

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 genioglossal (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 (Mg^{2+} ; 6 mM) perfusate was used to block calcium-mediated synaptic release while intracellular or extracellular cesium (Cs^+) and/or extracellular tetraethylammonium (TEA) or barium (Ba^{2+}) were used to block potassium conductances. In all cases, the addition of TEA to the high Mg^{2+} perfusate generated a larger increase in both input resistance (R_n) and the first membrane time constant (τ_0) than did high Mg^{2+} alone indicating a substantial nonsynaptic contribution to input resistance. With intracellular injection of Cs^+ , GG motoneurons with lower resistance ($<40 M\Omega$), on the average, showed a larger percent increase in R_n than cells with higher resistance ($>40 M\Omega$). There was also a significant increase in the effect of internal Cs^+ on R_n and τ_0 with age. The largest percent increase (67%) in the τ_0 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 R_n and τ_0) beyond those blocked by the TEA perfusate. Substitution of external calcium with 2 mM barium chloride produced a significant increase in both R_n and τ_0 at all ages studied. The addition of either intracellular Cs^+ or extracellular Ba^{2+} 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 R_n of the cell at most ages. Thus low resistance cells had, on the average, more Cs^+ - and Ba^{2+} -sensitive channels than their high resistance counterparts. There was also a disproportionately larger percent increase in τ_0 as compared with R_n for both internal Cs^+ and external Ba^{2+} . 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 R_n 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 leak 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 tetraethyl ammonium (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

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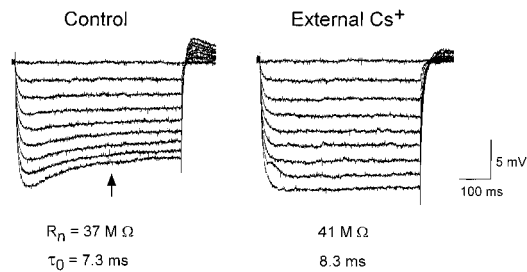


FIG. 1. Influence of extracellular cesium on the inward rectifying ("sag") current observed in *postnatal* (*P*) day 22 genioglossal motoneuron. Membrane potential response to a series of 8 hyperpolarizing current pulses (-0.05 to -0.4 nA, 500 ms duration) before (control) and after bathing with 5 mM cesium. In this and subsequent figures, the 1st trace is the membrane potential without injected current, each trace represents the average of 6 sweeps, and input resistance was calculated from a linear regression of V - I plots generated from the peak membrane responses to the hyperpolarizing current injections. Arrow in *left panel* denotes sag from a peak voltage. For this cell, the reduction of the sag current was accompanied by a small increase in the measured values of input resistance and 1st membrane time constant. This increase was due to the block of currents other than the sag. External cesium failed to produce a similar reduction in the inward current for many cells while generating a substantial increase in R_n and τ_0 (see Fig. 8).

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 (Ianssek 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 (*P*5–7, *P*13–15, and *P*19–24) 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 ($4^\circ C$) sucrose-artificial cerebral spinal fluid (ACSF) and quickly decapitated. The brain stem was removed and sectioned at $300 \mu m$ 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 ($21 \pm 1^\circ C$).

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_n), first membrane time constant (τ_0), rheobase (I_{rh}), and repetitive firing was measured in normal ACSF. The validity of using a long, hyperpolarizing current pulse (500 ms duration) to measure R_n and τ_0 in cells exhibiting an inward rectifying or "sag" current has been discussed in the first paper of this series. In a few cells, the sag 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_n and τ_0 than the measurements potentially contaminated by the sag current.

After making the control measurements in the first protocol, the

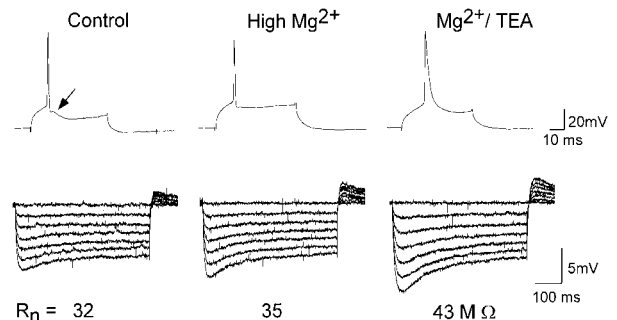


FIG. 2. Effect of high magnesium (Mg^{2+}) and tetraethylammonium (TEA) on action potential and input resistance (R_n). Recording from a *P*20 genioglossal (GG) motoneuron. *Top*: action potentials evoked by suprathreshold depolarizing current steps in control media, 6 mM Mg^{2+} (to block calcium-mediated synaptic transmission), and 6 mM Mg^{2+} with 20 mM TEA (to block calcium-independent potassium conductances). Bath application of Mg^{2+} abolished the afterdepolarization (ADP, arrow) and subsequent reduced the medium afterhyperpolarization (AHP) seen in the control action potential. There was a small increase in threshold for the generation of the action potential after the application of Mg^{2+} (0.4 nA, control rheobase vs. 0.5 nA for Mg^{2+}). The action potential was broadened by the addition of TEA. *Bottom*: membrane responses to a series of 6 hyperpolarizing current steps. High Mg^{2+} increased R_n slightly while the addition of TEA produced an even larger increase in R_n . Neither treatment affected the inward rectification (sag current) produced by hyperpolarizing current steps. Membrane potential -57 mV. In this figure and several others presented, there is a high-frequency noise evident in the voltage traces. This artifact was produced by the peristaltic pump used to ensure a constant flow through the recording chamber and does not impact the accuracy of the voltage measurement.

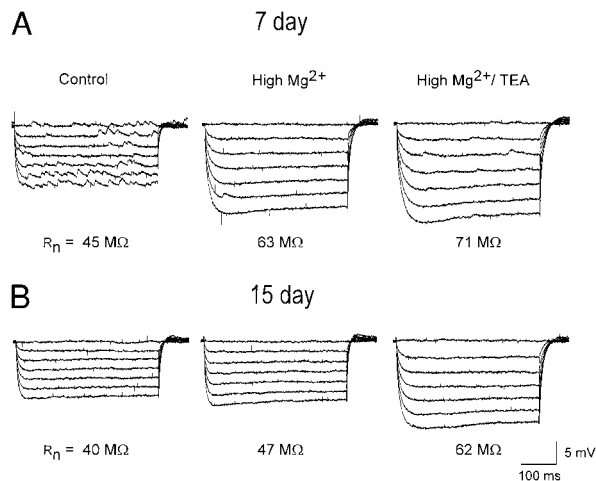


FIG. 3. Effect of external Mg^{2+} and TEA on input resistance of GG motoneurons from a P7 and P15 rat. These recordings are of membrane responses to a series of hyperpolarizing current steps in control, high (6 mM) Mg^{2+} , and high Mg^{2+} with 20 mM TEA. The inward rectification in both the P7 and P15 cells is smaller than that found in the P20 motoneuron (Fig. 2). The increase in input R_n 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_n and τ_0 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 μ M 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_n and τ_0 are presented in the text and tables as means \pm 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 action 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+} plus 20 mM TEA. The bottom panel shows the mem-

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

Postnatal Age, days	<i>n</i>	R_n , MΩ			<i>n</i>	τ_0 , ms		
		Control	High Mg^{2+}	Mg^{2+} /TEA		Control	High Mg^{2+}	Mg^{2+} /TEA
6–7	8	59.3 \pm 10.0	69.2 \pm 11.5*	90.4 \pm 14.3†	7	10.1 \pm 1.0	12.8 \pm 1.6*	19.7 \pm 4.5*
13–15	14	43.9 \pm 3.2	50.7 \pm 3.7‡	71.3 \pm 4.5‡	9	8.1 \pm 0.6	9.0 \pm 0.7	13.4 \pm 0.6‡
19–24	16	40.4 \pm 2.8	43.3 \pm 2.9*	60.9 \pm 4.3‡	10	7.4 \pm 0.8	7.7 \pm 0.6	12.9 \pm 1.4‡

Values are expressed as means \pm SE; *n* is total number of samples. R_n , input resistance; τ_0 , 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. * $P < 0.05$. † $P < 0.01$. ‡ $P < 0.001$.

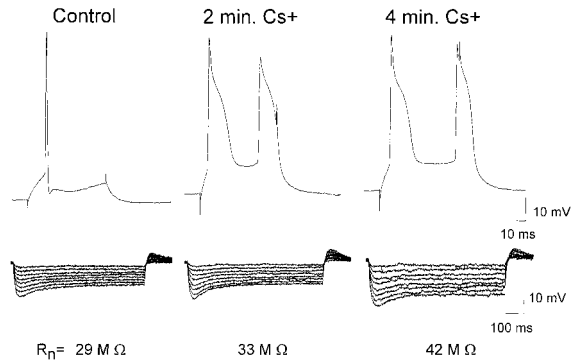


FIG. 4. Effects of intracellular cesium (Cs^+) injection on the action potential and R_n of a GG motoneuron from a P15 rat. *Top*: 3 records of the action potential. *Left*: spike recorded just after impalement (control); *middle*: after 2 min of injection (0.8 nA, 150 ms, 3.3 Hz); and *right*: after 4 min of Cs^+ injection. Note the increase in the half-width of the action potential from <2 to >10 ms and that it changed little after 2 min of injection. *Bottom*: the membrane response to 8 hyperpolarizing current steps of 0.5-nA increment. Below the waveforms is the input resistance calculated from the peak voltage. Membrane potential was held at its resting value of -71 mV during all tests with a bias current up to 0.48 nA. There was a 46% increase in the R_n of this cell.

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 high Mg^{2+} solution. 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

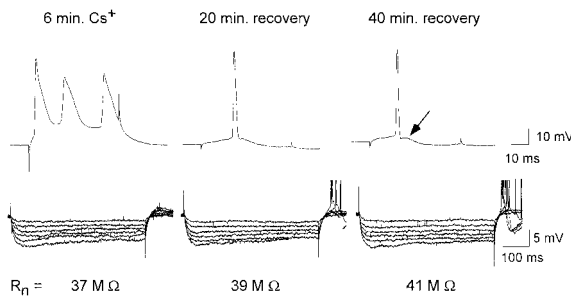


FIG. 5. Recovery of action potential after cessation of Cs^+ injection of a GG motoneuron from a P22 animal. *Top*: the change in action potential at the end of a 6-min injection and again after 20 and 40 min of recovery. The action potential is fully recovered by 40 min (ADP indicated by arrow) appearing 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.

TABLE 2. Effect of internal cesium (Cs^+) on R_n and τ_0 of developing GG motoneurons

Postnatal Age, days	n	R_n , MΩ		n	τ_0 , ms	
		Control	Cs^+		Control	Cs^+
5–6	17	55.9 ± 3.1	68.4 ± 4.6*	16	10.1 ± 0.9	13.1 ± 1.1*
13–15	37	39.5 ± 2.7	51.6 ± 2.7*	18	8.4 ± 0.8	14.0 ± 1.6*
19–23	19	37.1 ± 3.0	53.3 ± 3.5*	12	8.6 ± 0.8	12.3 ± 0.9*

Values are means ± SE; n is total number of samples. For age-dependent changes associated with internal cesium, see Fig. 7. * $P < 0.001$.

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 τ_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 τ_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 τ_0 at all ages. Over the developmental period studied, the absolute magnitudes and relative percents of change in R_n and τ_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 τ_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

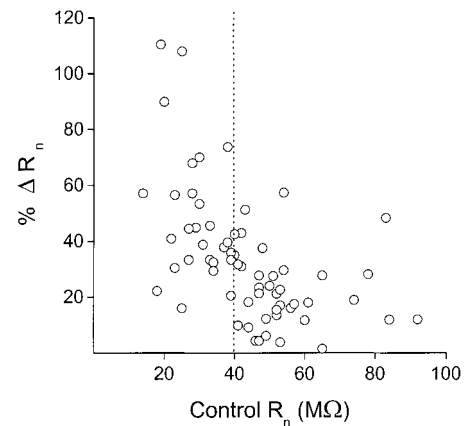


FIG. 6. Effect of intracellular Cs^+ on input resistance. Scatter plot of pooled data ($n = 65$) illustrating the effect of 2–4 min of Cs^+ injection on the percent increase in R_n as a function of control R_n . There was negative linear correlation ($R = -0.58$, $P < 0.001$). The motoneurons are arbitrarily 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.

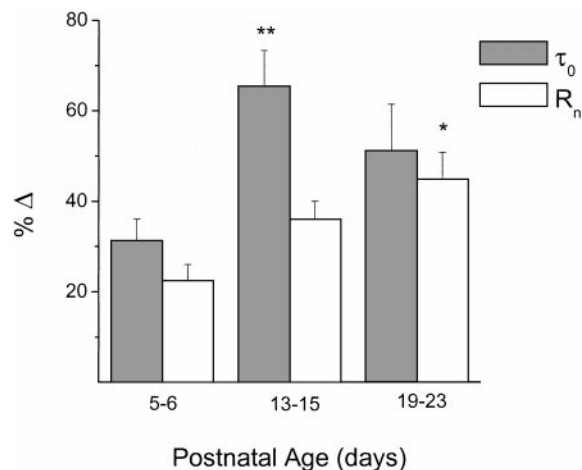


FIG. 7. Effect of intracellular Cs⁺ on the relative change in input resistance and time constant. Group data showing the changes in R_n and τ₀ produced by Cs⁺ injection as a function of postnatal age (see Table 2 for sample sizes). The effect of intracellular Cs⁺ on R_n increased with each succeeding week of postnatal development with the percent change at P19–23 becoming statistically greater than that at P5–6 ($P < 0.05$, *). In contrast, the effect of intracellular Cs⁺ on τ₀ increased significantly ($P < 0.01$, **) between P5–7 and P13–15. The effect of Cs⁺ on τ₀ was less at P20–25 and was not statistically different from that at P5–6. The disproportionate change in τ₀ relative to R_n at P13–15 will be examined further in Fig. 11.

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 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.

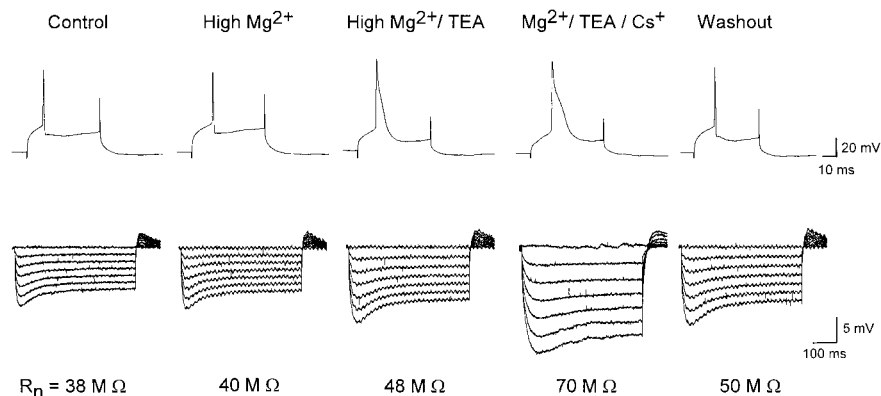


FIG. 8. Combined effects of high Mg²⁺, TEA, and extracellular Cs⁺ on the action potential and input resistance in a 24-day-old GG motoneuron. Data from an experiment on 1 GG motoneuron to illustrate the combined effects of 6 mM Mg²⁺, 20 mM TEA, and 5 mM external Cs⁺. *Top*: series of action potentials in response to depolarizing current pulses. *Bottom*: membrane responses to a series of 6 hyperpolarizing current pulses. Bath application of Mg²⁺ abolished the ADP while only slightly increasing R_n and the rheobase. Addition of TEA to the bath solution broadened the action potential and increased R_n. Neither Mg²⁺ nor TEA affected the inward rectification. The inclusion of 5 mM Cs⁺ in the bath containing Mg²⁺ and TEA further broadened the action potential and substantially increased R_n. External Cs⁺ also reduced the magnitude of the inward rectification. After 60 min of wash out, the action potential narrowed and the ADP returned; however, R_n failed to return to the control level. Membrane potential, -65 mV.

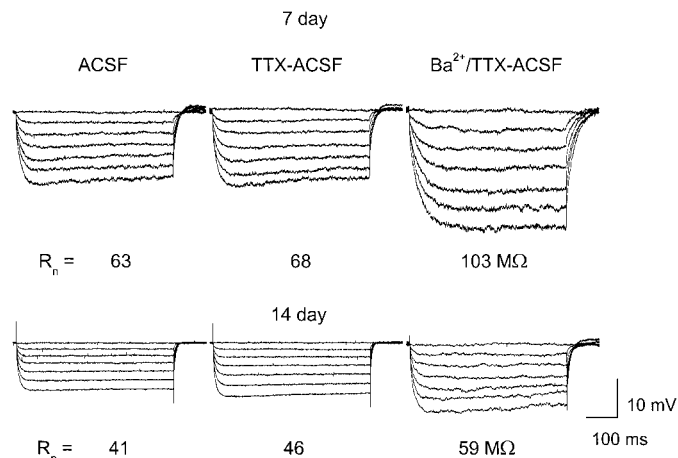


FIG. 9. Membrane responses of 2 GG motoneurons (P7 and P14) to a series of 6 hyperpolarizing current pulses applied before (1st column) and after the addition of 1 μM TTX (2nd column) and TTX plus 2 mM BaCl (3rd column) to the artificial cerebrospinal fluid (ACSF). In most cases, the R_n rose in response to block of the evoked synaptic release with TTX. In all cases, the addition of Ba²⁺ resulted in a further large increase in R_n. It was necessary to block sodium spiking with TTX to average membrane responses without spontaneous spiking.

When individual age groups were analyzed, intracellular cesium produced a significant increase in both R_n and τ₀ 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

TABLE 3. Effects of tetrodotoxin (TTX) and external barium (Ba^{2+}) on R_n and τ_0 of developing GG motoneurons

Postnatal Age, days	R_n , M Ω			τ_0 , ms		
	n	TTX		n	TTX	
		Control	Ba^{2+} -TTX		Control	Ba^{2+} -TTX
5-6	7	48.3 \pm 3.6	97.5 \pm 8.3*	7	10.7 \pm 4.4	22.8 \pm 1.8*
13-15	17	38.4 \pm 3.4	69.1 \pm 5.3*	15	7.9 \pm 2.0	20.4 \pm 5.3*
19-23	16	35.6 \pm 2.3	66.3 \pm 5.7*	16	9.0 \pm 4.1	19.1 \pm 7.6*

Values are means \pm 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^{2+} -sensitive component of R_n in the preceding study (data not shown).

The magnitude of the Cs^+ effect on both R_n and τ_0 varied with postnatal age. Figure 7 summarizes the effects of internal Cs^+ on R_n and τ_0 for the three age groups studied. The effect of Cs^+ injection on R_n increased with each succeeding week, while the effect on τ_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^+ injection and τ_m can be approximated by τ_0 , then the changes in τ_0 reflect changes in the specific membrane resistance (R_m , where $\tau_m = R_m \times C_m$). Thus the decreased resistance in GG motoneurons at 2 wk of age is due, in large part, to the proliferation of Cs^+ -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 (τ_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 τ_0 at 3 wk was reduced. These development trends were also evident in the absolute changes in R_n and τ_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^+ to determine what, if any, increases may be seen in R_n and τ_0 above that generated by external TEA. One effect of external Cs^+ was to reduce the inward rectification in some cells (Fig. 1). In the P24 GG motoneuron shown in Fig. 8, external Cs^+ greatly increased both R_n and τ_0 above that achieved with external TEA. In the experimental protocol, the high Mg^{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^+ , 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^+ was 61.4 \pm 18.9% (mean \pm SE) and 71.5 \pm 25.3% for R_n and τ_0 , respectively. Similar values of 61.0 \pm 29.3% and 69.0 \pm 7.0% were measured for R_n and τ_0 for two cells at P5-7. This large increase in R_n and τ_0 was observed in GG motoneurons irrespective of whether the motoneurons exhibited an inward current or not.

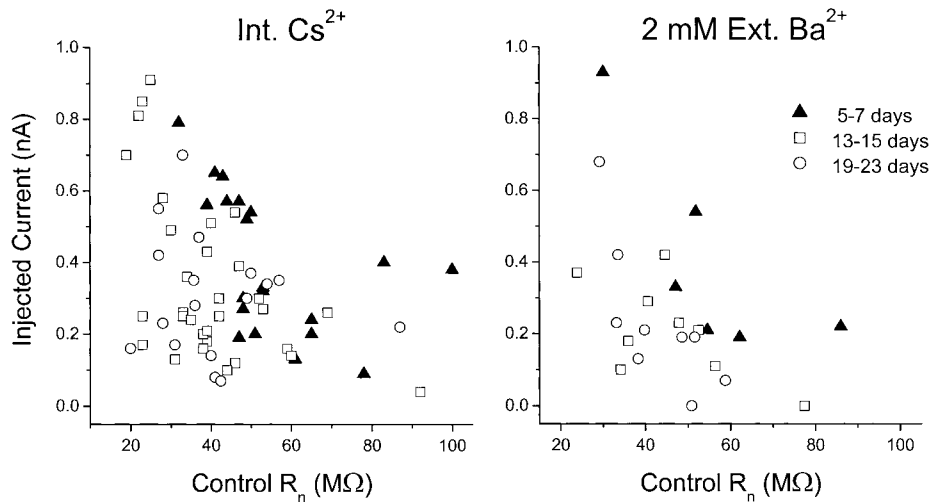


FIG. 10. The amount of injected current required to maintain the membrane potential at its initial value following blockade with internal cesium (left) and external barium (right) as a function of control input resistance. There was a negative correlation between current and resistance for the P5-7 ($P < 0.05$), P13-15 ($P < 0.01$) and the pooled data ($P < 0.005$) for the cesium treatment. There was only 1 negative correlation at P19-23 ($P < 0.02$) for the barium treatment, although the negative trend was apparent for P5-6. Some ages (P19-23 for cesium and P13-15 for barium) exhibited a more variable relationship potentially indicating a possible transition period. In general, these combined data suggest that low resistance cells have more cesium- and barium-sensitive channels than their high resistance counterparts. Equations of least-squares fit: cesium: P5-7: $y = 0.73x - 0.006$, $r = -0.58$, $n = 20$; P13-15: $y = 0.66x - 0.008$, $r = -0.54$; pooled: $y = 0.55x - 0.006$, $r = -0.36$, $n = 70$; barium: P19-23: $y = 0.88 - 0.015x$, $r = -0.75$, $n = 9$.

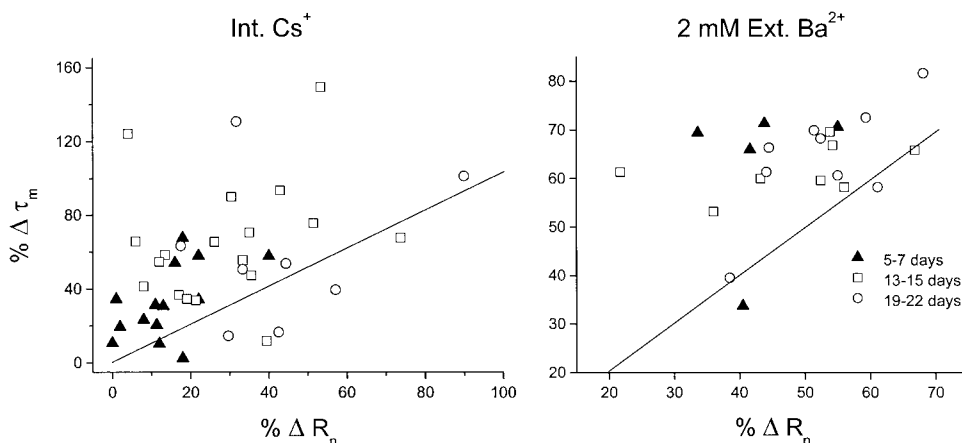


FIG. 11. Comparison of the percent change in τ_0 as a function of R_n for cells injected with intracellular cesium (left) and cells exposed to extracellular barium (right). A line with slope of 1 has been added to show how many cells exhibited a disproportionately larger (above line) increase in τ_0 as compared with R_n . According to Redman and colleagues (Redman et al. 1987), this kind of differential enhancement can only be simulated if the blocked conductances are located on the distal dendrites. Most cesium and barium treated cells lie above the unity line indicating a predominant dendritic localization of the blocked potassium conductances.

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 τ_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 τ_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 internal 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 ($P < 0.02$) 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 τ_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 τ_0 in response to the actions of barium on resting conductance. When expressed as a ratio of change in τ_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 τ_0 than R_n and a ratio < 1.0 . Figure 11 plots the percent change in τ_0 as a function of the percent change in R_n . Points lying above a unity line indicate a larger change in τ_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 τ_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 τ_0 of developing GG motoneurons

Postnatal Age, days	Cs^+		Ba^{2+}	
	<i>n</i>	Ratio	<i>n</i>	Ratio
5-7	7	5.2 ± 8.7	5	1.5 ± 0.5
13-15	18	4.4 ± 7.1	8	1.4 ± 0.6
19-22	8	1.6 ± 1.4	9	1.2 ± 0.2

Values are means \pm 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 (Núñez-Abades et al. 1993) and anatomy (Núñez-Abades and Cameron 1995; Núñez-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 τ_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 τ_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 τ_0 . In general, the cells having lower input resistance tend to exhibit more Cs^+ - and Ba^{2+} -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 Mg^{2+} 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 (Safonov 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 Ba^{2+} 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 effected 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 Mg^{2+} 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 τ_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 (Puil 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 τ_0 would be equal if the

channels were uniformly spread over the motoneuron membrane. The larger change in τ_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 τ_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 τ_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 de-

velopment. 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 τ_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 τ_0 as compared with R_n , suggesting a distal dendritic location for these conductances. Second, the ratio of percent change in τ_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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