Ionic Currents of the Lateral Pyloric Neuron of the Stomatogastric Ganglion of the Crab

JORGEO G OLOWASCH AND EVE MARDER
Department of Biology, Brandeis University, Waltham, Massachusetts 02254-9110

SUMMARY AND CONCLUSIONS

1. The lateral pyloric (LP) neuron is an important component of the network that generates the pyloric rhythm of the stomatogastric ganglion (STG) and is a direct target of many modulatory inputs to the STG. Our aim in this and the subsequent two papers is to describe the conductances present in this cell and to understand the role these conductances play in shaping the activity of the neuron.

2. LP neurons were studied in two-electrode voltage clamp (TEVC) in a saline solution containing tetrodotoxin (TTX) and picrotoxin (PTX) to isolate them pharmacologically from presynaptic inputs.

3. We identified six voltage-dependent ionic conductances. These include three outward currents that resemble a delayed rectifier current, a Ca\(^{2+}\)-activated K\(^+\) current and an A-current similar to those seen in many other preparations. LP neurons show three inward currents, a fast TTX-sensitive current, a hyperpolarization-activated inward current, and a Ca\(^{2+}\) current.

INTRODUCTION

To those who seek to understand how the nervous system generates behaviors, biophysical studies of the ion channels found in neurons of the CNS have provided an embarrassment of riches (Hille 1984). It is now patentely clear that the neurons that participate in the generation of complex behaviors will display huge numbers of ion channels, many of which are likely subject to modulation by transmitters and hormones (Kaczmarek and Levitan 1987). However, despite the profound insights that biophysics has provided into the structure, function, and modulation of single ion channels, relatively few attempts have been made to understand the contribution of the full complement of conductances displayed by a neuron to its overall excitability properties. Our aim is eventually to understand the biophysical and cellular mechanisms that underlie circuit modulation in the crab stomatogastric ganglion (STG). To this end, we have initiated a study of the ionic currents found in one of the most important neurons in the STG of the crab, Cancer borealis, the lateral pyloric (LP) neuron. This paper provides a general description and identification of the ionic currents expressed by this cell.

The LP neuron is multifunctional. It is an excitatory motor neuron that innervates several muscles that participate in the rhythmic constriction of the pyloric chamber of the foregut (Hooper et al. 1986). Additionally, the LP neuron makes a number of inhibitory connections within the neurtopil of the STG (Eisen and Marder 1982), where these connections play an important role in shaping the pyloric motor patterns produced by the STG. Many of the neuromodulators of the motor patterns produced by the STG markedly influence the number of LP action potentials fired within each cycle of the pyloric rhythm (Hooper and Marder 1987; Marder et al. 1986; Marder and Weissman 1992; Nusbaum and Marder 1988, 1989a,b). For these reasons, and because there is only a single LP neuron in each ganglion, we chose to study the currents of this neuron. Many of the currents found in neurons depend critically on both voltage and time, and the expression of each of the currents in a neuron contributes to, and is influenced by, the dynamical properties of the neuron's firing pattern. Therefore we first characterized as many as possible of the voltage- and time-dependent conductances found in the LP neuron. We then constructed a model neuron from these conductances (Buchholz et al. 1992). In the model neuron we can determine the role of each conductance in shaping the firing pattern of the neuron under a variety of dynamical situations that mimic the activity of the neuron under physiological conditions (Golowasch et al. 1992).

METHODS

Experiments were performed on dissected stomatogastric neuronal systems (cf. Selverston and Moulins 1987) from the crab, C. borealis, obtained from local fish markets and kept in seawater aquaria at 12°C. Data were collected from 10-30 experiments. STGs were desheathed, pinned on a silicone elastomer (Silgard)-lined Petri dish, and cells identified as described in Hooper et al. (1986). Inputs from anterior ganglia were blocked by placing a petroleum jelly (Vaseline) well around the desheathed stomatogastric nerve (snn) filled with isotonic (750 mOsm) sucrose. Preparations were superfused with Cancer saline through a homemade cooling device with the use of thermoelectric heat pumps (Melcor, NJ) to maintain the temperature at 9-11°C. The composition of Cancer saline was (in mM) 440 NaCl, 11 KCl, 13 CaCl\(_2\), 26 MgCl\(_2\), 5 maleic acid, 11 trisma base, and pH 7.4-7.5. When divalent cations were added, Ca\(^{2+}\) was removed, retaining at least 0.1 mM Ca\(^{2+}\) in the bath to maintain membrane stability. Low amounts of divalent cations (<200 mM) were added without compensation. Less than 0 mM concentrations of other substances (C6Cl, TEACI, etc.) were added to the bathing saline without compensation.

Unless otherwise specified, chemicals were obtained from Sigma (St. Louis, MO). They were all prepared immediately before use. Tetrodotoxin (TTX; Calbiochem) was dissolved in distilled water to obtain a stock solution of 10^-4 M and kept at 4°C. Charybdotoxin (CTX), which was kept frozen in aliquots of 1 mM, was a gift from Dr. Christopher Miller.

The LP neuron was impaled with two microelectrodes filled with either 0.6 M K\(_2\)SO\(_4\) + 20 mM KCl (10- to 25-M\(_\Omega\) resistance) or 2.5 M KCl (5- to 15-M\(_\Omega\) resistance). An Axoclamp 2A (Axon

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Instruments, CA) was used for either two-electrode current clamp (TECC) or for two-electrode voltage clamp (TEVC). Currents were filtered with an eight-pole Butterworth filter (901F, Frequency Devices, MA) and recorded on-line on a Gould 2400 chart recorder (Gould, Cleveland, OH). The voltage clamp was driven, and currents and voltages recorded, stored, and subsequently analyzed on a computer with the pClamp software from Axon Instruments (CA). When a leak subtraction procedure was chosen, the p/n protocol was used (Bezannou and Armstrong 1977), and the n subpulses were applied in the opposite direction from the test pulse, \( V_{\text{test}} \). For TEVC the gain was set to 100 \( V_{\text{test}} \) for the maximum (10,000 \( V_{\text{test}}/V_{\text{max}} \)), and capacitance compensation of the electrodes was used. Shielding of the electrodes or separating them with a grounded plate did not improve the recordings. Ground consisted of a calomel reference electrode (Fisher Scientific, NJ) connected to the bath through an agar bridge (4% agar in 0.6 M K2SO4 + 20 mM KCl).

To block outward currents, Cs+ was injected with the use of 5- to 10-nA current pulses of 0.5 s at 1 Hz through Cs2SO4 electrodes (0.6 M Cs2SO4 + 20 mM KCl).

Unless otherwise specified, experiments were done in the presence of 0.1 mM TTX and 10 \mu M picrotoxin (PTX). The impalement of the cells was done before TTX, PTX application for proper identification of the cells (Hooper et al. 1986). Impalement after TTX application in the bath rarely gave satisfactory input resistances (\( R_{\text{in}} \)), Impalement before TTX application gave \( R_{\text{in}} \) values between 5 and 20 M\( \Omega \) (Table 1). Cells with lower \( R_{\text{in}} \) were discarded.

## RESULTS

### LP neuron

Each STG contains one LP neuron. Figure 1 shows a drawing of an LP neuron filled intracellularly with Lucifer yellow. LP neurons have a large soma (70–100 \mu m diameter) and an extensive dendritic tree through a large-diameter neurite from which emerge a few thick branches and the axon. Fine neurites sprout directly from the thick central process, as well as from the secondary branches.

Typical activity patterns of the LP neuron and its functional antagonist, the pyloric dilator (PD) neuron, are shown in normal physiological saline in Fig. 2A (left). During rhythmic pyloric activity the LP neuron fires bursts of action potentials that are terminated by inhibitory synaptic potentials from other neurons in the pyloric network. PTX (10 \(-7 \text{M}\)) blocks the inhibitory glutamatergic synapses in the STG (Eisen and Marder 1982; Marder and Eisen 1984; Marder and Paupardin-Tritsch 1978). Figure 2A (right) illustrates that application of PTX effectively blocks the

### Table 1. Passive properties and maximum chord conductance values of the lateral pyloric neuron

<table>
<thead>
<tr>
<th>Condition</th>
<th>Average ± SD</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>( R_{\text{in}} ), M( \Omega )</td>
<td>11.6 ± 5.5</td>
<td>37</td>
</tr>
<tr>
<td>( V_{\text{m}} )</td>
<td>198.9 ± 42.2</td>
<td>15</td>
</tr>
<tr>
<td>( I_{\text{f}} )</td>
<td>1.15 ± 0.34</td>
<td>6</td>
</tr>
<tr>
<td>( V_{\text{max}} ) (TTX-PTX), mV</td>
<td>-48.9 ± 6.3</td>
<td>38</td>
</tr>
<tr>
<td>( g_{\text{leak}}, \mu S^* )</td>
<td>0.34 ± 0.10</td>
<td>11</td>
</tr>
<tr>
<td>( \Delta V_{\text{max}}, \mu V^* )</td>
<td>0.74 ± 0.24</td>
<td>9</td>
</tr>
<tr>
<td>( g_{\text{leak}}(V = -80 \text{ mV}), \mu S^* )</td>
<td>0.38 ± 0.11</td>
<td>17</td>
</tr>
</tbody>
</table>

PTX-PTX, tetrodotoxin-picrotoxin.

* \( g_{\text{leak}} = \frac{1}{R_{\text{in}} - R_{\text{leak}}} \); \( R_{\text{leak}} \) assumed to be -80 mV and j is 1 of the 3 currents.

**FIG. 1.** Drawing of a Lucifer yellow-filled LP cell.

**Passive properties of the LP neuron**

The LP neuron's complex geometry potentially poses space-clamp problems that could make it difficult to achieve good voltage control of the neuron. It has been shown that current injection into the soma of a cell with a geometry like that of the LP neuron (a large, thick neurite with thin processes emerging from it) will show voltage changes that attenuate little as current spreads into the smaller branches as long as the membrane resistance remains relatively constant, whereas the sharpest voltage attenuation usually occurs at the branch points (Graubard and Calvin 1979). This suggests that the soma and the major proximal neurite of the LP cell are nearly isopotential and can be effectively clamped from the soma.

Figure 3A shows simultaneous recordings from the soma and a neuropil process at a point several hundred microns from the soma. The slow membrane potential oscillations recorded at these two sites are virtually superimposable, although the fast action potentials are significantly larger at the neuropil recording site than at the soma. This suggests that the fast membrane potential (F) changes are subject to the low-pass filtering that the membrane capacitance imposes on the electrical signals across the membrane. It also indicates that the site of spike initiation is distant from the soma.

Rall and colleagues (Rall 1977) have suggested that cells with an electrotonic length constant, \( L \) (approximated by \( L = \pi \cdot (r_0/r_1 - 1)^{1/2} \)), where \( r_0 \) is the membrane time constant and \( r_1 \) the first equalizing time constant), smaller than two to three are electrotonically compact, as long as the membrane resistance remains relatively constant (i.e.,
at steady state). In the experiment illustrated in Fig. 3B, two electrodes were placed in an LP neuron, and the voltage responses to constant current pulses are shown. These can be fit by two exponentials (smooth lines in Fig. 3B). The shorter time constant, $\tau_1$, is an equalizing time constant, whereas the longer one, $\tau_0$, is the actual membrane time constant (Rall 1977). The equalizing time constant describes the charge movement along the branches of the cell.

FIG. 2. Isolation of LP cell. A: simultaneous intracellular recordings of LP and PD neurons in control saline (left) and in 10 µM PTX (right). B: intracellular recording from LP neuron before (left) and after (right) applying a sucrose block around the stn to remove afferent inputs. C: effect of bath application of 1 µM TTX on a LP cell beginning at the square. The baseline of the action potentials is at $V = -55$ mV.

FIG. 3. Cable properties of the LP neuron. A: simultaneous intracellular recordings from the soma and neuropil of the LP cell. Preparation in normal saline. Neuronal process impaled ~500 µm away from the cell body. Arrows indicate $V = -50$ mV. B: voltage responses of an LP cell to constant-current pulses of ~8 to +3 nA (steps of 1 nA) in TTX-PTX. Superimposed (smooth traces) are the 2-exponential fits, with $\tau_0 = 212$ ms and $\tau_1 = 11$ ms. Electronic length constant ($L_e$) = 0.74.
whereas the membrane time constant $\tau_m$ reflects the charge movement across the membrane. By this method, $\tau_m$ was $199 \pm 42$ (SE) ms. The values of $\tau_m$ and $\tau_l$ obtained for the LP cell indicate that this cell is electrotonically equivalent to a two-compartment model cell with an average electrotonic length constant, $L_m = 1.15$ (Table 1). This analysis applies to neurons that can be represented as an equivalent cylinder (Rall 1977), and we have assumed that this is the case for the LP neuron. In most LP cells we were able to measure only two time constants, indicating that after steady-state conditions are achieved we have reasonably good voltage control over most of the membrane in the soma and major processes in spite of their large size.

**Outward currents**

To characterize the voltage-dependent currents of the LP neuron, experiments were done in TEVC. As seen below, our data indicate that LP neurons display three distinct outward currents that resemble closely the delayed rectifier ($I_{K_\text{d}}$), transient A ($I_A$), and Ca$^{2+}$-activated outward ($I_{\text{out(Ca)}}$) currents described in other preparations. In fact, we employed the well-known properties of $I_{K_\text{d}}$ and $I_{\text{out(Ca)}}$ to isolate each outward current. When depolarizing voltage pulses are applied from a holding potential $V_h = -40$ mV to remove $I_{K_\text{d}}$ (see below), two outward current components can be seen (Fig. 4): a fast transient component that inactivates within the first 250 ms, and a slower component that persists for many seconds. Because of the poor voltage control in the first 20–30 ms (Fig. 4, top), we never made current measurements in the first 30 ms after the pulse onset. The transient component and part of the sustained component are blocked with 200 $\mu$M Cd$^{2+}$ (Fig. 5 and below). We are defining the remaining sustained current that reaches its maximum with a time course that becomes faster as the cell is more depolarized and shows no signs of voltage-dependent inactivation (Fig. 5A) as $I_A$. $I_A$ activates at $V$ values above around $-40$ mV (Fig. 5B), and its maximum chord conductance ($g_{A}$) is $0.34 \mu$S (Table 1). From the characteristic delayed onset and its voltage dependence, this current resembles other well-described delayed rectifiers (Adams et al. 1982; Czernasty et al. 1989; Hodgkin and Huxley 1952; Thompson 1977). As with most delayed rectifier currents, the steady-state activation $a_s(V)$ curve of Fig. 5B can be fit with a sigmoidal function. In the present case the following expression adequately fits the data

$$a_s(V) = \frac{2.0}{1 + \exp[(V + 22.4)/-25.91]}.$$

Like most delayed rectifier currents (cf. Cobbett et al. 1989; Czernasty et al. 1989; Stanfield 1983; Thompson 1977) the LP $I_A$ is partially blocked by tetraethylammonium (TEA). The block shows a dissociation constant, $K_D$, of 0.71 mM, a first order of binding and saturates around 10 mM (measured at $V_{\text{rest}} = +20$, $n = 4$). However, the block is not complete and only $\sim 65$% of the total $I_A$ is blocked with 10 mM TEA (see Golowasch 1990).

A large component of the outward current that is activated at voltages more positive than $V_{\text{rest}}$ is blocked by inorganic divalent cations like Cd$^{2+}$ (Fig. 6, B and D), Mn$^{2+}$,
and Co\textsuperscript{2+}. This component appears to be activated by Ca\textsuperscript{2+} as decreasing the extracellular Ca\textsuperscript{2+} concentration decreases the amplitude of part of the outward current until it reaches a plateau where only the Ca\textsuperscript{2+}-insensitive current is activated, namely \(i_{\text{Ca}}\) (Fig. 6, A and C). We call the outward current that is sensitive to Ca\textsuperscript{2+} concentrations \(i_{\text{Ca}(\text{Ca})}\) is blocked by increasing concentrations of Cd\textsuperscript{2+} (Fig. 6, B and D). The blocking effect of Cd\textsuperscript{2+} has a \(K_0\) of \(-30\) μM, assuming first-order binding (measured at +20 mV, \(n = 3\)). It is this property of Cd\textsuperscript{2+} that we have used to isolate \(i_{\text{Ca}(\text{Ca})}\) from \(i_{\text{Ca}}\) (Fig. 5), assuming that Cd\textsuperscript{2+} blocks all of the underlying Ca\textsuperscript{2+} current and that no significant fraction of \(i_{\text{Ca}(\text{Ca})}\) remains that can be activated by the existing background Ca\textsuperscript{2+} concentration.

The voltage dependence of \(i_{\text{Ca}(\text{Ca})}\) is similar to the voltage dependence of \(i_{\text{Ca}}\) but slightly displaced to more positive voltages: the current traces shown in Fig. 7A and the current-voltage (I-V) curve of the peak Ca\textsuperscript{2+}-activated outward current in Fig. 7C show that it activates at a V more depolarized than \(-30\) mV. However, the maximum chord conductance values, \(g\) of these two currents differ markedly (Table 1), leaving \(i_{\text{Ca}(\text{Ca})}\) as the predominant of the two currents. Like \(i_{\text{Ca}}\), \(i_{\text{Ca}(\text{Ca})}\) does not show signs of voltage-dependent inactivation for voltages more negative than \(-40\) mV because there is no difference in amplitude or kinetics in \(i_{\text{Ca}(\text{Ca})}\) activated from different \(V_s\) values (Fig. 7B). This may be, however, a reflection of the voltage-dependent properties of the underlying Ca\textsuperscript{2+} current (see below).

More direct evidence that \(i_{\text{Ca}(\text{Ca})}\) is a Ca\textsuperscript{2+}-sensitive current is shown in Fig. 8. Ca\textsuperscript{2+} was injected through a third microelectrode filled with CaCl\textsubscript{2}, and voltage pulses were applied to \(+20\) mV in control saline during the injection period. With endogenous inward Ca\textsuperscript{2+} currents intact (Fig. 8A), the injection of Ca\textsuperscript{2+} has a clear blocking effect on the
transient part of $i_{\text{Ca}}$, suggesting that Ca$^{2+}$ directly inactivates part of $I_{\text{Ca}}$. When the endogenous inward Ca$^{2+}$ currents are blocked with Cd$^{2+}$ (Fig. 8B), Ca$^{2+}$ injection with positive current pulses induces the activation of a large sustained outward current in response to a depolarizing voltage step to +20 mV, indicating direct Ca$^{2+}$ activation. Thus the apparent transient nature of the Ca$^{2+}$-activated outward current (Figs. 6–8) seems to be a reflection of the kinetic properties of the underlying Ca$^{2+}$ current and not a property of voltage-dependent inactivation of $I_{\text{Ca}}$ itself (cf. Knöpfel et al. 1990). This is best illustrated in Fig. 8B, where sustained Ca$^{2+}$ injection activated only a sustained outward current when all the Ca$^{2+}$ currents were blocked with Cd$^{2+}$.

$I_{\text{Ca}}$ is sensitive to TEA, which blocks it almost completely (Golowasch 1990), but insensitive to the bee venom Amapin, known to block a subset of the described Ca$^{2+}$-activated K$^+$ currents (Latorre et al. 1989). Nanomolar concentrations of CTX, known to block a set of Ca$^{2+}$-activated K$^+$ currents (Latorre et al. 1989), partially and irreversibly blocked $I_{\text{Ca}}$ (Golowasch 1990).

Like the originally described $i_{\text{K}}$ of molluscan neurons (Connor and Stevens 1971; Neher 1971), and those described in other species (Rogawski 1985; Cobbett et al. 1989), the LP $i_{\text{K}}$ shows a strong voltage and time dependence of both the activation (Fig. 9A and B) and inactivation processes (Fig. 9C and D). $i_{\text{K}}$ turns on at voltages around −40 mV (Fig. 9B) and steeply increases its activation between −40 mV and approximately +20 mV after which the activation begins to plateau. As previously described (Cobbett et al. 1989; Connor and Stevens 1971; Neher 1971) the voltage dependence of activation is well fit by a sigmoidal function of voltage. The voltage dependence of activation of the LP $i_{\text{K}}$ can be fit with a function, the parameters of which are

$$a(V) = \frac{1.1/(1 + \exp(V + 19.4)/-20.3)^3}{1}$$

Notice, however, that this function describes an approxi-
mation of the voltage dependence of the true activation because it corresponds to measurements of the peak current at 30 ms. As mentioned below, instantaneous tail current measurements in these cells are not possible, and therefore instantaneous activation and inactivation parameters cannot be measured.

The activation kinetics of $i_a$ depend on voltage. $i_a$ activates and reaches a peak more rapidly as the voltage is increased (Fig. 10 A). $i_a$ is maximally deactivated at voltages more negative than around $-90$ mV and completely inactivated at voltages of about $-40$ mV or higher in the steady state (Fig. 9 D). As described before (Cobett et al. 1989; Connor and Stevens 1971; Neher 1971), this inactivation process can be well fit by a sigmoidal function of voltage. The relation in the LP cell is

$$I(V) = 0.26 + 0.75/[1 + \exp(V + 61.3)/6.1]$$

where 0.26 corresponds to the unsubtracted contribution of $i_a$ to these measurements. The complete inactivation of $i_a$ at around $-40$ mV is the property we used to separate $i_a$ from the other two outward currents: when voltage pulses from $V_h = -40$ mV are applied, only $i_d$ and $i_{os}$ turn on, whereas $i_a$ remains inactive. $i_a$ inactivation is slow, taking several seconds for the current to decrease to baseline levels. This inactivation process shows two apparent time constants of inactivation...
FIG. 10. Time course of the release of the inactivation of \(i_{Na}\). In TTX-PTX + 200 \(\mu\)M Cd\(^{2+}\) + 10 mM TEA, \(V_m = -40\) mV. \(A\): currents were evoked by a pulse to \(+30\) mV from a prepulse potential of \(-80\) mV and of increasing duration. \(B\): peak currents obtained as in \(A\) at 30 ms after the onset of \(V_m\) plotted as a function of prepulse duration for different \(V_m\) levels (top to bottom: \(-100\), \(-90\), \(-80\), \(-70\), \(-60\), \(-50\), and \(-40\) mV). Normalized to the current obtained from a \(V_m = -90\) mV and 3 s of duration (51.9 \pm 7.1 nA, \(n = 5\)). Each point is the average \pm SD of 3 cells.

(9.4.1), each with a slight voltage dependence that becomes faster as \(V_m\) is more depolarized (see Golowasch 1990).

The inactivation of \(i_{Na}\) can be reversed by hyperpolarizing the cell. The time course of the release of the inactivation (deactivation) shows a strong dependence on voltage as shown in Fig. 10. In Fig. 10.4 we demonstrate the time and voltage dependence of the inactivation process by applying increasingly longer duration prepulses (\(V_{prep}\)) to \(-80\) mV from a \(V_m = -40\) mV. \(i_{Na}\) is then activated by stepping the voltage from \(V_{prep}\) to a \(V_m = -40\) mV (Fig. 10.4). Figure 10.5 shows that the release of inactivation occurs faster with more negative prepulse voltages, approaching saturation at voltages around \(-100\) mV.

4-Aminopyridine (4-AP) produced a partial and irreversible block of \(i_{Na}\) (see Golowasch 1990). 4-AP had limited usefulness for the isolation of ionic currents because it damaged the cells when exposed for too long times even at concentrations that failed to completely block \(i_{Na}\). Short applications were successful but produced incomplete blocking of \(i_{Na}\). The \(K_d\) of the 4-AP block of \(i_{Na}\) is \(\sim 1\) mM (measured with a fit assuming 1st-order binding 4-AP, \(n = 3\)) suggesting that a large fraction of \(i_{Na}\) is blocked with millimolar amounts of the drug. Unlike some other A-like currents reported in the literature (Cobett et al. 1989; Tasaki and Cooke 1986; Thompson 1977), \(i_{Na}\) in the crab is not blocked by TEA. In fact, TEA actually enhanced the apparent rate of activation and the amplitude of \(i_{Na}\), probably because TEA increased \(R_m\) by blocking \(i_{K}\) and \(i_{NaCa}\), thus allowing a better voltage control of the cell. Finally, \(i_{Na}\) is not affected by Ca\(^{2+}\) because neither its activation nor its inactivation characteristics were affected by changing the extracellular Ca\(^{2+}\) concentration (not shown).

When the three currents just described were measured in the same cell, the individual ratios of the maximum conductances \(g_{NaCa}/g_{Na}\) and \(g_{K}/g_{Na}\) were 2.2 \pm 0.8 and 1.1 \pm 0.4, respectively (\(n = 8\)). When the averages of the pooled maximum conductances from all measured cells (Table 1) were taken, regardless of whether they were measured in the same cell or not, and the same ratios \(g_{NaCa}/g_{Na}\) and \(g_{K}/g_{Na}\) were calculated, identical values were obtained (2.2 and 1.1, respectively).

**Inward currents**

When the LP is hyperpolarized, a "sag" in the voltage slowly develops that depolarizes the cell. This sag is clearly the result of a conductance activation because \(R_m\) decreases when the cell is hyperpolarized and the sag is generated (not shown). Underlying this sag is a current \(i_{Na}\) with the characteristic behavior of an inward rectifier (Angstadt and Calabrese 1988; Arbax and Calabrese 1987; DiFrancesco 1986; Edman et al. 1987; Parker and Miledi 1988), which carries current only inwardly and activates at membrane potentials more negative than \(V_m\) (not shown). The voltage dependence of \(i_{Na}\) activation indicates that it begins activating at \(-50\) to \(-60\) mV and continues to activate toward more negative voltages with no signs of voltage-dependent inactivation (Fig. 11.4, left). Its steady-state \(I-V\) curve shows the inward rectifying nature of this current (Fig. 11.4B). Judging from the tail currents obtained at \(V_m\) after activating \(i_{Na}\) at different voltages (Figs. 11.4A and 12.4), \(i_{Na}\) activation begins to saturate at around \(-110\) mV. Its extrapolated reversal potential is \(-20\) to \(-30\) mV from tail current measurements at different voltages after a prepulse to \(-120\) mV (Fig. 12).

\(i_{Na}\) is reversibly blocked by millimolar concentrations of Ca\(^{2+}\) (Fig. 11.4). Ba\(^{2+}\), which is known to block other inward rectifying currents (Benson and Levitan 1983; Uchimura et al. 1989), is less effective, as 12.5 mM Ba\(^{2+}\) is not enough to block \(i_{Na}\) completely, whereas 5 mM Ca\(^{2+}\) is sufficient.

The presence of a Ca\(^{2+}\)-activated current indicates the presence of a Ca\(^{2+}\) current. However, under our control conditions (TTX, PTX, and the rest of the currents intact) an inward current is not observed (see for example Fig. 4). To visualize small inward currents, the larger outward currents must be blocked. We used two methods for this purpose: 1) iontophoretic injection of Ca\(^{2+}\) into the cell; and 2) adding saturating concentrations of TEA to block \(i_{NaCa}\) and a large fraction of \(i_{K}\), and activating the current from a
FIG. 11. \( i_\text{L} \) isolation, time course, and voltage dependence. LP cell in TEVC and TTX + PTX. A: voltage pulses from a \( V_\text{p} = -40 \text{ mV} \) to a range between -110 and -50 mV. Left: control saline. Middle: in 5 mM Cs\(^+\). Right: after 20 min washout of Cs\(^+\). B: voltage dependence. I-V curve of the Cs\(^+\)-sensitive component obtained by subtraction of the currents measured in 5 mM Cs\(^+\) from control saline in A. Measurements taken at the end of the 20-s-long pulses.

holding potential where \( i_\text{L} \) is inactive (e.g., \( V_\text{p} = -40 \text{ mV} \)). By the use of the first of these methods (Fig. 13), a small inward current was revealed that activated at voltages more positive than \(-30 \text{ mV} \) (Fig. 13A). Two hundred micromolar of Cd\(^{2+}\) blocks most of this current (Fig. 13B). Figure 14A displays the net Cd\(^{2+}\)-sensitive current obtained from data in Fig. 13, A and B. It shows the presence of apparently two components, a transient and a sustained component.

In most preparations Ba\(^{2+}\) both permeates better than Ca\(^{2+}\) through Ca\(^{2+}\) channels (Bean 1989) and fails to activate Ca\(^{2+}\)-activated K\(^+\) currents. When Ba\(^{2+}\) was used as the charge carrier, an inward current was observed (Fig. 13C) that was also completely blocked by 200 \( \mu \text{M Cd}^{2+}\) (Fig. 13D), at least within the limits of the resolution of the present recording conditions. This Ba\(^{2+}\) current, like the Ca\(^{2+}\) current described above, was sensitive to Cd\(^{2+}\) and also shows two components: a transient one and a sustained component (Fig. 14B). This suggests that Ba\(^{2+}\) permeates through the same channels as Ca\(^{2+}\).

With the use of \( i_{\text{Ca}^{2+}}\) as an assay for \( i_\text{Ca} \), we tested a number of blockers of known Ca\(^{2+}\) currents. Ten millimolar of Mn\(^{2+}\) and 10 mM Co\(^{2+}\) block \( i_{\text{Ca}^{2+}}\) with an effectiveness similar to that of 200 \( \mu \text{M Cd}^{2+}\). Fifty to two hundred millimolar of Ni\(^{2+}\), which blocks some known Ca\(^{2+}\) currents (Susuki and Rogawski 1982; Tsien et al. 1988), did not affect \( i_{\text{Ca}^{2+}}\). Finally, the dihydropyridine nifedipine, known to block a subclass of Ca\(^{2+}\) currents (Bean 1989; Susuki and Rogawski 1982; Tsien et al. 1988), had no apparent effect on \( i_\text{Ca} \).

The TTX-sensitive Na\(^+\) current underlying action-potential generation cannot be studied under somatic voltage clamp in the LP neuron. The action potentials are generated far enough from the cell body so that the spikes are attenuated in the soma (Fig. 3A) and are not subject to
The results presented here provide a first description of the major voltage-dependent ionic currents seen in the LP neuron of the crab STG. Obviously, we do not mean to imply that there are no additional currents to be recorded in these neurons. Indeed, it is likely that the modulatory inputs, the action of which we removed, might amplify or activate other currents that were undetectable under the conditions we employed. We anticipate that, as work on these currents continues, it may be possible to identify several different classes of Ca$^{2+}$ currents or Ca$^{2+}$-activated currents. However, despite these caveats, we have been able to define those currents that are likely to contribute in a major way to the activity of the LP neuron, at least in the absence of exogenously applied neuromodulatory sub-

stances. Further work will be required to determine, for each of the many substances that can modulate the activity of the LP neuron (Golowasch and Marder 1992; Hooper and Marder 1987; Marder et al. 1986; Marder and Weimann 1992; unpublished observations; Nusbaum and Marder 1988, 1989a,b), the currents that each activates or influences.

**Outward currents**

We have identified three main outward currents that activate at membrane potentials more positive than $V_{rev}$ (−49 mV, Table 1): a delayed rectifier-like current, an A-like current, and at least one type of Ca$^{2+}$-dependent outward current.

We argue that $I_{a}$, $I_{Ca}$, and $I_{K}$ are K$^{+}$ currents, or at least predominantly carried by K$^{+}$ for several reasons. The pharmacology of these currents is consistent with that of well-known K$^{+}$ currents of a number of different species. Although we would have liked to measure the actual reversal potential of these currents from tail current measurements and study its dependence on extracellular K$^{+}$, the large capacitance of our neurons made this impossible because we were unable to clamp the voltage during the initial 10–20 ms after an abrupt potential change (see Fig. 4), and tail currents have substantially decreased by 20 ms after the pulse. Nevertheless, we tried to determine the reversal potentials of these currents by using tail currents of total currents measured 20 ms after the voltage pulse. These tail currents were sensitive to extracellular [K$^{+}$] and changed in the direction expected from the Nernst equation for K$^{+}$ (not shown). However, because we cannot determine the ionic selectivity of all the outward currents individually, for the calculation of maximum chord conductances we have assumed a reversal potential equal to a K$^{+}$ equilibrium potential of −80 mV for all three outward currents. This value for the K$^{+}$ equilibrium potential is taken from the fact that a γ-aminobutyric acid (GABA)-activated K$^{+}$ response in the same cell, with a reversal potential between −80 and −90 mV, behaves as a K$^{+}$ electrode (Marder and Paupardin-Tritsch 1978; Golowasch 1990).

The Ca$^{2+}$-dependent inactivation of $I_{Ca}$ at high intracellular Ca$^{2+}$ concentrations is interesting. By analogy with the Ca$^{2+}$ block of Ca$^{2+}$ currents (cf. Morad et al. 1988), it would suggest that Ca$^{2+}$ can bind to the intracellular face of the pore of the channels underlying $I_{Ca}$, thus reducing K$^{+}$ flux through them, and perhaps even permeate through them (Tsien et al. 1987).

Interestingly, cells from other related decapod crusta-

cceans such as the lobster *Panulirus interruptus* (Graubard and Hartline 1988, 1991) and the lobster *Homoerus americanus* (Tasaki and Cooke 1986) exhibit the same three outward currents as those here described for *C. borealis*, albeit with some differences.

1) In *C. borealis*, $I_{a}$ is partially and reversibly blocked by TEA, similarly to what is seen in the lobster *H. americanus* (Tasaki and Cooke 1986) as well as a number of other preparations (Cobbett et al. 1989; Frech et al. 1989; Jones and Adams 1987; Thompson 1977). As in these cases, the estimated $K_{d}$ varied between 0.5 and 1 mM, and, as in other
preparations, $i_{h}$ is not affected by Cd$^{2+}$ or by 4-AP. Furthermore, $i_{h}$ has the typical delayed onset that becomes faster at more depolarized voltages (Fig. 5) that characterizes and defines the delayed rectifier currents.

2) $i_{h}$ shows the typical voltage dependence of both activation and inactivation observed in other preparations: it usually activates at more positive voltages than $V_{res}$ but at more hyperpolarized voltages than does $i_{k}$ (compare Figs. 5B and 9B), which is also seen in the lobsters $P.\ interruptus$ (Graubard and Hartline 1991) and $H.\ americanus$ (Tsaki and Cooke 1986) as well as a number of other cells (Cobett et al. 1985; Neher 1971; Thompson 1977; Zbic and Weight 1985). On the other hand, $i_{k}$ inactivates at voltages below the threshold for its own activation (Fig. 9), although a small amount of overlap exists, similar to that observed in $H.\ americanus$ (Tsaki and Cooke 1986) and other preparations (Connor 1975; Neher 1971; Zbic and Weight 1985), so that the cell has to be held at a voltage lower than $V_{res}$ to allow the activation of this current with subsequent depolarizing pulses. The level of inactivation that can be released with hyperpolarization shows time and voltage dependence (Fig. 10), which is also characteristic of other described A-currents (Connor 1975; Neher 1971; Tsaki and Cooke 1986; Zbic and Weight 1985).

$i_{h}$ in the crab looks similar to the same current recorded in the cardiac ganglion of the lobster $H.\ americanus$ (Tsaki and Cooke 1986). Some differences exist between $i_{h}$ of $P.\ interruptus$ (Graubard and Hartline 1991) and $C.\ borea-
lis, namely that in \textit{P. interruptus}, it is inactivated at \(-50\) mV and the half inactivation voltage is \(-75\) mV, whereas, in \textit{C. borealis}, \(i_h\) is inactivated only \(85\%\) at \(-50\) mV, and the half inactivation voltage is \(-61\) mV (Fig. 9, C and D). This implies that the activation and inactivation curves overlap substantially in \textit{C. sapidus} and \textit{C. magister}'s axons, as in \textit{C. borealis}' LP cell, they are shifted to more hyperpolarized voltages [compare our Fig. 9, B and D, with Fig. 3 in Connor et al. (1977)]. Interestingly, the walking leg axons show a baseline membrane potential around \(-60\) to \(-70\) mV during tonic action-potential firing, which coincides with the region where the activation and inactivation curves of \(i_h\) overlap. This allows \(i_h\) to have a noticeable influence on the spike rates of the leg axons (Connor et al. 1977). The LP cell's \(i_h\) activation and inactivation curves also overlap somewhat in the voltage range of the baseline of the tonically firing action potentials (Fig. 9, B and D). This suggests that \(i_h\) in the LP cell of \textit{C. borealis} should also have some effect on the firing frequency of the cell (see Golowasch et al. 1992).

3) \(i_{Ca}^{LQP}\) is sensitive to divalent cations; Ca\(^{2+}\), which activates it (Figs. 6, A and C, and 8), and Cd\(^{2+}\), which blocks it (Fig. 6, B and D). As with other reported Ca\(^{2+}\)-activated K\(^+\) currents (cf. Jones and Adams 1987; Pan and Behebani 1990; Stanfeld 1983; Zbicz and Weight 1985), \(i_{Ca}^{LQP}\) in \textit{C. borealis} is sensitive to TEA. The sensitivity of this current to TEA contrasts sharply with that of the cells in the cardiac ganglion of \textit{H. americanus}, where \(i_{Ca}(Ca)\) is completely insensitive to TEA (named \(i_h\) by Tasaki and Cooke 1986) but is similar to the \(i_{Ca}^{LQP}\) of the STG cells of \textit{P. interruptus} (Graubard and Hartline 1991).

We found that the maximum conductance ratios for the outward currents calculated from currents in the same cell are strikingly similar to the ratios of the averages of pooled maximum conductances from many different cells. This indicates that these currents adjust themselves to a constant relationship characteristic of the crab LP cell so that the LPs identity is reflected in the specific balance of conductances that produce its unique patterns of activity. This raises the fundamental question of what molecular mechanisms underly this balance of conductances.

**Inward currents**

The inwardly rectifying current found in the LP neuron resembles the \(i_h\) current described elsewhere (Angstadt and Calabrese 1989; DiFrancesco 1986; DiFrancesco et al. 1986; DiFrancesco and Tormba 1988). It is activated by hyperpolarization, it is blocked by millimolar concentrations of Cs\(^+\), and it reverses at voltages between \(-20\) and \(-30\) mV. However, the LP neuron's \(i_h\) is about an order of magnitude slower than that in the heart. A complication in the identification of this current is the fact that both Cs\(^+\) and Ba\(^{2+}\) block it. The cardiac \(i_h\) is blocked only by Cs\(^+\) (DiFrancesco and Noble 1989), whereas other inward rectifiers are blocked by both ions (Benson and Levitan 1983; Uchimura et al. 1989). However, the inward rectifier blocked by both Cs\(^+\) and Ba\(^{2+}\) is a K\(^+\) current (Benson and Levitan 1983; Uchimura et al. 1989), whereas the LP \(i_h\) is clearly not a K\(^+\) current because its reversal potential is near \(-20\) mV and \(E_K\) is close to \(-80\) mV.

The Ca\(^{2+}\) current expressed by the LP cell appears to consist of two components: a transient and a sustained component (Figs. 13 and 14). Because both components are blocked by Cd\(^{2+}\), it is possible that these two components reflect the activity of a current with complex kinetics and voltage dependence. Alternatively, there may be multiple Ca\(^{2+}\) currents that we have not been able to separate pharmacologically.

It is hard to fit the LP \(i_{Ca}\) into the currently accepted classification scheme of Ca\(^{2+}\) currents (T, L, and N type) (Bean 1989; Tsien et al. 1988). The block by Cd\(^{2+}\) and no effect of Ni\(^{2+}\) make it unlikely that our \(i_{Ca}\) is a T-type current. Moreover, the T-type current is a low-threshold activated current that is usually completely inactivated (Tsien et al. 1988) at the \(V_h\) used here to trigger the Ca\(^{2+}\) currents (Fig. 13). This rules out a low-threshold T-type current as the identity of the \(i_{Ca}\) we have thus far measured in the LP cell. We have seen no effect of bath applying the dihydropyridine antagonist, nifedipine, usually used to characterize the L-type Ca\(^{2+}\) current (Bean 1989; Susuki and Rogawski 1989; Tsien et al. 1988). Thus it seems that the Ca\(^{2+}\) current activated in the LP cell is a high-threshold current.

**FIG. 14.** Net Cd\(^{2+}\)-sensitive Ca\(^{2+}\) and Ba\(^{2+}\) currents. Data obtained from Fig. 13 by subtraction of currents obtained in the presence of 200 \(\mu\)M Cd\(^{2+}\) from control currents. A: Ca\(^{2+}\) currents. B: Ba\(^{2+}\) currents.
current with kinetics similar to those of the N and L types but with little or no dihydropyridine sensitivity.

Although the TTX-sensitive Na⁺ current cannot be measured in the LP cell, its existence is evident because action potentials are readily abolished with 10⁻⁷–10⁻⁶ M TTX or STX (see Fig. 2C).

The approach we have taken in this work differs from that taken by many biophysicists. Most often, cells are chosen for study because 1) they are easy to voltage clamp, 2) they provide a source of a particularly interesting and novel current, 3) they have a particular current that is relatively easy to isolate because it is present in large amounts or activates in a voltage range significantly different from others, or 3) they provide a cell in which a particular current’s role in shaping the activity of the cell can be evaluated. The above strategy is obviously ideal for studying the properties of ion channels under optimal conditions. However, if one eventually wishes to understand how the neurons that participate in the generation of a specific behavior are modulated on the basis of their underlying currents, it is necessary to describe the currents in cells that are not ideally suited for the evaluation of all of their ionic currents, or possibly any of them. In recent years we have been looking for examples of attempts to characterize the currents that contribute to a neuron’s electrical signature, or individual properties (Baxter and Byrne 1991; McCormick and Pape 1990; Susuki and Rogawski 1989; Yamada et al. 1989).

We argue that it is important to attempt to characterize the major components leading to the cell’s electrical activity because the nonlinear voltage- and time-dependent processes in a neuron make it almost impossible to intuit what effects any one current will have on the behavior of a cell without an understanding of all of them, because any given current will play a different role depending on the constellation of currents generating rise to the dynamical properties of the neuron. For this reason, we have found it particularly useful to use models (Buchholz et al. 1992; Golowasch et al. 1992) that incorporate the activity of these currents, to allow us to evaluate the role of each in the behavior of the neuron.

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Present address of J. Golowasch: Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46 rue d’Ulm, 75005 Paris, France.

Address reprint requests to E. Marder.

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