that only a very small part of $I_{\text{fast}}$ could by blocked by 1 mM 4-AP. Thus it seems plausible that spike lengthening by such a small dose of 4-AP does not involve depression of $I_{\text{fast}}$. Furthermore, as the resting potential of neonatal motoneurons is about $-70$ mV and the spike duration about 1 ms (Viana et al. 1995), it is likely that at rest level $I_{\text{fast}}$ is largely inactivated and that any available fraction of $I_{\text{fast}}$ possesses activation and deactivation kinetics ($\sim 10$ and $20$ ms, respectively) that are much slower than the spike duration. For these characteristics we suggest that $I_{\text{fast}}$, rather than controlling the duration of a single action potential (which perhaps relies on a distinct Ca$^{2+}$-dependent K$^+$ current not investigated in the present study), has a role in determining the initial adaptation of firing (lasting about 40 ms) which is only moderately dependent on Ca$^{2+}$-activated K$^+$ currents (Sawczuk et al. 1997). The AHP after the first action potential, typically lasting about 100 ms and with <10 mV undershoot from rest (Viana et al. 1995), would, however, be long enough to remove part of $I_{\text{fast}}$ inactivation. Thus, the second action potential could be delayed by a more substantial $I_{\text{fast}}$ activation. A similar mechanism can be suggested to operate for the third and fourth spike observed within the initial 50 ms of a train (Sawczuk et al. 1995), particularly because $I_{\text{fast}}$ deactivation required about 20 ms. $I_{\text{fast}}$ would then be able to control firing frequency through a mechanism similar to the one proposed by Connor and Stevens (1971b) for $I_o$.

Only a small part of $I_{\text{slow}}$ could by activated during the first spike because of its slow kinetics. Nevertheless, because $I_{\text{slow}}$ deactivated with a time constant (40 ms) longer than first interspike interval (Sawczuk et al. 1995, 1997), a certain fraction of activated $I_{\text{slow}}$ would be present at the beginning of the second spike in a train. Likewise, further accumulation of $I_{\text{slow}}$ would occur during the spike train with summing properties after each successive spike.

Unfortunately, direct experimental test of $I_{\text{slow}}$ and $I_{\text{fast}}$ participation in spike frequency adaptation is presently difficult made difficult by the lack of highly selective blockers. For instance, although $I_{\text{fast}}$ is sensitive to 4-AP, even a 5 mM concentration of this substance could not block it completely. The same applies to TEA acting on $I_{\text{slow}}$. Along the same line it might be difficult to rely on holding potential changes to remove $I_{\text{fast}}$ inactivation selectively, as this approach would also affect the kinetic properties of voltage dependent inward currents. Thus it seems useful to employ computer stimulation studies (see for instance, Powers 1993), which in the future might provide a better understanding of the functional role of these currents in hypoglossal motoneurons, but they will require strictly quantitative data. The availabil-
ity of kinetic parameters pertaining to these currents is expected to be useful for this purpose.

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