Membrane Properties Related to the Firing Behavior of Zebrafish Motoneurons

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Buss, Robert R., Charles W. Bourque, and Pierre Drapeau. Membrane properties related to the firing behavior of zebrafish motoneurons. J Neurophysiol 89: 657–664, 2003; 10.1152/jn.00324.2002. The physiological and pharmacological properties of the motoneuron membrane and action potential were investigated in larval zebrafish using whole cell patch current-clamp recording techniques. Action potentials were eliminated in tetrodotoxin, repolarized by tetraethylammonium (TEA) and 3,4-diaminopyridine (3,4-AP)-sensitive potassium conductances, and had a cobalt-sensitive, high-threshold calcium component. Depolarizing current injection evoked a brief (approximately 10–30 ms) burst of action potentials that was terminated by strong, outwardly rectifying voltage-activated potassium and calcium-dependent conductances. In the presence of intracellular cesium ions, a prolonged plateau potential often followed brief depolarizations. During larval development (hatching to free-swimming), the resting membrane conductance increased in a population of motoneurons, which tended to reduce the apparent outward rectification of the membrane. The conductances contributing to action potential burst termination are hypothesized to play a role in patterning the synaptically driven motoneuron output in these rapidly swimming fish.

INTRODUCTION

Larval zebrafish swim with rapid undulating body movements (Budick and O’Malley 2000; Buss and Drapeau 2001; Fuiman and Webb 1988; Saint-Amant and Drapeau 1998) powered by the contraction of embryonic red and white myotomal muscle (Buss and Drapeau 2000). Myotomal muscle is driven by a motoneuron-evoked synaptic drive that alternates between ipsilateral and contralateral myotomes and propagates in a rostral to caudal direction (Buss and Drapeau 2002). The coordinated neural output generated during swimming is maintained in paralyzed preparations (Buss and Drapeau 2001, 2002), thus allowing an electrophysiological analysis of motoneuron activation. During fictive swimming, motoneurons receive a glutamatergic and glycinergic synaptic drive (Buss and Drapeau 2000; Drapeau and Legendre 2001; Drapeau et al. 2000; Nguyen et al. 1999), and their growth and development (Eisen 1999; Eisen and Melancon 2001; Lewis and Eisen 2001; Westerfield and Eisen 1988). However, there is a paucity of information on the physiological properties of motoneurons.

In this study, the physiological and pharmacological properties of the motoneuron action potential and membrane were investigated in larval zebrafish using whole cell patch current-clamp recording techniques. Fast sodium-dependent action potentials, a high-threshold calcium conductance, and repolarizing potassium conductances were observed, and their features were related to the motoneuron firing pattern during swimming.

METHODS

Experiments were performed on zebrafish (Danio rerio) larvae of the Longfin strain raised at approximately 28.5°C and obtained from a breeding colony maintained according to Westerfield (1995). All procedures were carried out in compliance with the guidelines stipulated by the Canadian Council for Animal Care and McGill University. The experimental methodology has been described (Buss and Drapeau 2001; Drapeau et al. 1999). Results are taken from 84 morphologically identified (dye-filled) motoneurons (located dorsal or lateral to the central canal) of zebrafish aged 1.9–2.5 (day 2), 3.0–3.5 (day 3), and 4.1–4.4 (day 4) days postfertilization, encompassing the period from hatching to free-swimming.

Experiments were performed at room temperature (approximately 22°C). Evan’s fish saline recording solution (Buss and Drapeau 2001; Drapeau et al. 1999) contained (in mM) 134 NaCl, 2.9 KCl, 2.1 CaCl₂, 1.2 MgCl₂, 10 HEPES, and 0–10 glucose, and osmolarity was adjusted (with glucose) to 290 mOsm and pH 7.8. Larvae were paralyzed by immersion in α-bungarotoxin (10 μM; n = 16) for 10–20 min or d-tubocurarine (15 μM; n = 68) for the duration of the experiment. Similar observations were made using α-bungarotoxin or d-tubocurarine. Patch-clamp electrodes (4–7 MΩ) were pulled from thin-walled Kimax-51 borosilicate glass and were filled with either a potassium gluconate (n = 70) or cesium gluconate solution (n = 14). The potassium gluconate solution was composed of (in mM) 116 d-gluconic acid potassium salt, 16 KCl, 2 MgCl₂, 10 HEPES, 10 EGTA, 4 Na₂ATP, and 0.2% sulforhodamine B (osmolarity 280–290 mOsm, pH adjusted to 7.2). In the cesium gluconate solution, potassium gluconate and KCl were replaced with cesium gluconate and CsCl. Tetrodotoxin (TTX; 1 μM), tetraethylammonium chloride (TEA; 5 mM), 3,4-diaminopyridine (3,4-AP; 1 mM), or cobalt chloride (1 mM) were dissolves in fish saline (constant osmolarity was 290 mOsm).

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maintained by replacing glucose with equivalent amounts of TEA, 3,4-AP, or cobalt) and applied by bath perfusion. The actions of TTX, TEA, 3,4-AP, cobalt, and cesium intracellular solution on the action potential and membrane properties were investigated in day 2 (newly hatched) larvae. A liquid junction potential of –10 mV was experimentally determined according to (Barry and Lynch 1991; Neher 1992) and records were corrected for this potential.

Current-clamp recordings were performed with an Axoclamp-2A patch clamp amplifier (0.01 headstage; 10-kHz low-pass filter) and digitized at 20–40 kHz. All chemicals were purchased from Sigma Chemical (St. Louis, MO). Voltage-clamp recordings were not obtained because the goal of this first study was to relate the firing behavior of larval motor neurons to their membrane properties, which is only directly revealed in current-clamp recordings. Analyses were performed using pClamp 8 or Axograph 4.4 software (Axon Instruments). Motoneurons were held at –75 mV by current injection and all measurements were made from this potential. Short (2 ms) or long (300 ms) depolarizing current pulses were used to evoke single spikes or bursts of action potentials, respectively. The steady-state membrane potential at the end of long current pulses (when firing had stopped) was used to construct I-V curves. Input resistances (RI) were calculated from the slopes of the I-V relation for the following voltages: –105 to –75 mV, –75 to –45 mV, and > –45 mV. The ratio of RI (–45 mV) to RI (–75 to –45 mV) was used as an index of outward rectification (rectification ratio), and the ratio of RI (–75 to –45 mV) to RI (–105 to –75 mV) as an index of inward rectification of the membrane; a ratio of 1 indicates a linear relationship. Membrane time constants (τfast and τslow) were determined by fitting the voltage response to long current pulses (resulting in hyperpolarizations of 15–25 mV) with a sum of exponential curves. Capacitance was calculated by dividing τfast by RI (–105 to –75 mV). τfast was assumed to represent the time to charge the motoneuron soma, whereas the smaller but longer lasting τslow was assumed to represent the charging of a small dendritic tree (consistent with anatomical descriptions) and possibly an active conductance. Rheobase was the smallest current that could initiate an action potential during long (300 ms) current pulses. Spike afterhyperpolarization measurements were performed on the single action potential evoked at rheobase; the steady-state potential following the spike was used as baseline. Instantaneous firing frequency was determined by taking the inverse of the time between the first and second, second and third, and third and fourth action potentials in a burst. Because of the wide range of motoneuron input resistances, injected current was normalized to rheobase current and firing frequencies are presented for current pulses 1.5 and 2.0 times rheobase current. Spike threshold, amplitude, rise time, and half-width measurements were made on single action potentials evoked during threshold short current pulses. Action potentials initiated on the decay of the short pulse; spike threshold was measured as the most negative potential reached during the decay prior to spike initiation. Rise time was defined as the time between the action potential upstroke and peak, and half-width as the time from peak amplitude to when the action potential had decayed to 50% of its amplitude. Results are presented as mean ± SE throughout the text. The term significant denotes a relationship with P < 0.01 determined using the Student’s and paired t-test on normally distributed data, the Mann-Whitney rank sum test and Wilcoxon signed-rank test for nonparametric comparisons, and the Spearman rank order test for correlations.

RESULTS

Active and passive properties

The resting membrane potential of motoneurons ranged from –63 to –79 mV (mean = –74 ± 0.4 mV) and did not change during development (Table 1). Nearly all (93%) motoneurons responded to sustained depolarization by firing a short (approximately 10–30 ms) burst of action potentials followed by a period of silence or erratic firing (Figs. 1, 5, 6, 7, and 8). However, five motoneurons (7%) responded by firing throughout the current injection. Four of these were day 2 motoneurons having a high-input resistance (640 ± 78 MΩ; –75 to –45 mV range) and small- to medium-sized amplitude action potentials (53 ± 9.3 mV), and one was a day 4 motoneuron with an average spike amplitude (79 mV) and a low input resistance (84 ± 11 MΩ). During the burst, instantaneous firing frequencies of ≤800 Hz were observed. Instantaneous firing frequencies accommodated rapidly during the first four spikes and significantly higher (P < 0.001) frequencies were observed when stimulation strength was increased from 1.5 to 2.0 times rheobase (Table 2). Instantaneous firing frequencies were significantly higher (P < 0.001) in day 3–4 versus day 2 motoneurons (Table 2). During the sustained depolarization, when the membrane is more positive to EK, a hyperpolarization following the action potential was observed in 90% of motoneurons. Measured at rheobase, the afterhyperpolarization can reach ≤13 mV and last ≤40 ms (Table 1).

Membrane potentials used for constructing I-V relationships were measured at the end of 300-ms current steps, when the initial burst of action potentials had terminated and a steady-state potential had been reached. Three distinct components were observed in the I-V relationship (Fig. 1C; Table 1), which included an inward rectification at potentials negative to VR and an outward rectification at potentials positive to spike threshold. Input resistances were calculated from the slopes of the I-V relation over these three voltage ranges, i.e., negative to VR (–75 to –105 mV), positive to VR (–75 to –45 mV), and near spike threshold (> –45 mV). From day 2 to day 3–4, there was a significant decrease in the input resistance of the membrane measured during subthreshold steps negative and positive to the resting potential, while there was no significant change in the input resistance at potentials near spike threshold (Table 1). Consistent with the decrease in input resistance negative to spike threshold, rheobase current increased signifi-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 2 (n = 47)</th>
<th>Day 3–4 (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vm (mV)</td>
<td>–74 ± 0.5</td>
<td>–73 ± 0.6</td>
</tr>
<tr>
<td>Spike threshold (mV)</td>
<td>–38 ± 0.6</td>
<td>–36 ± 1.1</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>75 ± 1.9</td>
<td>75 ± 1.6</td>
</tr>
<tr>
<td>Spike rise time (ms)</td>
<td>0.34 ± 0.02</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>Spike afterhyperpolarization (mV)</td>
<td>6 ± 0.4</td>
<td>5 ± 0.6</td>
</tr>
<tr>
<td>Spike afterhyperpolarization (ms)</td>
<td>11 ± 1.3</td>
<td>9.1 ± 1.9</td>
</tr>
<tr>
<td>Rheobase (pA)</td>
<td>120 ± 8*</td>
<td>222 ± 25*</td>
</tr>
<tr>
<td>Capacitance (pF)</td>
<td>15.6 ± 0.7</td>
<td>17.4 ± 1.3</td>
</tr>
<tr>
<td>τfast (ms)</td>
<td>3.4 ± 0.2*</td>
<td>2.6 ± 0.3*</td>
</tr>
<tr>
<td>τslow (ms)</td>
<td>36 ± 2.6</td>
<td>47 ± 5.8</td>
</tr>
<tr>
<td>τslow (%)</td>
<td>15 ± 1.4</td>
<td>13 ± 1.6</td>
</tr>
<tr>
<td>R fast (–105 to –75 mV) (MΩ)</td>
<td>255 ± 28*</td>
<td>180 ± 32*</td>
</tr>
<tr>
<td>R slow (–75 to –45 mV) (MΩ)</td>
<td>315 ± 31*</td>
<td>215 ± 36*</td>
</tr>
<tr>
<td>Rectification ratios</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rfast/Rslow (–45 mV/–75 mV)</td>
<td>0.26 ± 0.02*</td>
<td>0.45 ± 0.04*</td>
</tr>
<tr>
<td>Rfast/Rslow (–75 to –45 mV)</td>
<td>1.26 ± 0.03</td>
<td>1.20 ± 0.02</td>
</tr>
<tr>
<td>Rfast/Rslow (–105 to –75 mV)</td>
<td>1.9 ± 0.7</td>
<td>31* ± 215</td>
</tr>
</tbody>
</table>

Values shown are means ± SE. * Significant difference of means (P < 0.01) determined using the Student’s t-test or Mann-Whitney rank sum test.

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significantly from day 2 to day 3–4 (Table 1). Interestingly, while there was no significant change in the input resistance of the outwardly rectifying portion of the $I-V$ relationship, there was a change in the apparent outward rectification, as quantified by the rectification ratio ($\frac{R_{\text{IL}}>-45 \text{ mV}}{R_{\text{IL}}>-75 \text{ to } -45 \text{ mV}}$), which increased 73% from day 2 to day 3–4 (Table 1). Together, these findings indicate that the decrease in outward rectification was due to a conductance increase at membrane potentials around $V_m$.

In addition to the parallel developmental changes in input resistance and rheobase, there was also a strong negative relationship ($r = -0.96; P < 0.001$) between input resistance and rheobase (Fig. 2A). Figure 2A also shows that although mean input resistance ($R_{\text{IL}}-105 \text{ to } -75 \text{ mV}$ and $R_{\text{IL}}(-75 \text{ to } -45 \text{ mV})$), and rheobase values changed significantly from day 2 to day 3–4 (Table 1), their values overlap in many motoneurons at all ages. However, a population of motoneurons ($n = 11$) with input resistances $>125 \text{ M}\Omega$, and rheobase values $>250 \text{ pA}$ (values never observed in day 2 motoneurons), appeared at day 3–4. Motoneurons within this population had larger rectification ratios (means of $0.59 \pm 0.06$ vs. $0.27 \pm 0.01; P < 0.001$) and contained the neurons with the most linear $I-V$ relationships (Fig. 1) observed in this study (3 motoneurons with rectification ratios ranging from 0.79 to 0.86).

The developmental changes in input resistance and rheobase could be due to an increase in conductance per unit area of membrane (e.g., ion channel insertion) or because of a developmental increase in motoneuron size. However, capacitance (a measure of membrane area and motoneuron size) values

![Figure 1](image1.png)

**FIG. 1.** Motoneurons of day 2 (A) and day 4 (B) zebrafish respond to long depolarizing current pulses by firing a short burst of action potentials. C: $I-V$ curves of the day 2 (○) and day 4 (●) motoneurons shown in A and B. Both motoneurons have a similar input resistance at membrane potentials $>-30$ mV, whereas large differences are apparent at more negative membrane potentials. Traces shown are from a typical day 2 motoneuron and a day 4 motoneuron with a high rectification ratio.

![Figure 2](image2.png)

**FIG. 2.** There is a negative relationship between input resistance ($-75 \text{ to } -45 \text{ mV}$) and rheobase current (A) and a positive relationship between conductance ($-105 \text{ to } -75 \text{ mV}$) and capacitance (B). Day 2, ○; day 3, □; and day 4, △. B: linear regressions are fit to day 2 (–––; $R = 0.77$), day 3 (––; $R = 0.88$), and day 4 (···; $R = 0.90$) values.

In addition to the parallel developmental changes in input resistance and rheobase, there was also a strong negative relationship ($r = -0.96; P < 0.001$) between input resistance and rheobase (Fig. 2A). Figure 2A also shows that although mean input resistance ($R_{\text{IL}}-105 \text{ to } -75 \text{ mV}$ and $R_{\text{IL}}(-75 \text{ to } -45 \text{ mV})$), and rheobase values changed significantly from day 2 to day 3–4 (Table 1), their values overlap in many motoneurons at all ages. However, a population of motoneurons ($n = 11$) with input resistances $<125 \text{ M}\Omega$, and rheobase values $>250 \text{ pA}$ (values never observed in day 2 motoneurons), appeared at day 3–4. Motoneurons within this population had larger rectification ratios (means of $0.59 \pm 0.06$ vs. $0.27 \pm 0.01; P < 0.001$) and contained the neurons with the most linear $I-V$ relationships (Fig. 1) observed in this study (3 motoneurons with rectification ratios ranging from 0.79 to 0.86).

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<table>
<thead>
<tr>
<th>Table 2. Instantaneous action potential firing frequency in day 2 and day 3–4 motoneurons</th>
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<tbody>
<tr>
<td>Rheobase ($R$)</td>
</tr>
<tr>
<td>1st</td>
</tr>
<tr>
<td>1.5 × $R$</td>
</tr>
<tr>
<td>($n = 46$)</td>
</tr>
<tr>
<td>2.0 × $R$</td>
</tr>
<tr>
<td>($n = 20$)</td>
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overlapped considerably at all ages (Fig. 2B) and did not change significantly from day 2 to day 3–4 (Table 1), ruling out motoneuron size as the only changing variable. A decrease in the fast component of the membrane time constant ($\tau_{\text{fast}}$), which accounted for approximately 85% of the membrane charging, compensated for the decrease in input resistance (Table 1). We assumed that the fast time constant represents the charging of the motoneuron soma as larval neurons lack extensive dendrites and chose to use it to calculate cell capacitance during development (Fig. 3). However, $E_K$ (approximately $-97$ mV) is close to the resting potential, and closer examination revealed that the decay time-course of voltage transients following action potentials were faster in 68% of motoneurons than the decay of subthreshold voltage transients.

Action potentials were initiated during the decay of voltage transients generated following short 2-ms current injections (Fig. 3), indicating a remote spike initiation site. Mean spike threshold was $-37\pm0.6$ mV, ranged from $-49$ to $-24$ mV, and was correlated with input resistance ($r = -0.407; P < 0.001$; $-75$ to $-45$ mV range), and spike rise time ($r = -0.51; P < 0.001$). Spikes were generally slightly overshooting or slightly undershooting (mean amplitude $= 75 \pm 1.4$ mV; range, 30 to 95 mV). These findings are also consistent with an axonal spike initiation site. Spikes elicited by short 2-ms current injections were not followed by a noticeable hyperpolarization (Fig. 3). However, $E_K$ (approximately $-97$ mV) is close to the resting potential, and closer examination revealed that the decay time-course of voltage transients following action potentials were faster in 68% of motoneurons than the decay of subthreshold voltage transients.

TTX unmasked an outward conductance (Fig. 5A) that appeared as a transient depolarization (Fig. 5B), possibly of a regenerative nature, which lasted approximately as long as an action potential burst (Fig. 5A). In the presence of TEA and 3,4-AP, the outward rectification is reduced, and addition of cobalt reduces it further (Fig. 6). When applied alone, cobalt reduced the outward rectification, decreased the firing frequency of the action potential burst (33 ± 12% decrease; determined from the first inter-spike interval normalized to 1.5

![FIG. 3. Pharmacological properties of the motoneuron action potential.](http://jn.physiology.org/)

### TABLE 3. Actions of TEA and 3,4-AP on the motoneuron action potential

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TEA (n = 6)</th>
<th>3,4-AP (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike half-width</td>
<td>Control $= 0.87 \pm 0.15$ ms</td>
<td>Control $= 1.0 \pm 0.40$ ms</td>
</tr>
<tr>
<td></td>
<td>14 ± 3 times longer*</td>
<td>13 ± 3 times longer*</td>
</tr>
<tr>
<td>Spike rise time</td>
<td>Control $= 0.33 \pm 0.07$ ms</td>
<td>Control $= 0.5 \pm 0.07$ ms</td>
</tr>
<tr>
<td></td>
<td>2.7 ± 0.2 times longer*</td>
<td>2.0 ± 0.1 times longer*</td>
</tr>
<tr>
<td>Spike voltage threshold</td>
<td>$6 \pm 2$ mV decrease</td>
<td>$2 \pm 1$ mV decrease</td>
</tr>
</tbody>
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Values shown are means ± SE. *Significant difference of means ($P < 0.01$) determined using the Student’s $t$-test or Mann-Whitney rank sum test.
or 2.0 times rheobase), and blocked the erratically occurring or delayed clusters of action potentials that can follow the bursts (Fig. 6). Cobalt also reduced the level of membrane noise. Although TEA, 3,4-AP, and cobalt had the greatest effect on the outwardly rectifying portion of the $I-V$ relationship, they also had minor effects at more negative potentials. Rheobase current decreased 26 ± 3% in TEA and 3,4-AP, while it increased 35 ± 14% in cobalt. The action of cobalt was surprising as cobalt reduced outward rectification and thus would be expected to decrease rheobase. This finding suggests that cobalt is either blocking a low threshold calcium conductance or is producing a charge screening effect.

A high-threshold transient potential was observed in the presence of TTX, TEA, and 3,4-AP (Fig. 4) at membrane potentials between –10 and –25 mV. This depolarizing potential was blocked by cobalt and is thus defined as a calcium-dependent action potential. The calcium spike activated slowly, and 2-ms current pulses were ineffective, whereas 10-ms pulses activated a spike. At higher membrane potentials (>0 mV), a sustained train of cobalt-sensitive, calcium-dependent action potentials (occurring at 60–80 Hz) followed the initial calcium spike in 3 of 4 motoneurons (Fig. 6B).

It was anticipated that TEA and 3,4-AP would facilitate repetitive action potential firing if the outward rectification they reduce contributed to burst termination. Application of

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**FIG. 4.** A high threshold calcium conductance (*) is revealed when sodium and potassium conductances are antagonized. The traces in A and B–C are from 2 different motoneurons shown in Figs. 5 and 6, respectively. The calcium conductance, obscured by the rapidly developing outward rectification (A), is revealed when the potassium conductances are blocked by TEA and 3,4-AP (B) and cobalt (C). The peak of the regenerative calcium ion conductance was measured and plotted before (▼) and after (▼) it was blocked with cobalt (D). The steady-state outward rectification observed prior to pharmacological treatments (●) is shown in D.

**FIG. 5.** TTX blocks action potentials and reveals an outward rectification causing a transient depolarization. Control (A, ———; C, ●) and TTX (B, - - -; C, ▼). At steady-state values, TTX has little effect on the $I-V$ relationship (C). Day 2 motoneuron. *Burst of action potentials in A and a transient depolarization of comparable duration in B.

**FIG. 6.** A high-threshold, TTX-resistant, cobalt-sensitive oscillation (B) due to a calcium conductance (C) is observed in motoneurons after sodium and potassium conductances are antagonized with TTX, TEA, and 3,4-AP. Control (A, ———; D, ●); TTX, TEA, and 3,4-AP (B, - - -; D, ▼); TTX, TEA, 3,4-AP, and cobalt (C, - - -; D, ▼). TEA, 3,4-AP, and cobalt reduce the steady-state outward rectification observed in the $I-V$ relationship (D). Day 2 motoneuron.
TEA or 3,4-AP led to repetitive firing, at 20–70 Hz, following the initial prolonged action potential burst (Fig. 8). However, these potassium channel blockers do not block all potassium channels (e.g., the calcium-dependent potassium conductance observed in this study), and to further reduce potassium conductances, intracellular potassium ion was replaced with the less permeant ion cesium. With a cesium ion–based intracellular solution, short 2-ms current pulses initiated a prolonged after discharge (lasting 170–1,000 ms), resembling a plateau potential (5/14 motoneurons) that was not observed in cobalt solutions (Fig. 9). In motoneurons that did not initiate a plateau, the action potential was broadened similarly to action potentials observed in TEA or 3,4-AP (Fig. 9B). Motoneurons examined using cesium ion–based intracellular solutions had significantly larger mean half-widths (27 ± 3 ms), rise times (0.61 ± 0.05 ms), and a lower spike threshold (−43 ± 0.8 vs. −37 ± 0.6 mV), than those recorded with potassium gluconate–based intracellular solutions. The prolonged action potential was attenuated in cobalt (Fig. 9B; 57 ± 0.1% as long; n = 5) and completely blocked in TTX (data not shown). Together, these findings show that the conductances active during the outwardly rectifying portion of the I-V relationship are important for burst termination.

**DISCUSSION**

The action potential recorded from motoneurons of larval zebrafish is initiated distal to the soma, is generated by a TTX sensitive sodium ion conductance, and is terminated by a potassium ion conductance sensitive to TEA and 3,4-AP. I-V curves revealed an inwardly rectifying conductance at voltages negative to resting potential and an outwardly rectifying conductance at voltages near spike threshold. From hatching to the free-swimming stage, average membrane input resistance at potentials negative to spike threshold decreased, and I-V relationships became more linear even though the input resistance of the suprathreshold outwardly rectifying segment of the I-V curves did not change significantly. The decrease in input resistance was not accounted for simply by increases in motoneuron size, as reflected by less variable membrane capacitance estimates, but rather by an increase in specific membrane conductance. The population of motoneurons examined had a...
wide range of input resistances, capacitative values (sizes), and rheobase current thresholds, supporting the presence of graded motoneuron recruitment during swimming at these larval stages (Buss and Drapeau 2002).

Over 90% of motoneurons responded to long depolarizing current pulses by firing a brief (approximately 10–30 ms) burst of action potentials at all ages. A strong outward rectification, antagonized by voltage-activated potassium channel antagonists (TEA and 3,4-AP), intracellular cesium, and cobalt, contributes strongly to burst termination. The role of potassium conductances in burst termination is most clearly demonstrated in Fig. 9, where a sustained plateau potential appears when intracellular potassium ions are replaced with impermeant cesium ions. Although not tested directly, cobalt is likely preventing the activation of a calcium-dependent potassium ion (and/or chloride ion) conductance by blocking a voltage-activated calcium influx. A high-threshold, cobalt sensitive calcium conductance was also observed when sodium and potassium conductances were decreased in TEA, 3,4-AP, and TTX (Fig. 6). At positive potentials, the calcium spike could become repetitive with a long current pulse. Furthermore, the plateau potentials observed using cesium ion–based intracellular solutions were not observed and action potential half-widths were reduced in the presence of cobalt. Based on these observations, we hypothesize that the high-threshold calcium spike, activated by the overshooting depolarizing sodium-dependent action potentials, may provide an electrogenic contribution to action potential bursting.

This is the first study to examine the electrophysiological properties of motoneurons in a larval fish. With the exception of the lamprey, there have been few physiological investigations of fish locomotor motoneurons. Fish motoneurons generally have low resting membrane potentials: mean = −75 mV in goldfish (Fetcho 1992); mean = −75 mV in a Japanese teleost (Bando 1975); mean = −80 mV in 25% of stingray motoneurons (Williams et al. 1984); mean = −75 mV in lamprey (Buchanan 1993). These are similar to that observed in larval zebrafish with a mean = −74 mV. Depolarizing after potentials have been observed in goldfish, a Japanese teleost, and stingray motoneurons, while an afterhyperpolarization was observed in stingray motoneurons (Bando 1975; Fetcho 1992; Williams et al. 1984) and lamprey (see following text). In larval zebrafish, an afterhyperpolarization was not observed when action potentials were evoked by short pulses from the negative resting membrane potential but were observed when the membrane was held at more depolarized levels. The afterhyperpolarization of larval zebrafish motoneurons could be obscured by the fast membrane time constant, the proximity to $E_K$, or to calcium chelation by the intracellular solution. However, examination of the $I-V$ relationship after the calcium conductance was blocked with cobalt did reveal the presence of a calcium-activated conductance, which was likely mediated by potassium ions.

Interestingly, Buss and Drapeau (2001) reported a mean spike threshold of −46 ± 0.8 mV (corrected for the different junction potentials used in these papers) determined by measuring the amplitude at which rhythmic locomotor drive potentials initiated action potentials during fictive swimming. This value is approximately 9 mV closer to the resting potential than the spike threshold determined by using somatic current injections in this study (−37 ± 0.6 mV). The more negative spike threshold observed during fictive swimming could be because excitatory locomotor synapses are located electrically closer to the axonal spike initiation zone than the somatic patch electrode point current source. In addition, there could be a locomotor-related reduction in spike threshold, as reported in the cat (Krawitz et al. 2001).

Fin (Rovainen and Birnberger 1971) and myotomal (Tera-vainen and Rovainen 1971) motoneurons have been examined in detail in lamprey, where motoneurons to twitch fibers have lower input resistances than those to slow fibers (Tera-vainen and Rovainen 1971). The $I-V$ relation determined at potentials 20 mV positive and negative to the resting membrane potential is linear, and input resistances, action potential thresholds, and instantaneous firing frequencies are considerably lower in the lamprey (Buchanan 1993). Similar to larval zebrafish, the action potential is prolonged in TEA or 4-AP and blocked by TTX (Hess and El Manira 2001; Kemnitz 1997; Matsushita et al. 1993; Wallen et al. 1989). A calcium-activated potassium conductance, transient A-type current, and high-threshold calcium current has also been described in lamprey motoneurons (El Manira and Bussieres 1997; Grillner and Wallen 1985; Grillner et al. 2001; Hess and El Manira 2001; Hill et al. 1992; Matsushita et al. 1993; Wallen et al. 1989).

The duration of the action potential bursts observed during long depolarizing current pulses closely approximates the duration of the active period of a swim cycle (Buss and Drapeau 2001, 2002). The factors contributing to burst termination may include a high-threshold inactivating calcium spike and voltage- and calcium-dependent potassium conductances. Together these conductances help sculpt the period of action potential firing so that it is consistent with the period of rhythmic excitatory drive to the motoneurons. If the same conductances are present in interneurons of the central pattern generator for swimming, they could also underlie the generation of rhythmic locomotor network activity.

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