Time-Dependent Changes in Input Resistance of Rat Hypoglossal Motoneurons Associated with Whole-Cell Recording

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The effect of cellular dialysis associated with whole-cell recording was studied in 24 developing hypoglossal motoneurons in a rat brainstem slice preparation. In all cases, establishing whole-cell continuity with the electrode solution resulted in an increase in the input resistance measured in current clamp. The mean magnitude of this increase was 39.7% and the time course of the maximum effect was quite variable. There was no correlation found between the time to maximum effect and the magnitude of the increase in resistance. These data indicate that the passive membrane properties are not constant during whole-cell recording in mammalian motoneurons.

INTRODUCTION

The measurements of passive membrane properties, input resistance (R\(_i\)), and membrane time constant have been complicated by a large somatic shunt attributed in part to impalement by the sharp electrode (Rall 1993). This shunt can result in significant underestimates of the passive membrane properties (Durand 1984). Although the principal source of this shunt remains controversial (Campbell and Rose 1997), it has been suggested that whole-cell recording might circumvent the impalement-induced conductances (Blanton et al. 1989; Edwards et al. 1989). However, with whole-cell configuration there is a different set of problems associated with the dialysis of the intracellular compartment. One report in dentate granule cells (Staley et al. 1992) reported that there were no time-dependent changes in either R\(_i\) or R\(_m\) associated with the whole-cell recording configuration whereas other studies presume a significant washout effect (Spruston and Johnston 1992). Although whole-cell configuration is used extensively for studies of synaptic currents and passive properties, little information is available regarding the magnitude and time course of the washout for mammalian central neurons. This study provides the first evidence for a widely varying time course and magnitude of the washout effect in mammalian motoneurons. These changes in R\(_i\) will complicate the interpretation of synaptic currents measured at varying times after establishing whole-cell configuration (e.g., Singer and Berger 1999).

METHODS

Transverse slices were prepared from brainstems obtained from rats that were between postnatal day 11 and 15 in age. Hypoglossal motoneurons were retrogradely labeled by injecting rhodamine dextran, under ether-induced anesthesia, into the genioglossus muscle of the tongue two days before the experiment. On the day of the experiment, animals were initially induced with ether and deeply anesthetized with 1–2.5% isoflurane. Animals were transcardially perfused with a cold sucrose solution containing (in mM) 215 sucrose, 0.5 CaCl\(_2\), 2 KCl, 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 10 glucose, and 4 MgSO\(_4\) (pH = 7.39, 313 mOsm, bubbled with 97.5%O\(_2\)-2.5%CO\(_2\)). After 1 min of perfusion the rat was decapitated rapidly and its brainstem was removed and sliced into 300-μm coronal slices using a sapphire blade (DDK) mounted on a Leica VT1000S vibratome. The slicing chamber was cooled to −1°C and contained the sucrose solution described above. Slices were transferred to a holding chamber containing a low-calcium artificial cerebrospinal fluid (ACSF) composed of (in mM) 126 NaCl, 2 KCl, 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 1 MgCl\(_2\), 0.5 CaCl\(_2\), and 10 glucose (pH = 7.35, 300 mOsm, 30°C). After 40 min the slices were transferred to a second holding chamber that contained a normal ACSF (2 mM CaCl\(_2\)) at room temperature.

In the recording chamber, slices were continuously perfused with normal ACSF at room temperature (21 ± 1°C). Cells were visualized using either epifluorescence illumination or differential interference contrast (DIC) infrared video microscopy (Stuart et al. 1993). Conventional whole-cell patch-clamp techniques (Hamill et al. 1981) were used in current-clamp mode to assess the input resistance of hypoglossal motoneurons. Patch pipettes with a tip resistance between 5 and 12 MΩ were filled with a solution containing (in mM) 140 KMeSO\(_4\), 10 KCl, 5 NaCl, 2 MgCl\(_2\), 1 CaCl\(_2\), 10 HEPES, 10 BAPTA, and 2 ATP (pH = 7.35, 300 mOsm). KMeSO\(_4\) was chosen instead of the more commonly used K-gluconate because of reports that the former preserves neuronal excitability much more effectively than the latter (Velumian et al. 1997; Zhang et al. 1994). Current-clamp recordings were made with an Axopatch 1-D patch-clamp amplifier. The data were low-pass filtered at rates between 1 and 2 kHz and digitized at rates between 2.5 and 5 kHz.

Immediately after the whole-cell configuration was attained, the resting membrane potential was recorded. The value of the resting potential was monitored regularly throughout the session. All statistics are expressed as the mean ± SD and significance was assessed using the Student’s t-test.

RESULTS

This study is based on 24 developing hypoglossal motoneurons. These neurons had stable resting membrane potentials for at least 15 min and elicited a sustained discharge of action potentials in response to maintained current depolarization. The mean resting potential of these neurons was −64.4 ± 4.7 mV and the mean maximum firing frequency evoked by maintained depolarization was 37.6 ± 14.8 Hz. If a neuron was unable to elicit such sustained firing and/or its resting membrane potential became >5 mV more depolarized from its initial value, the recording was terminated.
Figure 1 shows typical recordings obtained from a hypoglossal motoneuron 0, 15, and 33 min after the whole-cell configuration had been obtained. The response of the neuron at the three times to a 300 pA maintained depolarization (1 s duration) is shown at the top of each panel. At each time the neuron responded with a sustained discharge of action potentials at a frequency of \(40\) Hz. The bottom panel illustrates responses to a series of hyperpolarizing current steps recorded immediately after the traces above. Each hyperpolarizing step (390 ms duration) was made from the resting membrane potential and its magnitude increased sequentially from 0 to \(-300\) pA in steps of \(-50\) pA. The “sag,” evident in the response to the larger hyperpolarizations, reflects activation of an inwardly rectifying cation channel and was observed in \(23\) of \(24\) neurons analyzed. The magnitude of the sag, determined by subtracting the peak potential from the membrane potential at 385 ms for the maximum step used, was \(2.3 \pm 2.6\) mV, which represents only a 10% decrease in resistance across the cells sampled.

\(R_n\) was determined from the slope of a linear fit of the relationship between the peak change in membrane potential (\(\Delta V_m\)) and the magnitude of the injected current. The top panel of Fig. 2 shows such relationships for the recordings illustrated in Fig. 1. The bottom panel of Fig. 2 shows how the \(R_n\) of the neuron illustrated in Fig. 1 changes with time after the whole-cell configuration had been obtained. Initially, the \(R_n\) was \(73\) M\(\Omega\) and increased gradually, reached a maximum of \(101\) M\(\Omega\) at 33 min, and remained at this value for \(-5\) min before it decreased rapidly. This decrease can be attributed to seal breakdown and rapidly resulted in the cell losing its ability to generate action potentials (not shown).

A time dependent increase in \(R_n\) was observed in all \(24\) of the neurons studied. The mean initial \(R_n\) was \(119.5 \pm 71.3\) M\(\Omega\) and increased in a time-dependent manner to a mean maximum value of \(162.8 \pm 103.8\) M\(\Omega\). The paired Student’s t-test revealed that this increase was highly significant (\(P < 0.0001\)).

The time course over which the increase in \(R_n\) occurred varied greatly between cells. Figure 3A shows the time-dependent increase in input resistance, normalized to the maximum value attained, plotted for two further neurons. As indicated by these representative cells, a number of neurons reached their maximum input resistance within 10 min whereas others took much longer. There was no relationship between the size of the change in \(R_n\) and the rate at which this change occurred. Table 1 summarizes the responses of the 24 cells recorded based on the time it took for the maximum \(R_n\) to be attained. Although some neurons reached a maximum in \(<10\) min, it should be noted that the recordings lasted much longer and were terminated only when the resting membrane potential changed by \(\pm 5\) mV or the cell no longer generated sustained discharge. Table 1 demonstrates that the rate of increase in input resistance was unrelated to either the resting membrane potential or maximum firing frequency observed in each group of neurons.

**DISCUSSION**

The introduction of patch-clamp recording has substantially improved our ability to resolve small synaptic currents (Ed-
wards 1995; Edwards et al. 1989) in the neurons of the mammalian CNS. One consequence of creating a continuity between the cytoplasm and the electrode solution is the movement of substances from the smaller volume of the cytoplasm into the larger volume of the electrode that are important for maintaining the resting membrane state. G-protein-regulated conductances in hypoglossal motoneurons are under the control of various neurotransmitters (Bayliss et al. 1997) and these currents would be altered by the dialysis. The values of membrane properties like $R_n$ and membrane time constant in the hippocampus have been found to be greater using either whole-cell (Staley et al. 1992) or perforated-patch (Spruston et al. 1994) than those found using the sharp electrode. The results described above agree with such findings. In identical preparations, $R_n$ determined using sharp electrode recording was $47 \pm 19 \, \text{M} \Omega$ (Núñez-Abades et al. 1993) as compared with $120 \pm 71 \, \text{M} \Omega$ that is reported here. Such an increase is in line with the approximate two- to fourfold increase found in hippocampal CA1 and CA3 neurons (Spruston et al. 1994).

In contrast to our study, significant time-dependent changes in $R_n$ were not observed in dentate granule cells of the rat hippocampus (Staley et al. 1992). However, closer inspection of their Fig. 6 reveals evidence for such an increase in their data. No time-dependent changes in $R_n$ were reported in a recent study using whole-cell recording in rat spinal ventral horn neurons (Thurbon et al. 1998). On the basis of the 4 of 10 neurons studied, these authors concluded “the results resolve the issue of a somatic shunt conductance for motoneurons, relegating it to a microelectrode impalement artifact.” Without electrode damage, there would be no somatic shunt and the motoneuron could be modeled using uniform resistivity. However, the majority of the cells from this study did require a somatic shunt to fit their model and these data are more consistent with a role for potassium conductances in generating the somatic shunt as proposed for cat cervical motoneurons studied in vivo (Campbell and Rose 1997). It is some of these conductances modulated by second messenger systems that could be the principal target of the washout phenomenon.

These results indicate that conventional whole-cell recording is not the best choice for experiments where $R_n$ is being measured. The rise in resistance is variable in both its absolute magnitude and its time course. One explanation for this increase in $R_n$ is that the membrane is slowly rescaling under the patch electrode. In this scenario, the magnitude of the instantaneous change in membrane potential would become much larger as the contribution of the RC time-constant associated with the electrode begins to overwhelm the time-constant associated with the membrane. As clearly indicated by Fig. 1, this was not found to be the case. Moreover, a preliminary report (Robinson and Cameron 1998) suggests that such increases in input resistance can apparently be avoided by using perforated-patch. In such recordings no rise in input resistance.

![Input Resistance Graph](http://example.com/image.png)
FIG. 3. Variation in time course of increase in $R_n$ and results obtained with perforated-patch recordings. A: 2 representative neurons selected to illustrate different time courses (0–10 min, >21 min) over which increase occurred. $R_n$ has been normalized to a maximum equal to 1.0. B: data obtained while using the perforated-patch variation of patch-clamp technique. Top: spiking patterns obtained to a depolarizing current injection at $t = 0$ and 13 min after whole-cell configuration was obtained. Middle: responses to hyperpolarizing currents at $t = 0$ and 13 min. Bottom: plots $R_n$, normalized to its maximum value, against time. In 4 neurons recorded using this technique, no increase in $R_n$ was ever observed.
TABLE 1.  Change in input resistance as a function of the time to maximum washout effect

<table>
<thead>
<tr>
<th>Time to Maximum $R_m$, min</th>
<th>N</th>
<th>$% \Delta R_m$</th>
<th>RMP, mV</th>
<th>Maximum Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>6</td>
<td>28.2 ± 10.4</td>
<td>−62.0 ± 5.4</td>
<td>33.2 ± 18.7</td>
</tr>
<tr>
<td>11–20</td>
<td>7</td>
<td>55.8 ± 38.8</td>
<td>−64.3 ± 3.7</td>
<td>45.0 ± 17.1</td>
</tr>
<tr>
<td>21 and above</td>
<td>11</td>
<td>35.8 ± 17.2</td>
<td>−66.0 ± 4.8</td>
<td>35.2 ± 9.7</td>
</tr>
</tbody>
</table>

Values are mean ± SD. $R_m$, input resistance; N, sample size; RMP, resting membrane potential.

was ever observed ($n = 4$). Conversely, it remained at a constant value ($n = 1$) or more typically ($n = 3$; Fig. 3B) decreased slowly over time, presumably a result of the incorporation of more Nystatin pores into the membrane patch.

There are increasing numbers of reports that examine membrane properties and/or synaptic currents using conventional whole-cell recording and it should be cautioned that these results might be very difficult to interpret if the neuronal populations studied exhibit a similar response to cellular dialysis.

This research was supported by National Institute of Child Health and Human Development Grant HD-22703 and by SIDS Foundation Megan's Run, Wilsonville, OR.

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Received 27 July 1999; accepted in final form 20 January 2000.

REFERENCES


