Role of Potassium Conductances in Determining Input Resistance of Developing Brain Stem Motoneurons

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Cameron, William E., Pedro A. Núñez-Abades, Ilan A. Kerman, and Tracy M. Hodgson. Role of potassium conductances in determining input resistance of developing brain stem motoneurons. J Neurophysiol 84: 2330–2339, 2000. The role of potassium conductances in determining input resistance was studied in 166 geniohyoid (GG) motoneurons using sharp electrode recording in brain stem slices of the rats aged 5–7 days, 13–15 days, and 19–24 days postnatal (P). A high magnesium (Mg2++; 6 mM) perfusate was used to block calcium-mediated synaptic release while intracellular or extracellular cesium (Cs+) and/or extracellular tetraethylammonium (TEA) or barium (Ba2+) were used to block potassium conductances. In all cases, the addition of TEA to the high Mg2++ perfusate generated a larger increase in both input resistance (Rin) and the first membrane time constant (τm) than did high Mg2++ alone indicating a substantial nonsynaptic contribution to input resistance. With intracellular injection of Cs+, GG motoneurons with lower resistance (<40 MΩ), on the average, showed a larger percent increase in Rin than cells with higher resistance (>40 MΩ). There was also a significant increase in the effect of internal Cs+ on Rin and τm with age. The largest percent increase (67%) in the τm due to intracellular Cs+ occurred at P13–15, a developmental stage characterized by a large reduction in specific membrane resistance. Addition of external Cs+ blocked conductances (further increasing Rin and τm) beyond those blocked by the TEA perfusate. Substitution of external calcium with 2 mM barium chloride produced a significant increase in both Rin and τm at all ages studied. The addition of either intracellular Cs+ or extracellular Ba2+ created a depolarization shift of the membrane potential. The amount of injected current required to maintain the membrane potential was negatively correlated with the control Rin of the cell at most ages. Thus low resistance cells had, on the average, more Cs+- and Ba2+-sensitive channels than their high resistance counterparts. There was also a disproportionately larger percent increase in τm as compared with Rin for both internal Cs+ and external Ba2+. Based on a model by Redman and colleagues, it might be suggested that the majority of these potassium conductances underlying membrane resistance are initially located in the distal dendrites but become more uniformly distributed over the motoneuron surface in the oldest animals.

INTRODUCTION

A critical event in the differentiation of mammalian motoneurons is the decrease in input resistance associated with the motoneurons innervating fast twitch muscle fibers during postnatal development (Navarrete and Vrbová 1993). In the first paper, we investigated the role of synaptic input in determining the membrane resistance. It was found that synaptic inputs accounted for a significant portion of the resting conductance of developing brain stem motoneurons. More specifically, glycine/GABA-mediated conductances were found to increase with age. There was a significant increase in the percent change of input resistance between the first and second week of postnatal life after synaptic blockade with either tetrodotoxin (TTX), high magnesium, or receptor blockers. However, the magnitude of this synaptic effect cannot account for the halving of the Rin observed during this time period. One alternative source of this decreased resistance would be nonsynaptic potassium channels.

There is a substantial body of evidence to suggest that specific membrane resistance is modulated by many voltage-sensitive channels, in addition to tonic synaptic activity (Rall et al. 1992). A variety of potassium channels including the delayed rectifier, inward rectifier, and A- and Ba- channels have been implicated in establishing the membrane resistance of mammalian motoneurons (Binder et al. 1996; Crill and Schwindt 1983). These channels are sensitive to a diversity of substances including internal and/or external tetraethylammonium (TEA), cesium, and barium in a wide variety of neurons (Hille 1992). Internal cesium reduces resting conductance in cat spinal motoneurons (Puil and Werman 1981), while external TEA has a similar effect in rat vagal motoneurons (Yarom et al. 1985). External TEA also prolongs the duration of the action potential by reducing the voltage-sensitive potassium conductances underlying the fast afterhyperpolarization (AHP) and the spike repolarization in cat lumbar (Schwindt and Crill 1980a) and rat hypoglossal motoneurons (Viana et al. 1993). In addition to depressing the fast voltage-sensitive potassium conductance, external barium decreases the potassium leak conductance (Schwindt and Crill 1980b). This barium-sensitive component of potassium leak conductance is modulated in rat hypoglossal motoneurons by thyrotropin-releasing hormone (Bayliss et al. 1992) and norepinephrine (Parkis et al. 1995) to change both motoneuron excitability and repetitive firing characteristics.

It has been proposed that the properties of the motoneuron membrane are not uniform. There are data to suggest that there is a difference in the specific membrane resistance between fast and slow motoneurons (Burke 1987; Burke et al. 1982). In...
addition, it has been suggested that there is a difference in the membrane resistivity between the cell body and dendrites of the same cell. The decreased resistance of the soma (somatic shunt) was first postulated by the Redman laboratory (Iansek and Redman 1973) in their study of cat lumbosacral motoneurons. More recently, work from the laboratory of P. K. Rose (Campbell and Rose 1997) has intracellularly injected cesium to assess the contribution of potassium channels to this somatic shunt in cervical motoneurons studied in vivo. These authors found an increase in the somatic time constant with injection of intracellular cesium while only a small decrease in the dendritic time constant. They concluded that the distribution of cesium-sensitive channels was concentrated in the somatic and proximal dendritic membrane. In contrast, studies of barium-sensitive conductances in lumbar sympathetic ganglion cells (Redman et al. 1987) have suggested a more distal dendritic location for the blocked potassium conductances. It would be interesting to know when the adult distribution of voltage-sensitive channels influencing membrane resistance is established during development and whether the patterns seen in these other neuron populations also applies to developing brain stem motoneurons. Thus we have examined the contribution of various potassium conductances to the membrane properties of input resistance and time constant to gain a better understanding of the changes occurring in the distribution of voltage-sensitive channels during the period when motoneurons differentiate. Some of these data have been presented in abstract form (Cameron 1998; Cameron et al. 1996).

**Methods**

Brain stem slices were prepared from male Sprague Dawley rats from three postnatal (P) age groups (P5–7, P11–15, and P19–24) as described in the preceding paper. In brief, rat pups were deeply anesthetized with halothane, tracheotomized, and ventilated with 100% O_2_. Anesthetized pups were transcardially perfused with cold (4°C) sucrose-artificial cerebral spinal fluid (ACSF) and quickly decapitated. The brain stem was removed and sectioned at 300 μm with a Vibraslice. The composition of sucrose-ACSF was as follows (in mM): 240 sucrose, 2 KCl, 1.25 Na_2HPO_4, 26 NaHCO_3, 10 glucose, 5 MgSO_4, and 1 CaCl_2. All slices were incubated in a holding chamber in normal ACSF consisting of (in mM) 126 NaCl, 2 KCl, 1.25 Na_2HPO_4, 26 NaHCO_3, 20 glucose, 2 MgSO_4, and 2 CaCl_2, at room temperature, bubbled with 95% O_2-5% CO_2 (pH 7.35). In experiments in which 6 mM MgCl_2, 20 mM tetraethylammonium chloride (TEA), or 5 mM CsCl (Sigma) was added to the bath solution, ionic strength was maintained by an equivalent decrease in the concentration of NaCl. In experiments in which 2 mM barium chloride was substituted for calcium chloride, 10 mM HEPES was substituted for both phosphate and bicarbonate buffers, and solution was bubbled with 100% O_2. This alternate buffer system was chosen to prevent barium from precipitating in the presence of phosphate buffer. The flow rates of normal and modified ACSF were kept constant at 1–2 ml/min using a perfusion pump. All membrane properties were measured at room temperature (21 ± 1°C).

Motoneurons were recorded from the ventromedial portion of the hypoglossal nucleus, a region shown to contain genioglossal (GG) motoneurons (Mazza et al. 1992). The criteria for a healthy cell and the protocols for measuring the membrane properties were described in the preceding paper. There were three separate protocols employed to block potassium conductances. In the first series of experiments, the role of synaptic inputs and nonsynaptic potassium conductances in determining membrane resistance was tested. Input resistance (R_i), first membrane time constant (τ_m), rheobase (I_r), and repetitive firing was measured in normal ACSF. The validity of using a long, hyperpolarizing current pulse (500 ms duration) to measure R_i and τ_m in cells exhibiting an inward rectifying or “sag” current has been discussed in the first paper of this series. In a few cells, this current could be blocked by the application of external cesium (Cs⁺, Fig. 1) with only a moderate change in the two membrane properties. However, in the majority of cases, the sag current was only partially blocked by external Cs⁺, and there was evidence that additional currents were being effected (see Fig. 8). Given this inconsistency, it was determined that exposure to external cesium would not provide any more consistent control value for measurement of R_i and τ_m than the measurements potentially contaminated by the sag current.

After making the control measurements in the first protocol, the
potentials were Mg$^{2+}$/TEA responses to a series of hyperpolarizing current steps in control, high (6 mM) Mg$^{2+}$, and high Mg$^{2+}$/20 mM TEA. The inward rectification in both the P7 and P15 cells is smaller than that found in the P20 motoneuron (Fig. 2). The increase in input resistance in response to 20 mM TEA is greater in the P15 than P7 motoneuron. Note the prominent synaptic activity in the control traces of the P7 neuron that is almost completely abolished by high Mg$^{2+}$. Time and voltage scales are the same for both top and bottom panels. Resting membrane potentials were −71 and −59 mV for the P7 and P15, respectively.

Calcium-dependent neurotransmitter release was blocked by the addition of 6 mM (high) magnesium to the perfusate. The membrane properties were measured at 5 and 10 min after the start of the perfusate with blocker. Following 10 min of high Mg$^{2+}$, the perfusate was switched to one containing the high Mg$^{2+}$ plus 20 mM TEA, and the measurements were repeated. Similar measurements were made after 5 and 10 min in the Mg$^{2+}$/TEA perfusate and after 10, 20, and 30 min of wash out. In a separate protocol, 5 mM Cs$^+$ was added to the Mg$^{2+}$/TEA perfusate to assess the contribution of potassium channels that were Cs$^+$ sensitive and TEA insensitive.

In the second protocol examining the effect of internal Cs$^+$, electrodes were filled with 3 M cesium acetate. A series of control measurements of the action potential $R_h$ and $\tau_m$ were made immediately after impalement. Then Cs$^+$ was injected into the cell using a 50-ms, 5-Hz positive pulse with an amplitude sufficient to evoke several action potentials (0.2–1 nA; duration, 2–4 min). Measurements were made at 2 and 4 min of injection and 10–40 min after injection was stopped.

In all protocols other than internal Cs$^+$, the membrane potentials of developing GG motoneurons were recorded with glass micropipettes filled with a 3:1 mix of 3 M potassium acetate and 3 M potassium chloride (resistance, 60–100 MΩ). To avoid activating other voltage-sensitive conductances, the initial membrane potential was maintained by injecting a constant (bias) current to offset any depolarizing shift of the membrane. This value of bias current required to oppose the depolarization generated by the block of potassium conductances was recorded for further analysis. The level of membrane hyperpolarization at which the time constants were calculated was insufficient to activate voltage-sensitive currents (inward rectification) noted with larger hyperpolarizations in some cells (Fig. 1B, preceding paper).

In the final protocol, the slices were exposed to external barium. Due to the spontaneous firing associated with external Ba$^{2+}$, control measurements were made both before and after the addition 1 µM tetrodotoxin (TTX) to the normal ACSF. After 10 min in TTX, an ACSF solution containing 2 mM BaCl was introduced into the bath and measurements made at 5 and 10 min and after 20–30 min of wash out. The measurement after 10 min in TTX (devoid of evoked synaptic release) was used as the control value for comparisons with the barium data. In most instances, cells returned to their control (TTX) values for input resistance and membrane time constant after 30 min of wash out.

The values of $R_h$ and $\tau_m$ are presented in the text and tables as means ± SE. A two-way ANOVA was employed to determine whether there were any significant interactions between the levels of treatment and levels of postnatal age. A pair-wise multiple comparison procedure (Tukey test) was performed to test the differences between means. If significant differences were indicated from the Tukey test, then a one-way ANOVA with repeated measures or a paired $t$-test was performed to determine the differences between age groups. Statistical significance was defined as $P < 0.05$.

**Results**

Recordings were made from genioglossal (GG) motoneurons located in the ventromedial portion of the hypoglossal nucleus. A total of 166 motoneurons met our acceptance criteria and fell into one of the following postnatal age groups: P5–7 ($n = 34$); P13–15 ($n = 68$); P19–24 ($n = 64$). Throughout the postnatal period studied, no differences were found in the mean resting membrane potential or action potential amplitude for these motoneurons. The mean membrane potential was −64.4 ± 0.2 mV.

**High magnesium and TEA blockade**

The goal of this experimental series was to determine what proportion of the resting conductance was mediated by potassium conductances as compared with that mediated by synaptic input examined in the previous paper. Like the previous study, high (6 mM) Mg$^{2+}$ was added to the perfusate to inhibit calcium-mediated synaptic transmission. Potassium conductances were inhibited by either TEA or Cs$^+$. Figure 2 shows one P20 motoneuron from the first experimental series illustrating the effect external Mg$^{2+}$ and TEA on membrane properties. The top panel presents three actions potentials evoked by a depolarizing current pulse (50 ms, 1 Hz) in the control bath solution, and in solutions containing high Mg$^{2+}$ and high Mg$^{2+}$/TEA, plus 20 mM TEA. The bottom panel shows the mem-

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**Table 1.** Effect of high Mg$^{2+}$ and TEA on input resistance and membrane time constant on developing genioglossal (GG) motoneurons

<table>
<thead>
<tr>
<th>Postnatal Age, days</th>
<th>$R_h$, MΩ</th>
<th>$\tau_m$, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>High Mg$^{2+}$</td>
</tr>
<tr>
<td>6–7</td>
<td>$n = 8$</td>
<td>59.3 ± 10.0</td>
</tr>
<tr>
<td>13–15</td>
<td>$n = 14$</td>
<td>43.9 ± 3.2</td>
</tr>
<tr>
<td>19–24</td>
<td>$n = 16$</td>
<td>40.4 ± 2.8</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; $n$ is total number of samples. $R_h$, input resistance; $\tau_m$, membrane time constant; Mg$^{2+}$, magnesium; TEA, tetraethylammonium. There were no significant age-dependent changes in absolute or percent terms associated with the high magnesium or TEA treatment in this sampling. * $P < 0.05$. † $P < 0.01$. ‡ $P < 0.001$.
brane response to a series of six hyperpolarizing current pulses. High Mg\(^{2+}\) media abolished the afterdepolarization (ADP; arrow) of the action potential observed in the control and increased \(R_n\). With the addition of TEA, the repolarization phase of the action potential was slowed and a more substantial increase in \(R_n\) was observed than that seen in Mg\(^{2+}\) alone. However, neither high Mg\(^{2+}\) nor Mg\(^{2+}\)/TEA had any major effect on the depolarizing sag produced by the inward rectification, as seen in the traces in the bottom panel.

Figure 3 shows the effects of blockade of calcium-mediated synaptic activity and potassium conductances on \(R_n\) for two earlier periods of development (P7 and P15). The control recording from a P7 animal was punctuated by significant spontaneous synaptic activity that was nearly abolished by the addition of TEA. These two cells were selected because they demonstrated a clear incremental effect of the addition of external TEA to the high Mg\(^{2+}\) perfusate; however, the magnitude of the change in \(R_n\) was not representative of the larger sample. Table 1 summarizes the group data for 38 GG motoneurons analyzed in the first series of experiments. The two-way ANOVA showed that there were no interactions between age and treatment for either \(R_n\) or \(\tau_0\) and that there were significant differences among treatments but not among the different ages. Further statistical analyses revealed significant differences in \(R_n\) between high Mg\(^{2+}\) and Mg\(^{2+}\)/TEA for each age group. Similar analyses for \(\tau_0\) revealed a significant difference between control and high Mg\(^{2+}\) only at the youngest age (P5–7), while the addition of TEA to the Mg\(^{2+}\) containing bath generated significantly larger values of \(\tau_0\) at all ages. Over the developmental period studied, the absolute magnitudes and relative percents of change in \(R_n\) and \(\tau_0\) with the addition of TEA to the high Mg\(^{2+}\) perfusate were at least twofold greater than that produced by Mg\(^{2+}\) alone.

Internal cesium blockade

The contribution of potassium conductances in determining \(R_n\) and \(\tau_0\) were also assessed before and after an intracellular injection of cesium (Cs\(^+\)). Internal Cs\(^+\) had a more profound effect on the shape of the action potential than did extracellular TEA. Figure 4 shows the effect of internal Cs\(^+\) on the action potential and \(R_n\) of a GG motoneuron from a P15 animal. The maximum spike half-width was achieved after 2 min of injection and showed little change at 4 min. In contrast to the spike width, the \(R_n\) continued to increase between 2 and 4 min of injection. The staggering in the further reduction of potassium conductance implies that the potassium channels governing

![Figure 5](http://jhp.physiology.org/)

**FIG. 5.** Recovery of action potential after cessation of Cs\(^+\) injection of a GG motoneuron from a P22 animal. Top: the change in action potential at the end of a 6-min injection and again after 20 and 40 min of recovery. The action potential is fully recovered by 40 min (ADP indicated by arrow) appearing similar to the control. Bottom: the response of the membrane to the current steps at each of the 3 epochs. Note that \(R_n\) continues to rise, even as the somatic potassium channels are recovering as reflected in the more rapid repolarization phase of action potential. This recovery at soma without accompanying change in \(R_n\) suggests that the potassium channels governing \(R_n\) are located in the distal dendrites. Control \(R_n\), 30 MΩ.

![Figure 6](http://jhp.physiology.org/)

**FIG. 6.** Effect of intracellular Cs\(^+\) on input resistance. Scatter plot of pooled data (\(n = 65\)) illustrating the effect of 2–4 min of Cs\(^+\) injection on the percent increase in \(R_n\) as a function of control \(R_n\). There was negative linear correlation (\(R = -0.58, P < 0.001\)). The motoneurons are arbitrarily divided (vertical line) into high resistance (>40 MΩ) and low resistance (<40 MΩ) cells. With a few exceptions, the low resistance cells exhibited a larger change percent in \(R_n\) with intracellular Cs\(^+\) than did their high resistance counterparts.

![TABLE 2](http://jhp.physiology.org/)

**TABLE 2.** Effect of internal cesium (Cs\(^+\)) on \(R_n\) and \(\tau_0\) of developing GG motoneurons

<table>
<thead>
<tr>
<th>Age, days</th>
<th>(R_n), MΩ</th>
<th>(\tau_0), ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–6</td>
<td>17</td>
<td>55.9 ± 3.1</td>
</tr>
<tr>
<td>13–15</td>
<td>37</td>
<td>39.5 ± 2.7</td>
</tr>
<tr>
<td>19–23</td>
<td>19</td>
<td>37.1 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\) is total number of samples. For age-dependent changes associated with internal cesium, see Fig. 7. * \(P < 0.001\).
repolarization of the somatic action potential are not the same as those governing the resistance of the cell. This conclusion is supported by the time course of blockade demonstrated in Fig. 5. A GG motoneuron from a 22-day-old rat was injected with 5 mM extracellular Cs$^+$ for 6 min. The action potential exhibited a slow repolarization and multiple spikes. Twenty minutes after the cessation of Cs$^+$ injection, the action potential was narrowing and, by 40 min, it has reached its control width (showing an ADP, arrow). The membrane resistance of this cell continued to increase even after the injection had halted. With recovery of the somatic potassium channels governing repolarization (delayed rectifier, $I_{kV}$), another subset of potassium channels must be responsible for the increased resistance.

The one-way ANOVA with repeated measures revealed a significant difference between control and cesium treatments.

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When individual age groups were analyzed, intracellular cesium produced a significant increase in both $R_n$ and $\tau_0$ at all ages studied (Table 2). No age-dependent changes were found in absolute values of either membrane property; however, when expressed as a percent change from control, there was a developmental change (see Fig. 7).

The percent change from control values of $R_n$ varied greatly among the 65 motoneurons studied in this protocol and plotted in Fig. 6. We found a negative linear relation between control input resistance and percent change in $R_n$ with intracellular Cs$^+$ ($r = -0.58$, $P < 0.001$). A vertical line arbitrarily divides the motoneurons into a low resistance (<40 MΩ) and a high resistance (>40 MΩ) group. When divided in such a fashion, with few exceptions, low resistance cells tend to show larger percent increases (>30%) in $R_n$ with intracellular Cs$^+$ than most high resistance cells (<30%). Thus Cs$^+$-sensitive conductance is greater in the lower resistance than the higher resistance cells.
TABLE 3. Effects of tetrodotoxin (TTX) and external barium (Ba\textsuperscript{2+}) on \(R_n\) and \(\tau_0\) of developing GG motoneurons

<table>
<thead>
<tr>
<th>Postnatal Age, days</th>
<th>(R_n) (\text{M\Omega})</th>
<th>(\tau_0) ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TTX Control</td>
<td>Ba\textsuperscript{2+}-TTX</td>
</tr>
<tr>
<td>5–6</td>
<td>48.3 ± 3.6</td>
<td>97.5 ± 8.3*</td>
</tr>
<tr>
<td>13–15</td>
<td>38.4 ± 3.4</td>
<td>69.1 ± 5.3*</td>
</tr>
<tr>
<td>19–23</td>
<td>35.6 ± 2.3</td>
<td>66.3 ± 5.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\) is total number of samples. There were no significant age-dependent changes in absolute or percent terms associated with the barium treatment. * \(P < 0.01\).

resistance cells. No equivalent relationship was found for the Mg\textsuperscript{2+}-sensitive component of \(R_n\) in the preceding study (data not shown).

The magnitude of the Cs\textsuperscript{+} effect on both \(R_n\) and \(\tau_0\) varied with postnatal age. Figure 7 summarizes the effects of internal Cs\textsuperscript{+} on \(R_n\) and \(\tau_0\) for the three age groups studied. The effect of Cs\textsuperscript{+} injection on \(R_n\) increased with each succeeding week, while the effect on \(\tau_0\) was characterized by a prominent increase in membrane time constant between the first and second week of postnatal life. If we assume that the specific capacitance of the cell \(C_m\) remains constant during the Cs\textsuperscript{+} injection and \(\tau_m\) can be approximated by \(\tau_{ip}\), then the changes in \(\tau_{ip}\) reflect changes in the specific membrane resistance \(R_m\). Thus the increased resistance in GG motoneurons at 2 wk of age is due, in large part, to the proliferation of Cs\textsuperscript{+}-sensitive channels. Alternatively, as the animal matures, the distribution of cesium-sensitive potassium channels may shift from a distal distribution to a more uniform distribution or near the soma. The contribution of this subset of potassium channels to specific membrane resistance \(\tau_0\) appears to be maximal at 2 wk of age, while the contribution to \(R_n\) was greatest at 3 wk. Given the doubling of membrane surface area between 2 and 3 wk (Núñez-Abades and Cameron 1995), the increased numbers of cesium-sensitive channels at 3 wk responsible for the \(R_n\) are distributed over a larger surface area. As a result of this growth, the cesium-sensitive component of \(\tau_0\) at 3 wk was reduced. These development trends were also evident in the absolute changes in \(R_n\) and \(\tau_0\) (Table 2). This interpretation may be overly simplistic, and an alternative will be presented in the discussion.

External cesium blockade

We studied the effect of adding external Cs\textsuperscript{+} to determine what, if any, increases may be seen in \(R_n\) and \(\tau_0\) above that generated by external TEA. One effect of external Cs\textsuperscript{+} was to reduce the inward rectification in some cells (Fig. 1). In the P24 GG motoneuron shown in Fig. 8, external Cs\textsuperscript{+} greatly increased both \(R_n\) and \(\tau_0\) above that achieved with external TEA. In the experimental protocol, the high Mg\textsuperscript{2+} blocked the ADP present in the control action potential and increased \(R_n\) slightly. With the addition of TEA, the action potential broadened, and there was a further increase \(R_n\). Neither of these treatments greatly affected the inward rectification. Finally, with the addition of external Cs\textsuperscript{+}, there was a further increase in \(R_n\) and enhanced broadening of the action potential. This pattern occurred in five of five experiments. Within 60 min of wash out, the action potential narrowed to control width and the ADP returned; however, \(R_n\) failed to return to control levels. For 13 cells tested at P20–24, the mean percent change by external Cs\textsuperscript{+} was 61.4 ± 18.9% (mean ± SE) and 71.5 ± 25.3% for \(R_n\) and \(\tau_0\), respectively. Similar values of 61.0 ± 29.3% and 69.0 ± 7.0% were measured for \(R_n\) and \(\tau_0\) for two cells at P5–7. This large increase in \(R_n\) and \(\tau_0\) was observed in GG motoneurons irrespective of whether the motoneurons exhibited an inward current or not.
External barium blockade

External barium blocks some potassium conductances and the leak current in spinal motoneurons (Schwindt and Crill 1980b). Because barium increased the spontaneous activity of the slice, it was necessary to block spontaneously generated action potentials with TTX if the membrane properties were to be measured. Figure 9 shows the effect of Ba$^{2+}$ on the membrane responses of a P7 and a P14 GG motoneuron. At each age, there was a significant increase in $R_n$ and $\tau_0$ of the TTX-treated cells after perfusion with 2 mM barium chloride. The effect of external Ba$^{2+}$ on 40 GG motoneurons is summarized in Table 3. The magnitude of this effect on $\tau_0$ was relatively consistent between the different ages. There was a larger absolute change in $R_n$ at P5–6 than at the two older ages. Based on the intracellular cesium data, one might predict that the magnitude of the barium-sensitive component would be larger at P13–15, but this was not the case. A plot of the percent change in resistance with Ba$^{2+}$ as a function of the control $R_n$ yielded no correlation (data not shown).

With the addition of barium to perfusate or cesium to the intracellular compartment, almost all cells were found to depolarize. To avoid activating any voltage-sensitive channels, a bias current was applied to counteract this drift of the membrane potential. Figure 10 presents a plot of the bias (injected) current as a function of the control $R_n$ for intracellular cesium and extracellular barium. There is a negative correlation between injected current and input resistance at P5–6 and P13–15 and for the pooled data for cesium. A similar negative correlation was found in cells at P19–23 for external Ba$^{2+}$. These correlations may reflect that there are more cesium- and barium-sensitive channels controlling resting membrane potential in the low resistance motoneurons as compared with the high resistance cells. Alternatively, the lack of a strong correlation in some age groups was a result of the wider range of injected currents associated with low resistance than high resistance cells.

Distribution of cesium- and barium-sensitive channels

It was evident from Fig. 7 that the mean percent change in $R_n$ and $\tau_0$ with intracellular cesium was not equivalent. Similar to the first paper, we have applied the analysis of Redman and colleagues (Redman et al. 1987) to our data on cesium and barium blockade. Starting with a simple model of a small spherical ganglion cell, the model would predict a proportional change in $R_n$ and $\tau_0$ in response to the actions of barium on resting conductance. When expressed as a ratio of change in $\tau_0$ to $R_n$, the ratio would equal 1.0. With the addition of a dendrite to the model, changes in specific resistance of the dendrite are not reflected by a proportionate change in the $R_n$ result in larger changes in $\tau_0$ than $R_n$ and a ratio <1.0. Figure 11 plots the percent change in $\tau_0$ as a function of the percent change in $R_n$. Points lying above a unity line indicate a larger change in $\tau_0$ than $R_n$. Most of the cells from both treatments (Cs$^+$ and Ba$^{2+}$) are found above the line. In the context of the Redman model, this outcome is interpreted to mean that most of the blocked conductances reside in the dendritic tree and not at the cell body. This conclusion is supported by the earlier observation (Fig. 5) that suggested that the cesium-sensitive component of $R_n$ was located in the distal dendrites.

On closer inspection, there is a developmental trend in the data for both cesium and barium blockade. One alternative to the scatter plot is to quantitate the proportion of change in $R_n$ as compared with that in $\tau_0$ by calculating the ratio. A proportionate change in the two membrane properties would yield a ratio of 1.0, while values exceeding 1.0 would predict a more distal dendritic distribution. When the means and standard deviations were calculated at each age in response to intracellular cesium and external barium (Table 4), the oldest age group demonstrated the smallest mean and standard deviation. Because of the large standard deviations, none of these differences reached statistical significance; however, there was a trend for the ratios to decrease (approaching 1.0) with age. This reduction suggests that the conductances that were predominantly in distal dendrites at the younger ages actually become more uniformly distributed by the oldest age.

**TABLE 4.** Effects of intracellular cesium (Cs$^+$) and external barium (Ba$^{2+}$) on the ratio of percent change in $R_n$ and $\tau_0$ of developing GG motoneurons

<table>
<thead>
<tr>
<th>Postnatal Age, days</th>
<th>Cs$^+$</th>
<th>Ratio</th>
<th>Ba$^{2+}$</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–7</td>
<td>7</td>
<td>5.2 ± 8.7</td>
<td>5</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>13–15</td>
<td>18</td>
<td>4.4 ± 7.1</td>
<td>8</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>19–22</td>
<td>8</td>
<td>1.6 ± 1.4</td>
<td>9</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$ is total number of samples. There were no significant treatment or age-dependent changes.
DISCUSSION

This is the first study to examine the role of potassium conductances in determining the input resistance of developing mammalian motoneurons. The input resistance is the critical parameter in establishing the order of motoneuron recruitment in a spontaneous motor behavior (Cameron et al. 1991). During postnatal development, the pattern of activity in the cat phrenic motor nucleus was dramatically altered after the mean input resistance (and specific membrane resistance by inference) was reduced by half. Similar to the cat motoneurons, the rat genioglossal (GG) motoneurons studied in vitro undergo similar developmental changes in both their electrophysiology (Nuñez-Abades et al. 1993) and anatomy (Nuñez-Abades and Cameron 1995; Nuñez-Abades and Abades et al. 1994). The major findings of this study on GG motoneurons is that increases in the Cs⁺-sensitive conductances make the substantial contribution to the decrease in $R_n$ and $\tau_0$ found during postnatal development. From data in the preceding paper, synaptic blockade generated a 21 and 36% increase in $R_n$ and a 29 and 38% increase in $\tau_0$ at 1 and 2 wk of age, respectively. At these same ages, internal cesium blockade of potassium channels produced a comparable 22 and 31% increase in $R_n$ but a larger 30 and 67% increase in $\tau_0$. In general, the cells having lower input resistance tend to exhibit more Cs⁺- and Ba²⁺-sensitive conductances than their higher resistance counterparts. Initially, these potassium conductances are predicted to be located in the distal dendrites but become more homogeneously distributed with time.

Extracellular TEA blockade

The various contributions of synaptic inputs and potassium currents have been dissected using a variety of ionic/pharmacological manipulations. In the previous paper, almost all of the synaptically mediated conductances (evoked and spontaneous) were blocked by high magnesium. By applying the high magnesium prior to the application of external TEA, we wanted to assess the role of nonsynaptically mediated potassium conductances. The size of the external TEA response after high Mg²⁺ was taken as evidence for a large role for voltage-sensitive potassium conductances. Extracellular TEA increases motoneuron input resistance in rat vagal motoneurons (Yarom et al. 1985) while it specifically suppress both the delayed-rectifier and A-currents in spinal motoneurons (Safarova and Vogel 1995). In rat hypoglossal motoneurons, external TEA effectively blocks the other inward rectifying channels but not the $I_{h}$ (sag) current (Bayliss et al. 1994), the $I_{h}$ being more sensitive to Cs⁺ than either Ba²⁺ or TEA.

It has been proposed that the $I_{h}$ current in rat hypoglossal motoneurons is active at membrane potentials more negative than −65 mV (Bayliss et al. 1994) and, thereby, contributes to the membrane resistance. In fact, the current-voltage ($I-V$) plots from this report demonstrate little $I_{h}$ current at potentials more positive than −80 mV. In the present study, small current steps were used for the measurement of time constant to avoid activating the inward rectifier. Based on the linearity of the semi-log plots at small (−0.05 nA) current steps, there was little apparent sag contamination. Bayliss and colleagues also described a 10-fold increase in the amplitude of this $I_{h}$ current between $P2$ and $P65$. Given an order of magnitude increase with postnatal development in this current, it is not surprising that little sag was detectable in our youngest age group. In the present study, the sag current was sensitive to external cesium, but, in many instances, the value of input resistance was only minimally affected by blockade of the sag current (Fig. 1). When sag was detected, the voltage was measured at the peak response prior to the initiation of the inward current. As a result, we do not believe our measurements of $R_n$ to be significantly impacted by the sag current.

In the first series of experiments, high Mg²⁺ was relatively effective at depressing the calcium-mediated potassium current underlying the medium AHP. However, this channel was not apparently the source of the decrease resistance. The delayed rectifier can be blocked by either external TEA or cesium (Hille 1992; Schwindt and Crill 1981). It is interesting to note that there was a synergistic effect between external TEA and cesium on the delayed rectifier as evidenced by the increase in the duration of the action potential repolarization. This depression of the delayed rectifier was accompanied by an increase in the calculated $R_n$. Given the generalized nature of our blockers, the increment in resistivity resulting from the addition of external cesium cannot be attributed to a known set of potassium channels. It is interesting to note that combination of magnesium and TEA lead to an approximately 40% increase in $R_n$ and a 70% increase in $\tau_0$. After adding cesium to the external solution, input resistance increased by 60–70%, and the membrane time constant increased by 60–70%. According to the Redman model, the TEA- and Cs⁺-sensitive potassium channels have different patterns of distribution over the motoneuronal membrane of these developing brain stem motoneurons.

Intracellular cesium

In cat lumbosacral motoneurons, intracellular cesium resulted in a prolongation of the falling phase of the action potential, a large reduction in the amplitude of the AHP, and a reduction in resting membrane conductance, up to half its original value (Puill and Werman 1981). Recovery of the action potentials from the cesium was dose dependent and could take from 4 to 35 min. However, changes in conductances were not, in most instances, reversible, especially with large injections. We also observed that there was a partial recovery from intracellular cesium. Figure 4 demonstrates that intracellular cesium blocked the delayed rectifier in the present experiments. However, as the cesium effect lessened in the cell body (presumably due to the diffusion of cesium into the dendrites), the somatic action potential recovered while the $R_n$ remained elevated (Fig. 5). This persistent, reduced conductance suggests that either the delayed rectifier is not involved in establishing resting conductance and/or the resistance of a cell is determined predominantly by the resistivity of the dendrites.

The effects of intracellular cesium were recently studied in cat cervical motoneurons (Campbell and Rose 1997). These investigators concluded that the increased conductance of the soma (somatic shunt) is due to tonic activation of voltage-dependent potassium channels located on or near the soma. The present study suggests a less uniform distribution for cesium-sensitive channels at the younger ages for brain stem motoneurons that is subject to change during development. Based on a model by Redman and colleagues (Redman et al. 1987), the percent change in $R_n$ and $\tau_0$ would be equal if the

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channels were uniformly spread over the motoneuron membrane. The larger change in \( \tau_0 \) relative to \( R_n \) in the present study with intracellular cesium mimics the response in sympathetic ganglion cells when exposed to external barium. Our observations at the younger ages are consistent with the conclusion of Redman and colleagues that the blocked conductances reside in the more distant compartments of the dendrites.

The interpretation of the present data set is not trivial. During development, the size of the motoneuron (especially between weeks 2 and 3), the number of voltage-dependent channels, and the distribution of these channels are all changing. The changes can interact to produce complex effects on \( R_n \) and \( \tau_0 \). This problem is most evident in the summary of data presented in Fig. 7. One major conclusion from these data are that cesium-sensitive channels constitute a major component of the reduction in \( R_n \) occurring between 1 and 2 wk after birth. However, the doubling of membrane surface area between 2 and 3 postnatal weeks (Núñez-Abades and Cameron 1995) should produce a 50% reduction in \( R_n \), assuming that all other membrane characteristics remain the same. This is not the case; in fact, the \( R_n \) is approximately the same at both ages (Núñez-Abades et al. 1993). One possible explanation as to why the anticipated decrease in \( R_n \) did not occur is a simultaneous increase in specific membrane resistivity. However, assuming that the measurement of the slowest time constant approximates the specific resistivity, there is no evidence for such an increase. The situation becomes more complicated when it becomes apparent that the distribution of the voltage-dependent channels may be changing (Fig. 11). In the oldest animals, these data points are closer to the unity line than at earlier stages of development, suggesting a more uniform distribution (Table 4). It is not clear, without extensive modeling, how this redistribution of channels might impact the measurements of \( R_n \) and \( \tau_0 \) in the present study.

Given the potential limits of the analyses, there is one interesting interpretation of our data. Based on the greater Cs\(^+\) sensitivity of the low resistance motoneurons, we propose that there is a differential proliferation of Cs\(^+\)-sensitive potassium channels in the motoneurons innervating fast- versus those innervating slow-twitch muscle fiber types. This differential in Cs\(^+\)-sensitive channels might be more easily demonstrable in a muscle with a more diverse fiber type composition than GG muscle like the diaphragm (Brozanski et al. 1993). This selective proliferation of channels roughly coincides with the elimination of polyneuronal innervation (Redfern 1970) and may result from an induction signal derived from the maturing muscle.

**Barium-sensitive conductances**

When iontophoresed onto a cat lumbosacral motoneuron, extracellular barium depressed the delayed rectifier and leak conductance (Schwindt and Crill 1980b). In neostriatal cells, barium-sensitive conductances were primarily associated with the linear conductances making up the somatic shunt while cesium-sensitive conductances preferentially acted on the inward rectifier (Reyes et al. 1998). We applied extracellular barium onto developing motoneurons in vitro to assess what role that the leak conductance, in particular, played in producing the decrease in membrane resistance during postnatal development. If part of the reduced resistance of the second week of development was a result of a proliferation of the leak conductance, then we would expect that external barium would produce a larger increase in the resistance of motoneurons at 13–15 days. Statistical analyses failed to demonstrate any difference between age groups in absolute or percent change of either membrane property. However, like the response to intracellular cesium, there was a larger percent increase in \( \tau_0 \) associated with external \( Ba^{2+} \) at P13–15 than that noted for \( R_n \).

There are three conclusions about barium-sensitive channels that paralleled those for cesium-sensitive channels. First, there was a proportionately larger increase in \( \tau_0 \) as compared with \( R_n \), suggesting a distal dendritic location for these conductances. Second, the ratio of percent change in \( \tau_0 \) to \( R_n \) approached 1.0 with increasing age, suggesting that the distribution of barium-sensitive channels was more uniform in the older animals. Third, there was a negative correlation between control \( R_n \) and current injected to maintain membrane potential constant. The block of the barium-sensitive leak conductance generated a larger depolarizing shift in cells with low \( R_n \). Thus GG motoneurons with a low resistance were found to have more \( Ba^{2+} \)- and Cs\(^+\)-sensitive leak conductances than cells with high resistance. We would propose that the proliferation of leak channels in low resistance cells may be responsible, in part, for the lower specific membrane resistance found at P13–15.

A recent study (Talley et al. 2000) presented anatomical evidence in support of a differential distribution of leak channels in brain stem and spinal cord motor nuclei of the adult rat including the hypoglossal nucleus. These authors measured the relative expression levels of TASK-1, a two-pore domain potassium channel possessing properties that fit the behavior of a leak channel. They used in situ hybridization to demonstrate that TASK-1 mRNA was localized to the soma and proximal dendrites of hypoglossal motoneurons. These data revealed that some hypoglossal motoneurons were more heavily labeled than others, a pattern evident in other motor nuclei as well. Finally, these authors noted a lower density of labeling in brain stem and spinal cord motoneurons of younger animals (P7) than found in the adult. Thus this report supports the ideas that leak channels are proliferating with age and that some adult motoneurons (presumptive low resistance) have more expression of these channels than others (presumptive high resistance).

The barium-sensitive component of membrane resistivity is modulated by thyrotropin-releasing hormone (Bayliss et al. 1993, 1997), norepinephrine (Parkis et al. 1995), and serotonin (Hsiao et al. 1997) through a G-protein–coupled mechanism (Bayliss et al. 1997). These studies suggest that the modulation of the leak channel is important for altering the excitability of brain stem motoneurons and lowering the threshold for their repetitive firing. If all leak channels are under the regulation of the neuromodulators, then low resistance cells, with their greater number of leak channels, would show greater changes in excitability as compared with high resistance cells. This hypothesis requires more rigorous testing.

Based on the data from these two companion papers, we would propose that there is both a synaptically mediated and a nonsynaptically mediated component contributing to the changes occurring in membrane resistance during postnatal development. A major part of this nonsynaptically mediated...
conductance is mediated by potassium currents. Although all the specific potassium channels involved are not known, it is clear that there is a substantial contribution of the cesium-sensitive conductances to the developmental process of motoneuron differentiation. Future studies will be necessary to determine 1) what signals identify a motoneuron as predestined to become a low or high resistance cell, 2) what factors direct the formation of synaptic connections to specific regions of the motoneuron membrane, and 3) what processes determine the number of potassium channels will be expressed, where the channels will be inserted into the membrane, and how this distribution may be altered during development.

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