Dopamine Modulation of Two Delayed Rectifier Potassium Currents in a Small Neural Network

Matthias Gruhn,1 John Guckenheimer,2 Bruce Land, and Ronald M. Harris-Warrick1

1Department of Neurobiology and Behavior and 2Department of Mathematics, Cornell University, Ithaca, New York

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INTRODUCTION

Nonactivating delayed rectifier–type potassium channels generate a current, \( I_{K(V)} \), that is responsible for the fast repolarization of the membrane potential after action potentials (APs) in spiking neurons. It helps to determine the spike width and postspike hyperpolarization, and can help shape the maximal spike frequency of neurons (Rudy and McBain 2001; Shevchenko et al. 2004). Recently, \( I_{K(V)} \) channels have also been implicated in the prevention of AP generation in amacrine cells in the mouse retina and even in apoptosis-related K⁺ efflux (Ozaita et al. 2004; Pal et al. 2003). In vertebrates, \( I_{K(V)} \) is encoded by members of the Kv1, Kv2, and Kv3 subfamilies of potassium channel genes (Coetzee et al. 1999; Dodson and Forsythe 2004) and in invertebrates by the shab, shaw, and some splice variants of the shaker subfamilies of potassium channel genes (Covarrubias et al. 1991; Kim et al. 1998; Tsunoda and Salkoff 1995a,b). Nonactivating delayed rectifier channels are expressed in the somatodendritic as well as in the axonal and synaptic compartments of both mammalian and invertebrate cells (Dodson and Forsythe 2004; Martinez-Padron and Ferrus 1997). Within the same cell or cell population, different types of delayed rectifier channels can be expressed in parallel, and create specific mixed conductance–voltage relationships (Baranauskas et al. 1999; Covarrubias et al. 1991; Rothman and Manis 2003).

The stomatogastric ganglion (STG) of the spiny lobster Panulirus interruptus has been a valuable model system for the study of ionic currents and their contribution to shaping the rhythmic output of a neural network (Calabrese 2004; Golowasch et al. 1992; Graubard and Hartline 1991; Harris-Warrick 2002; Harris-Warrick et al. 1992; Hartline 1979). The STG contains the pyloric network of 14 neurons that controls rhythmic contractions of the posterior crustacean foregut (Harris-Warrick et al. 1992). In recent years, we have accumulated knowledge about many of the currents that take part in determining the unique firing properties of neurons in this small network, including a rapidly inactivating potassium (A) current (Baro et al. 1996b; Golowasch and Marder 1992; Graubard and Hartline 1991; Kim et al. 1997), the H-current (\( I_h \)) (Harris-Warrick et al. 1995b; Peck et al. 2004), voltage-sensitive calcium current (Johnson et al. 2003), and a Ca-dependent K⁺ current (Graubard and Hartline 1991; Kloppenburg et al. 1999). A detailed analysis of the nonactivating delayed rectifier–type current in the STG, however, is still missing (Kloppenburg et al. 1999).

All of the currents mentioned above are subject to modulation. Neuronomodulators such as biogenic amines or neuropeptides play crucial roles in shaping the output of the pyloric network (Ayalai and Harris-Warrick 1999; Marder and Thrumalai 2002). Dopamine (DA), for example, modulates the motor patterns of the pyloric circuit in the STG by its differential effect on pyloric neurons and synapses (Harris-Warrick et al. 1998). Generally, in the presence of DA, the cycle frequency of the pyloric circuit is modestly decreased (Ayalai and Harris-Warrick 1999). At the same time, the spike frequency of several neurons is increased, whereas other neurons are inhibited (Flamm and Harris-Warrick 1986a,b). The observed changes are in part the result of modulatory effects on \( I_A \), \( I_{K(Ca)} \), \( I_h \), and \( I_{Ca} \) in the different neurons (Harris-Warrick et al. 1995a,b; Johnson et al. 2003; Kloppenburg et al. 1999; Peck et al. 2001) as well as its effects on synaptic transmission at

Address for reprint requests and other correspondence: M. Gruhn, Universitäts zu Köln, Zoologisches Institut, Tierphysiologie, Weyertal 119, 50923 Köln, Germany (E-mail: mgruhn@uni-koeln.de).

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chemical and electric synapses (Ayali et al. 1998; Johnson and Harris-Warrick 1990; Johnson et al. 1995).

Here we describe the properties of the delayed-rectifier–type current $I_{K(V)}$ in the pyloric network of the STG and its cell-specific modulation by dopamine. Partial results of this work have been published in abstract form (Gruhn and Harris-Warrick 2003; Gruhn et al. 2004).

**METHODS**

**Animals**

Spiny lobsters (*Panulirus interruptus*) were obtained from Don Tomlinson Fishing (San Diego, CA) and maintained at 16°C in artificial seawater tanks for ≤4 wk. Chemicals, unless stated otherwise, were obtained from Sigma Chemicals (St. Louis, MO).

**Dissection and identification of neurons**

Animals were anesthetized on ice for ≥30 min. The stomatogastric ganglion (STG) was dissected along with the commisural and esophageal ganglia, as described by Selverston et al. (1976), and pinned out in a Sylgard-coated dish. The desheathed preparation was continuously superfused with 16°C oxygenated *Panulirus* saline at 3 ml/min, with the following composition (in mM): 479 NaCl, 12.8 KCl, 13.7 CaCl$_2$, 3.9 Na$_2$SO$_4$, 10 MgSO$_4$, 2 glucose, 11.1 Tris-base, and 5.1 maleic acid, pH 7.35 (Mulloney and Selverston 1974). Extracellular recordings from identified motor nerves were made with bipolar suction electrodes. Individual pyloric neurons were identified through intracellular recording with glass microelectrodes (10–20 MΩ; 3 mM KCl). Criteria for identification were a 1:1 correlation of intra- and extracellular spikes in pyloric neurons and identified motor nerves, characteristic phasing of neuron activity in the pyloric motor pattern, and the characteristic membrane potential oscillations in the pyloric rhythm.

**Electrical recordings in single pyloric neurons and block of currents**

Pyloric neurons were isolated from most synaptic input by the application of 0.1 μM tetrodotoxin (TTX), to block action potential propagation and neuromodulatory input from other ganglia, and 5 μM picrotoxin (PTX), to block glutamatergic synapses within the pyloric network. CsCl (5 mM) was used to block $I_h$ and $I_A$ was removed by holding cells at −40 mV at which the current is inactivated. To isolate $I_{K(V)}$, the perfusion saline additionally contained 0.8 mM CdCl$_2$ to block $I_{KC}$. $I_{Ca}$ as well as remaining synaptic inputs. This concentration appeared to block Ca-dependent currents in the lateral pyloric (LP) neuron more effectively than 0.5 mM CdCl$_2$. We did not detect any signs of toxicity at this concentration because stable recordings of $I_{K(V)}$ with constant holding currents were possible over several hours.

Two-electrode voltage-clamp (TEVC) recordings were performed with an Axoclamp 2B amplifier using pClamp8 software (both Axon Instruments, Foster City, CA). For current injection, glass microelectrodes with resistance of 8–12 MΩ were used. Linear leak was subtracted digitally with a p/8 protocol (Armstrong and Bezanilla 1974).

$I_{K(V)}$ characterization and DA effect

Voltage steps of 500-ms length were given in 5-mV increments between −30 and +45 mV from the holding potential of −40 mV. Current amplitude was measured as steady-state current at the end of each step. The pharmacology of $I_{K(V)}$ was tested by application of 4-aminopyridine (4-AP, 4–20 mM), tetraethylammonium chloride (TEA, 5–100 mM), and quinidine (100 μM to 1 mM), which were all dissolved in perfusion saline. When using 50 and 100 mM TEA, the NaCl concentration was lowered to avoid a change in osmolarity. In all 4-AP experiments, recordings were started 30 min after superfusing the blocker because of a temporary and reversible leak current evoked by 4-AP previously reported in pyloric dilator (PD) neurons (Kloppenburg et al. 1999). TEA and quinidine were superfused for ≥10 min before recording their effects on $I_{K(V)}$. The dose dependency of the block was tested by superfusing 4-AP, TEA, and quinidine at a specific concentration until a stable level of block was observed, and then stepping up the blocker concentration and repeating this procedure. 4-AP-, TEA-, and quinidine-sensitive currents were analyzed after digital subtraction from control currents.

Dopamine (DA, 0.1 mM) was freshly dissolved in saline containing 0.8 mM CdCl$_2$, 5 mM CsCl, 0.1 μM TTX, and 5 μM PTX immediately before application. $I_{K(V)}$ was measured before DA perfusion, 5 min after beginning perfusion, and 20–30 min after the end of perfusion (wash). Current was converted into conductance $g$, assuming $E_g = −38$ mV (Hartline and Graubard 1992). Cells showing a continuous reduction in current under control conditions or a lack of reversibility of the DA effect were discarded from the analysis.

Conductance was normalized against conductance at the +40-mV step for each cell and then averaged. For the studies of blockers and DA, conductance values were normalized against the conductance of the control value at the +40-mV step of the same cell and then averaged. Error values throughout text and figures are given as SDs.

**Mathematical separation of two components of $I_{K(V)}$**

The conductance was plotted as a function of voltage and was modeled as the sum of an initial saturable Boltzmann component and an exponential component. This was done because the data points we could reliably obtain for the second component represented only the rising phase of its Boltzmann relation and this could not be reliably fit by a second Boltzmann relation. Because the early rising phase of a Boltzmann relation resembles an exponential relationship, we used the estimated exponential to subtract the second component away and get a good Boltzmann fit for the first phase. We certainly believe that both currents represent typical channels with saturable activation, but we were unable to fit the second component with a full Boltzmann relation attributed to its depolarized activation range.

The analysis was performed with the following formula

$$g = g_{max}/[1 + e^{(V-V_{1/2})/s}] + p1 \times e^{(V-V_{2/2})/p2}$$

where $g$ and $V$ are the experimental conductance and voltages, with $V$ in millivolts. Five parameters were estimated from a curve-fitting process. $V_{1/2}$ is the voltage of half-activation of the lower threshold, saturable, Boltzmann-fitted component; $s$ is its slope; $g_{max}$ is its maximum conductance; and $p1$ and $p2$ are parameters of the high-threshold, nonsaturable current component. They reflect the quasi-exponential activation of a current that did not start to saturate at +45 mV and could not be fit by a Boltzmann relation, and thus do not represent biophysically realistic parameters. Estimation of the parameters and their errors was performed by repeated nonlinear curve fits of the conductance data. The value of 45 in the second term of the equation was chosen arbitrarily to scale the value of the coefficients for the conductance so that they have values that are not too far from one; it has no intrinsic physiological meaning.

Uncertainty of the parameter estimate could come from two sources: noise in the experimental data and failure of the curve fit to find a true minimum in the five-dimensional parameter landscape. Parametric error estimates resulting from experimental noise were handled by formal error propagation techniques. Many repetitions allowed us to estimate the uncertainty in the parameters arising from convergence of.
the nonlinear curve fit to false minima in the five-dimensional parameter landscape. Generally, the uncertainty in parameters was dominated by false minima, but showed a strong central tendency clustered around certain values.

Curve fitting and error analysis were carried out using Matlab (mathworks.com) and the Matlab Statistics toolbox, particularly the function \textit{nlinfit}. Formal error propagation was estimated using the Statistics toolbox routine \textit{nlparci}. Full details and the program are available at http://www.nbb.cornell.edu/neurobio/land/PROJECTS/MKG23curvefit/index.html.

The curve fit was performed several hundred times for each data set, using different starting estimates for the parameters. Each of five starting parameter estimates was drawn from a normal distribution with a mean determined through initial fitting with Kaleidagraph (v. 3.09, Synergy Software) and with SD of 30% of the mean. The distribution of each parameter (over many fitting runs) was plotted, so that a central tendency could be judged by eye. For the majority of cells, it was judged that there was a reasonable central tendency, and error ranges were calculated in a nonparametric fashion by finding the parameter values that included 95% of the computed parameter values.

Current traces were filtered at 500 Hz before export from pClamp8 (Axon Instruments) to Photoshop 6.0 (Adobe) for preparation for the figures. Plots were prepared with Origin (v. 6.1, Origin Lab, Northampton, MA).

Statistical analysis was carried out by ANOVA followed by protected $t$-test of specific cell pairs or DA-control pairs. Significant changes were accepted at $P < 0.05$; error bars in the figures represent the SD.

\textbf{RESULTS}

\textit{I}_{K(V)} consists of two components in pyloric neurons

We performed two-electrode voltage clamp on the six different pyloric cell types to characterize their delayed rectifier-type potassium currents [$I_{K(V)}$]. The cells were held at $-40 \text{ mV}$ to inactivate $I_A$, whereas $I_{Na}$ was blocked with 0.1 $\mu$M TTX, $I_{Ca}$ and $I_{K(Ca)}$ were blocked with 0.8 mM CdCl$_2$, and $I_h$ was blocked with 5 mM CsCl. All pyloric cell types expressed an $I_{K(V)}$ that consists of two current components with very different voltage activation ranges. In a majority of neurons among all cell types, this results in a $g/V$ curve that has a marked bulge in the range of $-10$ to $+10 \text{ mV}$, followed by a rising increase in current at higher voltages (e.g., Fig. 1E). However, even within a single cell type, there were marked differences in the relative proportions of the two current components. Based on their respective relative voltages of activation the two components were termed low-voltage–activated (LVA) and high-voltage–activated (HVA) $I_{K(V)}$.

Figure 1 shows three different pyloric dilator (PD) neurons, two cells (Fig. 1, A and C) representing extreme current compositions, and one cell (Fig. 1B) representing the majority of cells. Figure 1, D–F shows the corresponding conductance–voltage plots; Figure 1, A and D shows a neuron possessing a majority of LVA current. In the current traces, the additional current at each step increases nonlinearly up to the maximal activation, above which the additional voltage steps evoked

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Current and voltage traces showing variability of delayed rectifier potassium current [$I_{K(V)}$] in 3 different pyloric dilator (PD) neurons. Currents (A–C) were measured during 500-ms voltage steps from a holding potential of $-40 \text{ mV}$ to between $-30$ and $+40 \text{ mV}$ in 5-mV steps. D–F: corresponding conductance–voltage plots. A and D: a PD possessing a majority of low-voltage–activated (LVA) current; the $g/V$ plot shows that this current approaches saturation at high voltages. B and E: a mix of both LVA and high-voltage–activated (HVA) currents. Resulting $g/V$ plot (E) shows a marked bulge in the voltage range where the LVA component is saturating and the HVA component is just beginning to be expressed. C and F: a PD neuron that has primarily the HVA component. Here, the current steps increase continuously with voltage throughout the voltage range that we could obtain, giving rise to an apparently exponential $g/V$ relation (F).}
\end{figure}
currents that increased linearly with voltage as a result of the increased driving force on the current. The g/V plot shows that this current approaches saturation at high voltages. Figure 1, B and E shows the most typical results, with a mix of both LVA and HVA currents. The current increases and appears to saturate at intermediate voltages as in Fig. 1A, but then begins to increase a second time with higher voltage steps as the HVA component becomes activated. The resulting g/V plot shows a marked bulge in the voltage range where the HVA component is saturating and the HVA component is just beginning to be expressed. Finally, Fig. 1, C and F shows a PD neuron that has primarily the HVA component. Here, the current increases non-linearly with each voltage step throughout the voltage range that we could obtain, giving rise to the apparently exponential g/V relation seen in Fig. 1F. The majority of PD cells (n = 69) showed both currents in clearly visible, albeit variable amounts; only a minority showed primarily LVA (n = 6) or HVA (n = 13) currents.

Similar variability in the relative amounts of LVA and HVA components in I_{K(V)} was seen in the other pyloric neurons. In the lateral pyloric (LP) neuron, 23 cells (68%) showed a clear composite current, whereas 11 cells predominantly showed either the LVA (n = 8) or the HVA (n = 3) component. In pyloric (PY), ventricular dilator (VD), and inferior cardiac (IC) neurons, 51, 73, and 54% of the respective cell type showed the composite of two currents, whereas the remaining cells split into equal numbers showing either current predominantly. Of the 10 anterior burster (AB) cells measured, nine showed both components and only one had no clear HVA component.

To separate these two currents, we fit the conductance–voltage relation with a formula that combines the Boltzmann function for the LVA component with an exponential function for the HVA component (see METHODS). We believe that the second component is a normal saturable current that activates at depolarized levels but could in theory be fit by a Boltzmann relation. However, repeated attempts to fit the data as the sum of two Boltzmann components were unsuccessful; the HVA component is still rising quasi-exponentially at the highest voltages we could clamp the neuron, and these data were not adequate to constrain the fit by a Boltzmann function. Thus to subtract out this current and allow a detailed study of the LVA component, we were forced to approximate it by an arbitrary exponential relation that does not yield any biophysically realistic parameters beyond measures of amplitude at different voltages. This formula gave a good fit to the majority of g/V plots for all cell types, although the currents could not be adequately separated in many of our neurons. We subsequently separated the Boltzmann (LVA) and exponential (HVA) components using a mathematical program (Matlab; see METHODS) (Fig. 2). The LVA component activates at voltages around −25 mV and saturates around +20 mV, whereas the HVA component is very small at voltages <0 mV and does not begin to saturate at voltage steps up to +45 mV. As a test for the accuracy of the Boltzmann fit of the LVA current, we subsequently refit the separated conductance–voltage relation to a single Boltzmann relation; its parameters did not differ significantly from the values for the LVA current obtained from the composite analysis of the combined currents. The mathematical separation also allowed us to determine the amplitude of the HVA component, but not its Boltzmann parameters. We chose to measure the fitted conductance at +40 mV where the current was well clamped in all cells and cell types, to compare its amplitude among cell types and during dopamine application. The values for all the fitted parameters of the LVA as well as the conductance at +40 mV of the HVA are given in Table 1. Generally, the LP neuron has the greatest LVA conductance (0.35 ± 0.12 μS), with PD being second (0.29 ± 0.11 μS). The PY (0.25 ± 0.06 μS) and VD (0.25 ± 0.08 μS) neurons have similar but smaller amounts of this conductance, whereas the IC (0.21 ± 0.08 μS) and AB (0.19 ± 0.04 μS) neurons have the smallest amounts among the pyloric neurons. The LVA values for LP are significantly greater than those for all other cells. LVA in the LP and PD neurons are also significantly larger than the two neurons with the smallest conductance (IC and AB). The significance values were tested by one-way ANOVA, followed by protected t-test of individual cell type pairs (P < 0.05). The V_{1/2} of this current ranges from −7.9 mV in AB to −12.3 mV in LP, whereas the slope value ranges from −7.1 mV in PY to −10.2 mV in AB cells. There is a large degree of overlap in the range of parameters between different neurons.

FIG. 2. Separation of I_{K(V)} into LVA and HVA components. A: average g/V plot of I_{K(V)} in 69 PD neurons after superfusing the ganglion with Panulirus saline containing 5 mM CsCl, 0.1 μM tetrodotoxin (TTX), 5 μM picrotoxin (PTX), and 0.8 mM CdCl2 for a minimum of 30 min. Note the clear bulge around 10 mV. B: average total conductance from A (●) and mathematically separated LVA (○) and HVA (▲) components. Error bars mark SD.
When measured at +40 mV, the LP neuron has the greatest HVA conductance (0.29 ± 0.27 μS), closely followed by the VD neuron (0.27 ± 0.16 μS). The PD and PY neurons have similar but smaller amounts (0.20 ± 0.15 and 0.16 ± 0.19 μS, respectively), whereas the IC and AB neurons have the smallest amounts of HVA conductance (0.11 ± 0.05 and 0.06 ± 0.03 μS, respectively). Thus with the exception of the VD neuron, the pyloric neurons have the same rank order for both LVA and HVA currents. The ratio of LVA to HVA conductance was compared between the neurons (Table 1). At the lower end of the spectrum were the LP and VD neurons with LVA to HVA ratios of 1.2:1 and 0.9:1, respectively.

Although we have referred to the LVA and HVA components as separate currents, it is formally possible that there is only a single current with LVA-like characteristics. In this case, the exponentially rising high-threshold component would appear as an artifact of poor space clamp of additional LVA currents, we studied their sensitivity to three known potassium channel blockers: TEA, quinidine, and 4-AP. In PD neurons, bath application of 5–100 mM TEA reduces both components of the measured I_{K(V)} down to about 10% of the initial value in a dose-dependent manner.

Figure 3, A and B shows a PD neuron in control and in 50 mM TEA. The g/V plot in Fig. 3C shows the average normalized dose response to TEA for four PD neurons. The remaining current at each TEA concentration was digitally subtracted from the control current to determine the TEA-sensitive part of the conductance (Fig. 3D). This shows that both components are equally blocked at all concentrations of TEA used because the bulge from the control graph is visible in all the TEA-sensitive conductance traces. Unfortunately, we were unable to clearly separate the LVA from the HVA components of the currents in these experiments, so a more quantitative analysis of the block of the two components was not possible.

We also tested whether other potassium channel blockers would differentially affect the two current components. Quinidine has been shown to differentially block two delayed rectifier–type potassium currents in *Drosophila* larval muscles in a dose-dependent manner (Singh and Singh 1999). The more sensitive of these two currents is encoded by the *shab* gene, whereas the second remains unidentified. We tested whether the two delayed rectifier–type currents in the spiny lobster are also differentially affected by quinidine. We applied quinidine over a concentration range from 100 μM to 1 mM. At equilibrium in 100 μM quinidine, 35% of the total current was blocked. The block reaches 60% at 500 μM quinidine, and in

### Table 1. Properties of LVA and HVA components of I_{K(V)} in pyloric neurons

<table>
<thead>
<tr>
<th>Neuron type</th>
<th>g_{max}, LVA, μS</th>
<th>V_{1/2} LVA, mV</th>
<th>Slope LVA</th>
<th>HVA Amplitude at +40 mV, μS</th>
<th>LVA:HVA Ratio at +40 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD (n = 69)</td>
<td>0.29 ± 0.11</td>
<td>−9.5 ± 3.0</td>
<td>−8.3 ± 2.2</td>
<td>0.20 ± 0.15</td>
<td>1.5:1</td>
</tr>
<tr>
<td>LP (n = 23)</td>
<td>0.35 ± 0.12</td>
<td>−12.3 ± 2.9</td>
<td>−7.5 ± 2.2</td>
<td>0.29 ± 0.27</td>
<td>1.2:1</td>
</tr>
<tr>
<td>PY (n = 21)</td>
<td>0.25 ± 0.06</td>
<td>−10.8 ± 3.9</td>
<td>−7.1 ± 1.0</td>
<td>0.15 ± 0.19</td>
<td>1.6:1</td>
</tr>
<tr>
<td>VD (n = 16)</td>
<td>0.25 ± 0.08</td>
<td>−9.6 ± 1.8</td>
<td>−8.3 ± 1.8</td>
<td>0.27 ± 0.16</td>
<td>0.9:1</td>
</tr>
<tr>
<td>IC (n = 7)</td>
<td>0.21 ± 0.08</td>
<td>−9.2 ± 3.6</td>
<td>−9.6 ± 2.7</td>
<td>0.11 ± 0.05</td>
<td>1.9:1</td>
</tr>
<tr>
<td>AB (n = 9)</td>
<td>0.19 ± 0.04</td>
<td>−7.8 ± 2.5</td>
<td>−10.2 ± 1.7</td>
<td>0.06 ± 0.03</td>
<td>3.2:1</td>
</tr>
</tbody>
</table>

Values are means ± SD. LVA, low-voltage activated; HVA, high-voltage activated.
one case after application of 1 mM quinidine ≤80% of \(I_{K(V)}\) was blocked. Figure 4 shows an example of \(I_{K(V)}\) in a PD cell and its block by application of 500 \(\mu M\) quinidine (Fig. 4, A and B). The concentration-dependent block of \(I_{K(V)}\) averaged from three PD neurons and the respective g/V plots are shown in Fig. 4C. Analysis of the quinidine-sensitive current revealed that low concentrations of quinidine preferentially but only partially block the LVA current, with little detectable effect on the HVA current. This block is more complete at higher quinidine concentrations, but at concentrations \(\leq 500\) \(\mu M\), both LVA and HVA currents are reduced. Thus the LVA and HVA currents show a different concentration dependency of block by quinidine.

4-AP is a known blocker of the transient potassium current \(I_A\), but it also blocks two delayed rectifier–type currents in \textit{Drosophila} (Singh and Singh 1999). We applied 4-AP in concentrations of 4, 10, and 20 mM. With 4 mM 4-AP the block of \(I_{K(V)}\) rarely exceeded 25%; even at 20 mM 4-AP, the total block never exceeded 50%. Figure 5 shows an example of \(I_{K(V)}\) in a PD cell and its block by application of 10 mM 4-AP (Fig. 5, A and B). The concentration dependency of 4-AP block averaged from five PD neurons is shown in Fig. 5C, and the 4-AP–sensitive current is shown in Fig. 5D. At the lowest concentration used, 4 mM, 4-AP clearly differentially blocks the LVA current component more than the HVA component; the 4-AP–sensitive current predominantly saturates at +10 to +20 mV, as the isolated LVA component does. However, at higher concentrations, the HVA component is partially blocked as well (Fig. 5D). Thus the HVA and LVA currents also show a different dose dependency of block by 4-AP.

We also performed double-blocker experiments, testing the effect of sequential application of 4-AP or quinidine with TEA, or TEA with 4-AP or quinidine. The partial block of \(I_{K(V)}\) by either 4-AP or quinidine alone could always be enhanced by the application of TEA, demonstrating that quinidine and 4-AP fail to block all \(I_{K(V)}\). On the other hand, the TEA block was more complete and occluded any additional block by 4-AP or quinidine. In an experiment where we sequentially applied 4-AP and quinidine, currents not blocked by 4-AP could be further reduced by quinidine, whereas currents not blocked by quinidine could not further be reduced by 4-AP (data not shown). These experiments demonstrate that TEA is the most nonspecific of the \(K^+\)-channel blockers tested on the LVA and HVA currents. Low concentrations of quinidine and 4-AP selectively block the LVA component, but quinidine appears to be less specific than 4-AP, blocking a significant amount of the HVA current at the highest concentrations. Although we were unable to separate the blocked currents mathematically because of poor fits of the respective g/V plots in the presence of blockers, these results argue further that the LVA and HVA
components are independent potassium currents and not the result of poor space clamp of a single distributed current.

Dopamine modulates $I_{K(V)}$ in selected pyloric neurons

Dopamine (DA) has a profound effect on the firing pattern of the actively cycling pyloric network. DA excites the AB, LP, PY, and IC neurons, increasing their maximal spike frequency during their bursts, while inhibiting the PD and VD neurons (Flamm and Harris-Warrick 1986a,b). To see whether modification of $I_{K(V)}$ could contribute to these effects, we tested the effect of 0.1 mM DA on $I_{K(V)}$ in all pyloric cell types. DA had no effect on $I_{K(V)}$ in the PD neuron, as previously reported (Kloppenburg et al. 1999). DA also did not significantly affect $I_{K(V)}$ in the LP, VD, and IC neurons (data not shown, $n = 6, 5,$ and 3, respectively).

However, the major pyloric pacemaker neuron, the AB interneuron, showed a reversible increase in steady-state $I_{K(V)}$ of $\approx 40\%$ (average $22 \pm 18\%$, $n = 4$) in the presence of DA. Figure 6, A–C shows the current traces under control conditions, after a 5-min application of 0.1 mM DA and during wash after DA. The $g/V$ plot in Fig. 6D shows that the effect is noticeable at $-15$ mV, and becomes statistically significant at voltage steps above $+5$ mV. The mathematical separation of the two currents in the four cells measured was unsuccessful in these cases, as a result of the small size of the HVA component, which could not be reliably fit. Nonetheless, it appears that both components are enhanced by DA.

A majority of the PY neurons also showed a significant and reversible increase in steady state $I_{K(V)}$ during DA application. There are eight PY neurons in P. interruptus, which show varying excitatory responses to DA (Johnson et al. 2004). DA was applied to nine PY cells that showed a clear bulge under control conditions. Figure 7, A–C shows sample current traces from a typical experiment, whereas the normalized $g/V$ plot of the averaged DA effect from all nine PY cells is given in Fig. 7D. The DA enhancement becomes significant above 0 mV. Although there was variability in strength of the DA effect, eight out of nine PY neurons showed a DA-dependent reversible increase in steady-state total $I_{K(V)}$ of between 17 and 38% (average $29 \pm 8\%$, $n = 8$); one PY neuron showed no effect (Fig. 7E).

We were able to separate the PY neuron composite currents into their LVA and HVA components. This analysis demonstrated that DA increases the conductance of both components (Fig. 8, A and B). The LVA $g_{\text{max}}$ is increased by $26 \pm 35\%$. Boltzmann analysis of the LVA component revealed that the DA effect was limited to the maximal conductance: the $V_{1/2}$ and the slope parameters of this component were not affected by DA in PY neurons (Table 2). The HVA conductance at $+40$ mV was also enhanced, by $330 \pm 480\%$; however, this very large increase predominantly arises from a very large effect in one neuron that had a very small initial HVA current, with most neurons showing $<100\%$ increase (Fig. 8D). The increase in the LVA component is significant above $-5$ mV.
whereas the increase in the HVA current is significant above +10 mV. The enhancement of the LVA component washes out more poorly than the HVA component. When comparing DA actions on the PY neurons, it is clear that there is a continuum of responses among PY neurons. In two cells either the LVA or the HVA component actually decreased during DA, despite a net increase in total $I_{K(V)}$ conductance in those cells (Fig. 8, C and D).

**FIG. 5.** 4-Aminopyridine (4-AP) selectively blocks the LVA component of $I_{K(V)}$. Current traces of $I_{K(V)}$ under control conditions (A) and after perfusion with 10 mM 4-AP (B). C: normalized $g/V$ relationship of the total conductance in 5 PD neurons under control conditions (■), after equilibration with 4 mM 4-AP (●), 10 mM 4-AP (▲), and 20 mM 4-AP (▲). D: normalized $g/V$ relationship of 4-AP-sensitive conductance after equilibration with 4 mM 4-AP (■), 10 mM 4-AP (●), and 20 mM 4-AP (▲). Shape of the $g/V$ plot shows selective but incomplete block of the LVA component at 4 mM 4-AP and a less selective block of both LVA and HVA components at higher concentrations.

**FIG. 6.** Dopamine (DA) enhances $I_{K(V)}$ in the anterior burster (AB) neuron. A–C: current traces of an AB neuron under control (A), after 5-min perfusion with 0.1 mM DA (B) and after 30-min wash (C). D: normalized $g/V$ relationship of $I_{K(V)}$, total conductance under control (■), DA (●), and wash (▲) for 4 AB neurons. Asterisk at the +10-mV step marks the voltage above which the DA trace differs significantly from the control and wash.
FIG. 7. DA enhances $I_{K(V)}$ in most pyloric (PY) neurons. A–C: current traces of a PY neuron under control (A), after 5-min perfusion with 0.1 mM DA (B) and after 20-min wash (C). D: normalized $g/V$ relationship of $I_{K(V)}$ total conductance under control (●), DA (○), and wash (▲) for 9 PY neurons. Asterisk at the 0-mV step on the DA trace marks the voltage from which it differs significantly from the control. Error bars mark SD. In some pyloric neurons such as this one, the total current appears to show some inactivation at high voltages, which is eliminated by DA. This may reflect a small contamination of the current with $I_A$, which was not completely eliminated by holding at $-40$ mV. DA reduces $I_A$ in PY neurons (Harris-Warrick et al. 1995), possibly explaining this modest effect. E: percentage change in steady-state $I_{K(V)}$ at +40 mV after perfusion with DA in 9 different PY cells.

FIG. 8. DA effect on separated LVA and HVA components of $I_{K(V)}$ in PY neurons. g/V relationships of the LVA component (A), and HVA component (B) under control (●), after 5-min perfusion with 0.1 mM DA (○) and after 15-min wash (▲). Asterisk marks the voltages in DA above which the increase becomes significant ($P < 0.05$). C and D: percentage change of the LVA (C) and HVA (D) components under DA in all 9 PY neurons at +40 mV. Note that the cells are grouped according to the percentage change in conductance. Shades of gray correspond to the same neurons in C and D.
TABLE 2. Effect of dopamine on the low-voltage–activated component of $I_{K(V)}$ in PY neurons

<table>
<thead>
<tr>
<th>Component Values Alone</th>
<th>g_{\text{max}} \mu S</th>
<th>V_{1/2} \text{ mV}</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>PY control</td>
<td>0.22 ± 0.08</td>
<td>−11.5 ± 3.2</td>
<td>−6.8 ± 2.5</td>
</tr>
<tr>
<td>DA</td>
<td>0.26 ± 0.08*</td>
<td>−9.7 ± 3.4</td>
<td>−7.5 ± 2.9</td>
</tr>
<tr>
<td>Wash</td>
<td>0.24 ± 0.07</td>
<td>−9.1 ± 2.8</td>
<td>−8.2 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. Significant difference denoted by *, $P < 0.05$.

**DISCUSSION**

$I_{K(V)}$ is composed of two components

After blocking $I_{\text{Na}}$, $I_{\text{Ca}}$, $I_{K(Ca)}$, and $I_{h}$ and inactivating most of the transient potassium current $I_{A}$, we found that the majority of pyloric neurons express an $I_{K(V)}$ that appears to be made up of at least two components, resulting in a $g/V$ curve with a pronounced bulge in the range of −10 to +10 mV. The first LVA component activates at voltage steps above −25 mV, whereas the second HVA component is small below 0 mV and does not saturate at voltage steps up to +45 mV. These two components, both blocked by TEA, are clearly K$^+$ currents. The finding that $I_{K(V)}$ is composed of two separate currents is in accord with reports from several vertebrate and invertebrate systems, where multiple delayed rectifier–type potassium currents coexist in one cell type (Baranauskas et al. 1999; Covarrubias et al. 1991; Martínez-Padron and Ferrus 1997; Rothman and Manis 2003; Singh and Singh 1999).

We were able to fit the $g/V$ relationship for the pyloric $I_{K(V)}$ with a formula that combined a Boltzmann relation for the saturable LVA component with an exponential equation for the HVA current that approximates the initial quasi-exponential rise of a second Boltzmann component. We were unable to fit the second component with a Boltzmann relation because the current did not begin to saturate at the highest voltage we could hold, +45 mV. These fits were then mathematically separated to generate the $g/V$ plots of the individual LVA and HVA components. The LVA component was usually well fit by the Boltzmann relation. However, in neurons with small amounts of HVA current, the error in fitting the HVA component could be large. Therefore in such neurons, the values for the separated HVA component need to be interpreted with caution. Because we could not fit the HVA component with a Boltzmann relation, the only parameter we could determine is an estimate of its amplitude at a particular voltage, which we set at +40 mV.

The $V_{1/2}$ and slope values of the Boltzmann parameters for the LVA current varied only slightly between cell types, which indicates that the LVA current has similar properties in all the pyloric neurons. However, the maximal conductance ($g_{\text{max}}$) of the LVA current and the conductance of the HVA component at +40 mV varied significantly between the cell types. The LP, PD, and PY neurons generally had the largest, and IC and AB had the smallest amounts of both the LVA and the HVA components. The VD neuron did not follow this pattern: it showed the third smallest LVA $g_{\text{max}}$, but the second largest HVA $g_{\text{max}}$. These results indicate a cell type–dependent differential expression of the two $I_{K(V)}$ currents. A similar finding has been reported for $I_A$ in the lobster (Baro and Harris-Warrick 1998; Baro et al. 1997).

There remains another potential explanation for the presence of the HVA component. The high-threshold current could be an artifact as a result of poor space clamp and the apparently exponential recruitment of the same LVA current located in distant, poorly clamped regions of the neuropil. With our current data, we are unable to completely exclude this possibility. Several arguments, however, make this an unlikely explanation. First, we have performed a numerical analysis of a two-compartment neuron model, where the soma compartment is voltage clamped and coupled to the neuropil compartment by a variable coupling conductance. In all cases where realistic parameters for $V_{1/2}$ and slope were used, the contribution of the neuron current appears to be concave down for voltages >0 mV, and thus does not resemble the experimental result with its convex-up shape that appears at higher voltages. Second, both LVA and HVA currents are blocked equally by TEA at all concentrations, whereas at low concentrations, both 4-AP and quinidine preferentially block the LVA component, with little effect on the HVA component (Figs. 3–5). Third, neither $I_{K(Ca)}$ nor $I_A$ in the pyloric neurons shows a $g/V$ curve with an exponential component at high voltages; these currents can be well fitted with a single Boltzmann relation (Baro et al. 1997; Harris-Warrick et al. 1995a; Kloupenburg et al. 1999). We measured $I_{K(Ca)}$ in a number of cells that were subsequently used for $I_{K(V)}$ measurements (Gruhn, unpublished observations); in all cases, $I_{K(Ca)}$ was a typical saturating current, whereas in nearly all the neurons $I_{K(V)}$ showed its exponential HVA component at high voltages. Although these arguments are not completely conclusive, they strongly suggest that there are two separate components of $I_{K(V)}$ in these neurons.

Possible molecular basis for multiple $I_{K(V)}$ components in pyloric neurons

In *Drosophila melanogaster*, $I_{K(V)}$ is primarily encoded by the shab and shaw potassium channel genes (Covarrubias et al. 1991; Tsunoda and Salkoff 1995a,b). All the pyloric neurons express the lobster homologs of shab and shaw (Baro et al. 1996a; French et al. 2004). In many species, Shab/Kv2 channels are activated at lower threshold voltages (−40 to −20 mV) than Shaw/Kv3 channels (threshold between −30 and 0 mV; Elkes et al. 1997; Johnstone et al. 1997; Ono et al. 1999; Pak et al. 1991; Panofen et al. 2000; Rashid et al. 2001; Rudy and McBain 2001; Wicher et al. 2001). Furthermore, Kv3/ Shaw currents often do not saturate at voltages below +80 mV (Johnstone et al. 1997; Panofen et al. 2000). This raises the possibility that the LVA and the HVA components of $I_{K(V)}$ in *Panulirus* could be encoded by the lobster shab and shaw homologs, respectively.

Unfortunately, the pharmacological evidence is less clear. Quinidine is a fairly selective Shab antagonist at 100 µM in *Drosophila* larval muscle, where it blocks 89% of Shab and approximately 35% of an as yet unidentified additional delayed rectifier–type K current ($K_F$) (Singh and Singh 1999). This resembles the relatively selective block of the LVA current at low concentrations in pyloric neurons (Fig. 4). However, 4-AP also selectively blocked the LVA component at low concen-
trations in our pyloric neurons (Fig. 5), whereas in the *Drosophila* larval preparation, 5 mM 4-AP, blocks the Shab current and the unidentified K^F_ current equally (Singh and Singh 1999). In *Xenopus* oocytes, 4-AP is a much more selective antagonist of *Drosophila* than Shab (Tsunoda and Salkoff 1995b; Covarrubias et al. 1991). Thus at present the LVA and HVA components of I_{K(V)} in the lobster cannot be readily assigned to specific genes, although we suggest that the LVA component may be a Shab current and the HVA component a Shaw current. Further experiments will be needed to confirm this hypothesis.

Dopamine enhances I_{K(V)} in a subset of pyloric neurons

Dopamine affects the pyloric network by increasing the firing frequency of some neurons while reducing it in others (Flamm and Harris-Warrick 1986a,b). The observed changes are in part explained by modulatory effects on I_{K(V)}, I_{K(Ca)} and I_{Ca} in the different neurons (Harris-Warrick et al. 1995b; Johnson et al. 2003; Kloppenburg et al. 1999; Peck et al. 2001). We found that DA reversibly increased the total I_{K(V)} conductance in the AB neuron and in a subset of the eight PY neurons by 22 and 29%, respectively. Although we were unable to mathematically separate the LVA and HVA components in the AB neuron, the DA-induced increase becomes significant at voltages where the LVA component is activated and the HVA component is still very small, and continues above the range where the LVA component is saturated. This suggests that both components are enhanced by DA in the AB neuron. We were able to separate the LVA and HVA components in the PY neurons and showed a significant DA-induced increase in the conductance of both components. DA appears to elicit a continuous spectrum of effects in the PY neurons investigated, from strong enhancement of I_{K(V)} to very weak reduction in this current (Fig. 7E). Similar continuous variability in DA modulation of the firing properties among the eight PY neurons has been observed (B Johnson, unpublished observations), arguing that the eight PY neurons are not easily subdivided into two subpopulations (Hartline et al. 1987).

The relationship between the DA-induced increase in I_{K(V)} in the AB and PY neurons and the previously observed DA-evoked increase in firing frequency in these cells (Flamm and Harris-Warrick 1986a,b; Harris-Warrick et al. 1998) remains unclear. Because the I_{K(V)} components, and in particular the HVA component, are activated only at suprathreshold voltages, they most likely play roles in determining the repolarization of action potentials and the spike frequency during bursts. The HVA component will be partially activated only in the physiological voltage range; in this it is similar to I_{h}, whose full activation requires hyperpolarization well below −100 mV. In cortical inhibitory neurons and auditory neurons, Kv3.1 channels show extremely rapid activation and deactivation (Macica et al. 2003; Rudy and McBain 2001; Rudy et al. 1999). Increases in these currents accelerate peak spike frequency by facilitating rapid repolarization of the action potential, thus reducing inactivation of sodium channels and decreasing the minimal interval between spikes. In the pyloric neurons, however, the kinetics of I_{K(V)} activation and deactivation are much slower and do not act rapidly enough to increase maximal spike frequency. Modeling of the role of I_{K(V)} in PY neurons (Harris-Warrick et al. 1995b) suggested that increasing this current would decrease spike frequency because of its slow deactivation rate. In addition, increasing I_{K(V)} in oscillating AB neuron models does not accelerate spike frequency and modestly slows the period of the AB oscillations (Guckenheimer et al. 1992 and J. Guckenheimer, unpublished observations). Thus it is possible that the DA-evoked increases in I_{K(V)} actually oppose the increase in spike frequency in AB and PY neurons caused by the other modulatory effects of DA on I_{A}, I_{K(Ca)} and I_{h} (Harris-Warrick et al. 1995a,b; Johnson et al. 2003; Peck et al. 2001; JH Peck, ST Nakanishi, R Yapple, and RM Harris-Warrick, unpublished observations). These opposing actions would act in concert to constrain the DA-induced increase in excitability to within certain bounds. This in turn would increase the reliability of the DA effect and reduce the risk that the preparation will become “overmodulated” and dysfunctional.

One way to directly determine the function of I_{K(V)} and assess the influence of the DA modulatory effect on I_{K(V)} in PY and AB neurons would be to selectively block the current and look for changes in firing and DA responsiveness. However, at present, there are no specific blockers for I_{K(V)}, or its LVA or HVA components, in lobster neurons. 4-AP and quinidine also reduce I_{A} (Graubard and Hartline 1991; Tierney and Harris-Warrick 1992; Gruhn, unpublished observations), whereas TEA also blocks I_{K(Ca)} (Kloppenburg et al. 1999). Because DA also affects I_{K(V)}, I_{h}, and I_{Ca} in PY and AB neurons (Johnson et al. 2003; Peck et al. 2001; Peck, unpublished observations), the DA-evoked changes in activity result from a complex interaction of DA’s effects on all these currents.

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