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JN 94:2888-2900, 2005. First published Jul 13, 2005; doi:10.1152/jn.00434.2005

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

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Submitted 28 April 2005; accepted in final form 29 June 2005

Gruhn, Matthias, John Guckenheimer, Bruce Land, and Ronald M. Harris-Warrick. Dopamine modulation of two delayed rectifier potassium currents in a small neural network. *J Neurophysiol* 94: 2888–2900, 2005. First published July 13, 2005; doi:10.1152/jn.00434.2005. Delayed rectifier potassium currents [$I_{K(V)}$] generate sustained, noninactivating outward currents with characteristic fast rates of activation and deactivation and play important roles in shaping spike frequency. The pyloric motor network in the stomatogastric ganglion of the spiny lobster, *Panulirus interruptus*, is made up of one interneuron and 13 motor neurons of five different classes. Dopamine (DA) increases the firing frequencies of the anterior burster (AB), pyloric (PY), lateral pyloric (LP), and inferior cardiac (IC) neurons and decreases the firing frequencies of the pyloric dilator (PD) and ventricular dilator (VD) neurons. In all six types of pyloric neurons, $I_{K(V)}$ is small with respect to other K^+ currents. It is made up of at least two TEA-sensitive components that show differential sensitivity to 4-aminopyridine and quinidine, and have differing thresholds of activation. One saturable component is activated at potentials above -25 mV, whereas the second component appears at more depolarized voltages and does not saturate at voltage steps up to $+45$ mV. The magnitude of the components varies among cell types but also shows considerable variation within a single type. A subset of PY neurons shows a marked enhancement in spike frequency with DA; DA evokes a pronounced reversible increase in $I_{K(V)}$ conductance of $\leq 30\%$ in the PY neurons studied, and on average significantly increases both components of $I_{K(V)}$. The AB neuron also shows a reversible 20% increase in the steady state $I_{K(V)}$. DA had no effect on $I_{K(V)}$ in PD, LP, VD, and IC neurons. The physiological roles of these currents and their modulation by DA are discussed.

INTRODUCTION

Noninactivating 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). Noninactivating 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 noninactivating delayed rectifier-type current in the STG, however, is still missing (Kloppenburger et al. 1999).

All of the currents mentioned above are subject to modulation. Neuromodulators such as biogenic amines or neuropeptides play crucial roles in shaping the output of the pyloric network (Ayali and Harris-Warrick 1999; Marder and Thirumalai 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 (Ayali 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

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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 commissural 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_2SO_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 M Ω ; 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 $I_{K(\text{Ca})}$ and 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 M Ω 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 (Kloppenborg 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 $E_K = -86$ 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

$$g = g_{\max} / \{1 + e^{[(V - V_{1/2})/s]}\} + p1 \times e^{-[(V - 45)/p2]}$$

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 *nlinfit*. Formal error propagation was estimated using the Statistics toolbox routine *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.

RESULTS

$I_{K(V)}$ 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 mV to inactivate I_A , whereas I_{Na} was blocked with $0.1 \mu\text{M}$ 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 $+10$ mV, followed by a rising increase in current at higher voltages (e.g., Fig. 1*E*). 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. 1*B*) 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

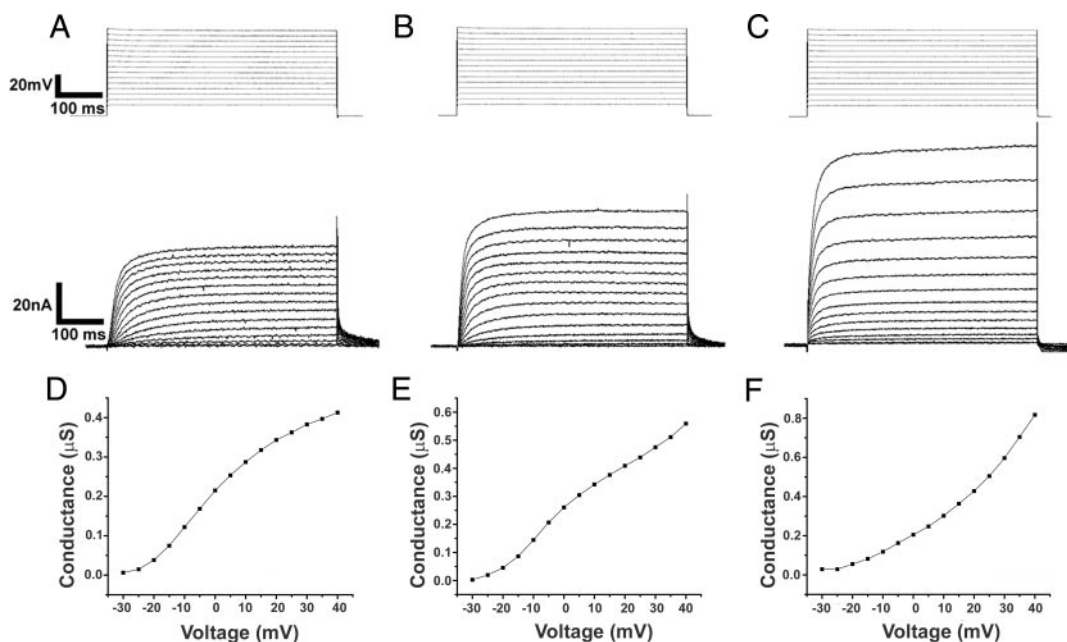


FIG. 1. Current and voltage traces showing variability of delayed rectifier potassium current [$I_{K(V)}$] in 3 different pyloric dilator (PD) neurons. Currents (*A–C*) were measured during 500-ms voltage steps from a holding potential of -40 mV to between -30 and $+40$ mV in 5 -mV steps. *D–F*: corresponding 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*).

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. 1*A*, 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 LVA 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 nonlinearly with each voltage step throughout the voltage range that we could obtain, giving rise to the apparently exponential g/V relation seen in Fig. 1*F*. 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

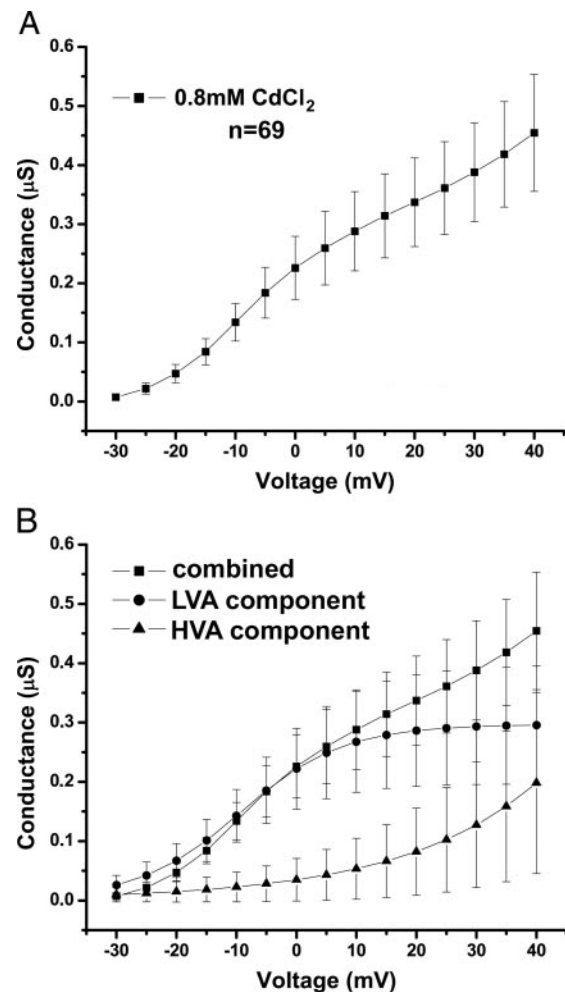


FIG. 2. Separation of $I_{K(V)}$ into LVA and HVA components. *A*: average g/V plot of $I_{K(V)}$ in 69 PD neurons after superfusing the ganglion with *Panulirus* saline containing 5 mM CsCl, 0.1 μ M tetrodotoxin (TTX), 5 μ M picrotoxin (PTX), and 0.8 mM CdCl₂ for a minimum of 30 min. Note the clear bulge around 10 mV. *B*: average total conductance from *A* (■) and mathematically separated LVA (●) and HVA (▲) components. Error bars mark SD.

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 μ S), with PD being second (0.29 ± 0.11 μ S). The PY (0.25 ± 0.06 μ S) and VD (0.25 ± 0.08 μ S) neurons have similar but smaller amounts of this conductance, whereas the IC (0.21 ± 0.08 μ S) and AB (0.19 ± 0.04 μ S) 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.

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

Neuron type	$g_{\max}$ LVA, μS	$V_{1/2}$ LVA, mV	Slope LVA	HVA Amplitude at +40 mV μS	LVA:HVA Ratio at +40 mV
PD ($n = 69$)	0.29 ± 0.11	-9.5 ± 3.0	-8.3 ± 2.2	0.20 ± 0.15	1.5:1
LP ($n = 23$)	0.35 ± 0.12	-12.3 ± 2.9	-7.3 ± 2.2	0.29 ± 0.27	1.2:1
PY ($n = 21$)	0.25 ± 0.06	-10.8 ± 2.9	-7.1 ± 2.0	0.16 ± 0.19	1.6:1
VD ($n = 16$)	0.25 ± 0.08	-9.6 ± 1.8	-8.2 ± 1.8	0.27 ± 0.16	0.9:1
IC ($n = 7$)	0.21 ± 0.08	-9.2 ± 3.6	-9.6 ± 2.7	0.11 ± 0.05	1.9:1
AB ($n = 9$)	0.19 ± 0.04	-7.8 ± 2.5	-10.2 ± 1.7	0.06 ± 0.03	3.2:1

Values are means $\pm$ SD. LVA, low-voltage activated; HVA, high-voltage activated.

When measured at +40 mV, the LP neuron has the greatest HVA conductance ($0.29 \pm 0.27 \mu\text{S}$), closely followed by the VD neuron ($0.27 \pm 0.16 \mu\text{S}$). The PD and PY neurons have similar but smaller amounts (0.20 ± 0.15 and $0.16 \pm 0.19 \mu\text{S}$, respectively), whereas the IC and AB neurons have the smallest amounts of HVA conductance (0.11 ± 0.05 and $0.06 \pm 0.03 \mu\text{S}$, 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 varied depending on the cell type. It was highest in the AB neuron with a ratio of 3.2:1, attributed primarily to the very small HVA component in most of these neurons. The IC, PY, and PD neurons had LVA:HVA ratios between 2:1 and 1.5:1 (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 current from distant, poorly clamped regions of the neuropil. We do not favor this interpretation for two major reasons. First, if this interpretation is true, it should hold for other currents as well. It is known that other currents, such as I_A and $I_{K(\text{Ca})}$, are distributed in the neuropil as well as the somata of pyloric neurons (e.g., Baro et al. 2000), yet in previous voltage-clamp studies of these currents, we have found their g/V curves to show normal saturation with voltage (Baro et al. 1997; Kloppeburg et al. 1999). To test this directly, we measured $I_{K(\text{Ca})}$ and $I_{K(V)}$ in the same neuron in a subset of 13 PD neurons. $I_{K(\text{Ca})}$ was on average fourfold greater than $I_{K(V)}$ and its g/V relation always approached saturation at higher voltage steps, with no evidence of a second exponential component. In contrast, in 12 out of the 13 PD neurons measured, the shape of the g/V plot of $I_{K(V)}$ resembled that in Fig. 1E with its exponential rise at higher voltage steps (data not shown). Second, we performed a numerical analysis of a two-compartment neuron model of the effects of currents in the distal neuropil. In this model, the soma compartment is voltage clamped and coupled to the neuropil compartment by a variable coupling conductance. Both compartments contain an LVA-type $I_{K(V)}$ with identical voltage dependency but variable maximal conductance. Because in voltage clamp we measure the conductances at steady state, we can explicitly solve the differential equations for the apparent $I_{K(V)}$ measured in the soma, including any contribution from the neuropil compartment. We then calculated the contribution of the neuropil $I_{K(V)}$

to the g/V curve measured in the soma over a wide range of coupling and conductance parameters, constrained only by the values of $V_{1/2}$ and slope derived from our Boltzmann analysis of the LVA component. In all cases, the contribution of the neuropil current never behaves like a rising exponential function at voltages >0 mV. Instead, it appears to be concave down in this voltage range, such that the composite current measured in the soma never has a bulge or second inflection point, as seen in the experimental observations. We carried out further analysis relaxing our constraints, and found that an exponentially rising HVA-like component could be obtained, but only with unrealistically small values of the slope parameter that are never seen in real ion channels. Details of this model are available on request.

The two components of $I_{K(V)}$ show partially different sensitivity to potassium channel blockers

To compare the pharmacological profiles of the two 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

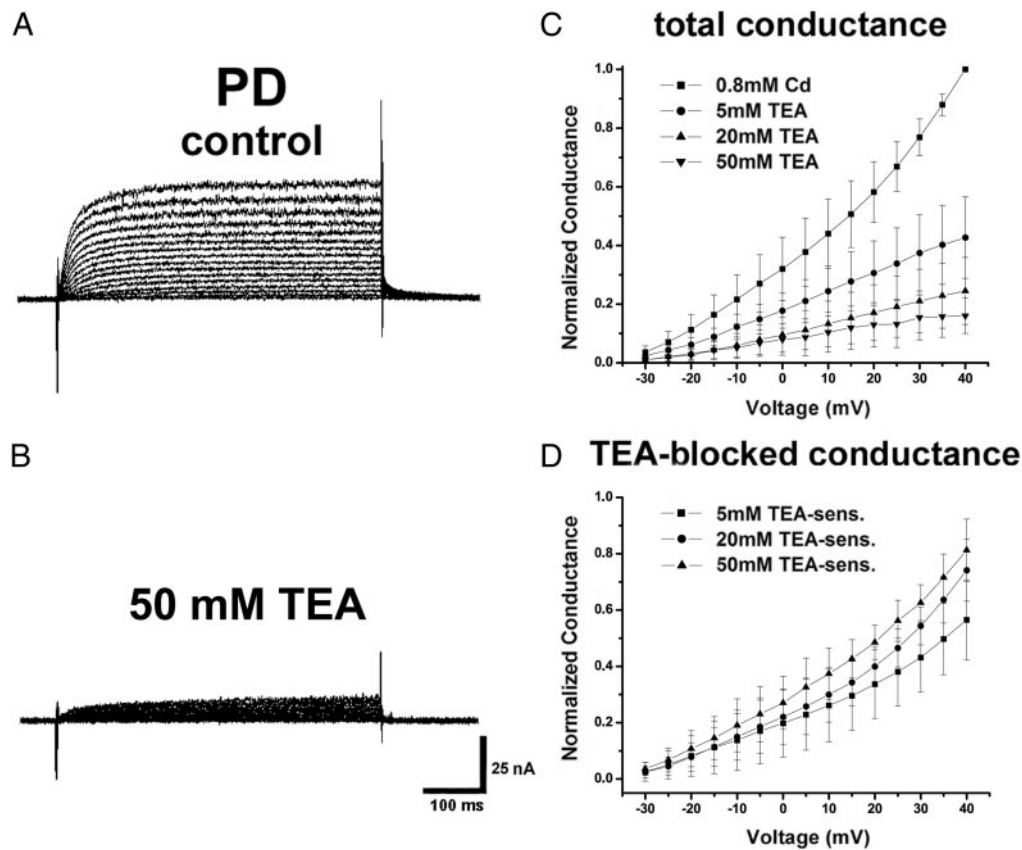


FIG. 3. Tetraethylammonium chloride (TEA) blocks $I_{K(V)}$. *Current traces* of $I_{K(V)}$ under control conditions (A) and after perfusion with 50 mM TEA (B). Measurements made as in Fig. 2. *C*: normalized g/V relationship of the total conductance in 4 PD neurons under control conditions (■) and after equilibrating with 5 mM TEA (●), 20 mM TEA (▲), and 50 mM TEA (▼). *D*: normalized g/V relationship of TEA-sensitive conductance after equilibration with 5 mM TEA (■), 20 mM TEA (●), and 50 mM TEA (▲). These were calculated by digital subtraction of TEA-blocked currents from control values and normalized against the maximum control conductance for each cell. Note that the shape closely follows that of the control conductance (C) at all TEA concentrations. Error bars mark SD.

one case after application of 1 mM quinidine $\leq 80\%$ 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 μ 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 $> 500 \mu$ 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 *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

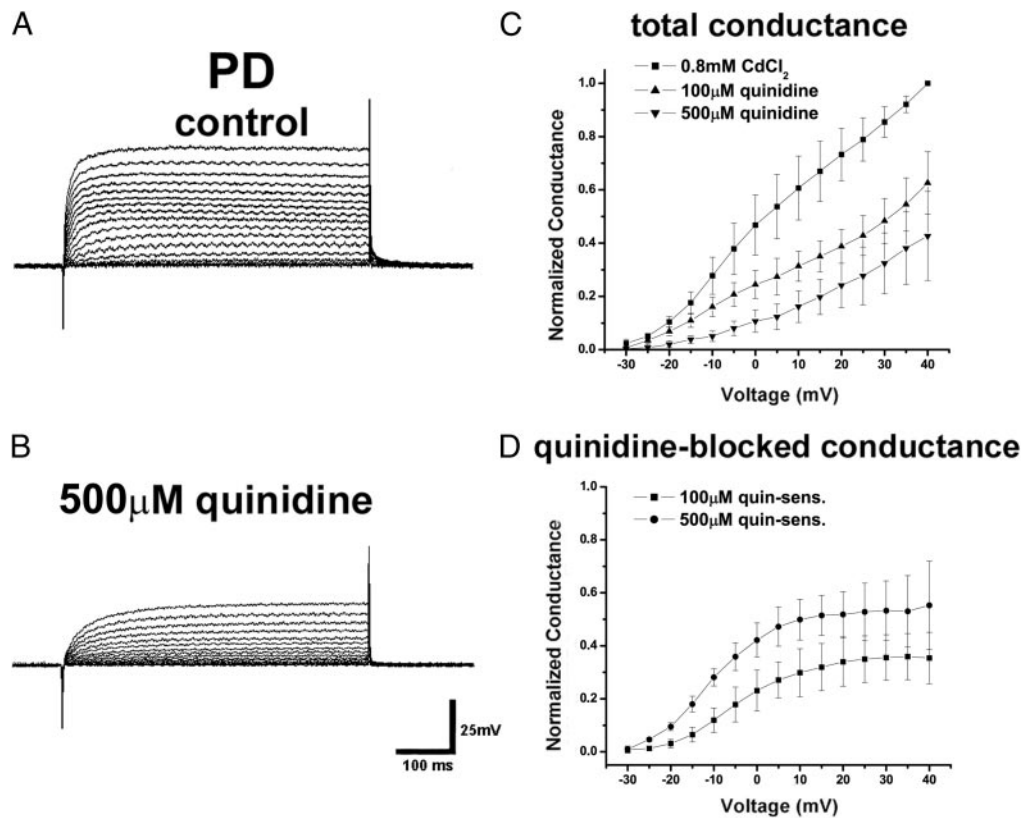


FIG. 4. Quinidine selectively reduces the LVA component of $I_{K(V)}$. *Current traces* of $I_{K(V)}$ under control conditions (A) and after perfusion with 500 μ M quinidine (B). Measurements made as in Fig. 2. *C*: normalized g/V relationship of the total conductance in 3 PD neurons under control conditions (■) and after equilibration with 100 μ M quinidine (●) and 500 μ M quinidine (▲). *D*: normalized g/V relationship of quinidine-blocked conductance after equilibration with 100 μ M quinidine (■) and 500 μ M quinidine (●), calculated as in Fig. 3. Shape of the g/V plot shows a selective block of the LVA component at 100 μ M quinidine, but quinidine also affects the HVA component at higher concentrations.

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 (Kloppenborg et al. 1999) ($n = 4$). 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 $\leq 40\%$ (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 $g_{\max}$ 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,

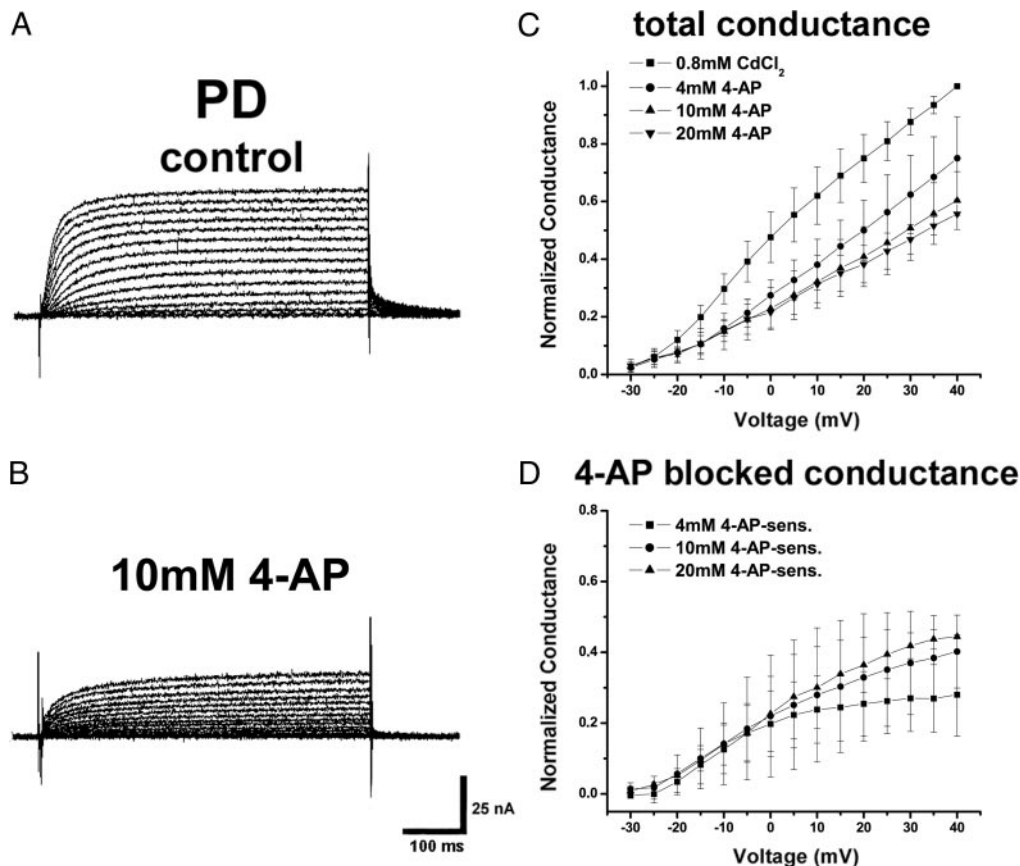


FIG. 5. 4-Aminopyridine (4-AP) selectively blocks the LVA component of $I_{K(V)}$. *A*: Current traces of $I_{K(V)}$ under control conditions (*A*) and after perfusion with 10 mM 4-AP (*B*). *C*: normalized g/V relationship of the total conductance in 5 PD neurons under control conditions (■), after 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.

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*).

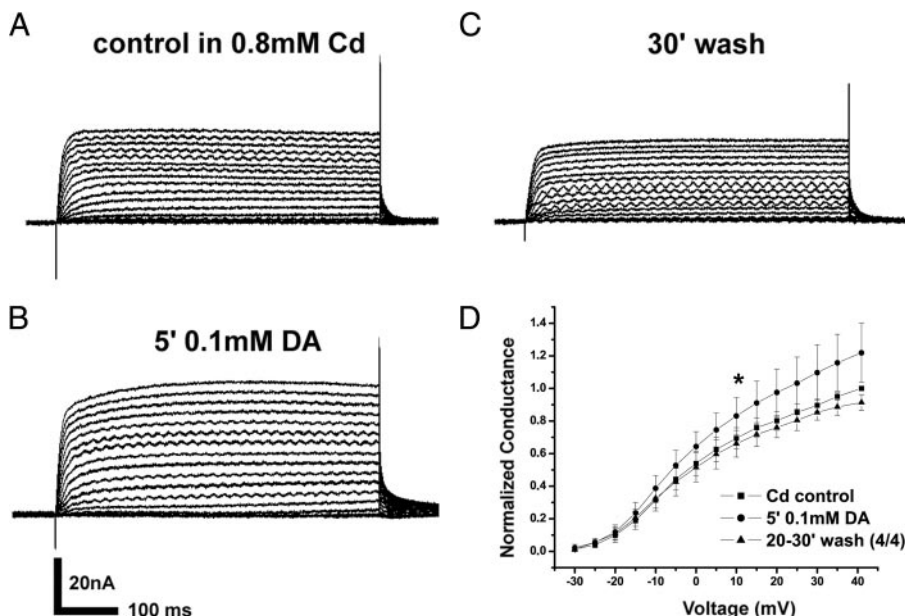


FIG. 6. Dopamine (DA) enhances $I_{K(V)}$ in the anterior burster (AB) neuron. *A–C*: current traces of an AB neuron under control (*A*), after 5-min perfusion with 0.1 mM DA (*B*) and after 30-min wash (*C*). *D*: normalized g/V relationship of $I_{K(V)}$ total conductance under control (■), DA (●), 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.

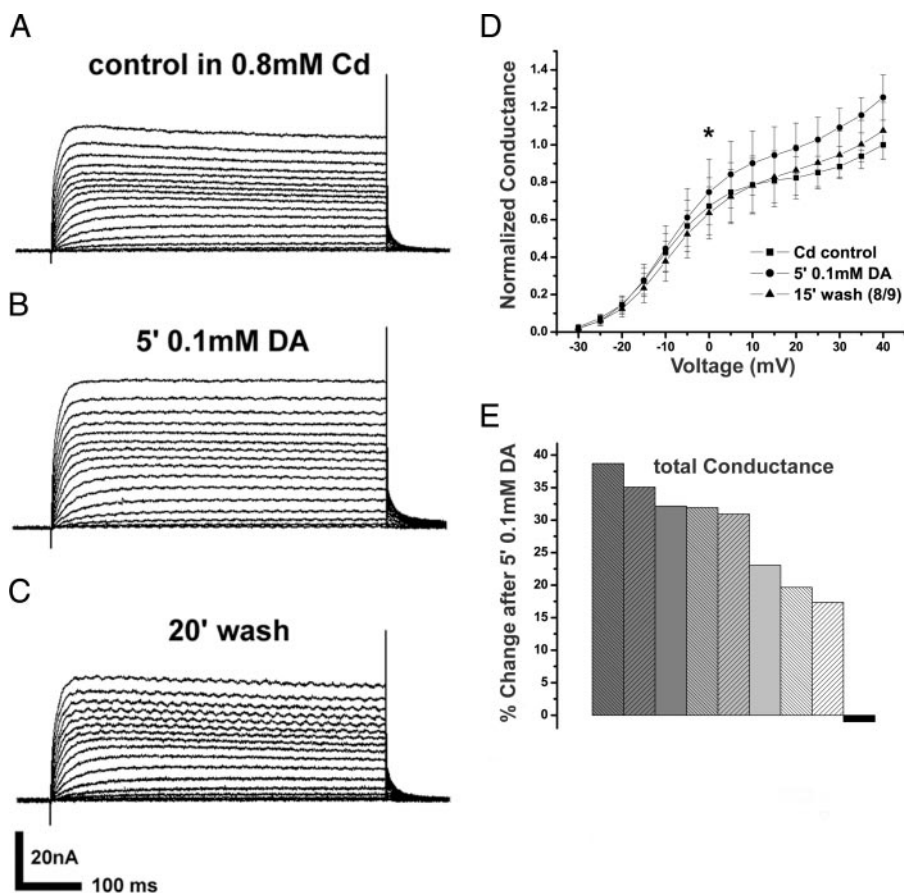


FIG. 7. DA enhances $I_{K(V)}$ in most pyloric (PY) neurons. *A–C*: current traces of a PY neuron under control (*A*), after 5-min perfusion with 0.1 mM DA (*B*) and after 20-min wash (*C*). *D*: normalized g/V relationship of $I_{K(V)}$ total conductance under control (■), DA (●), and wash (▲) for 9 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.

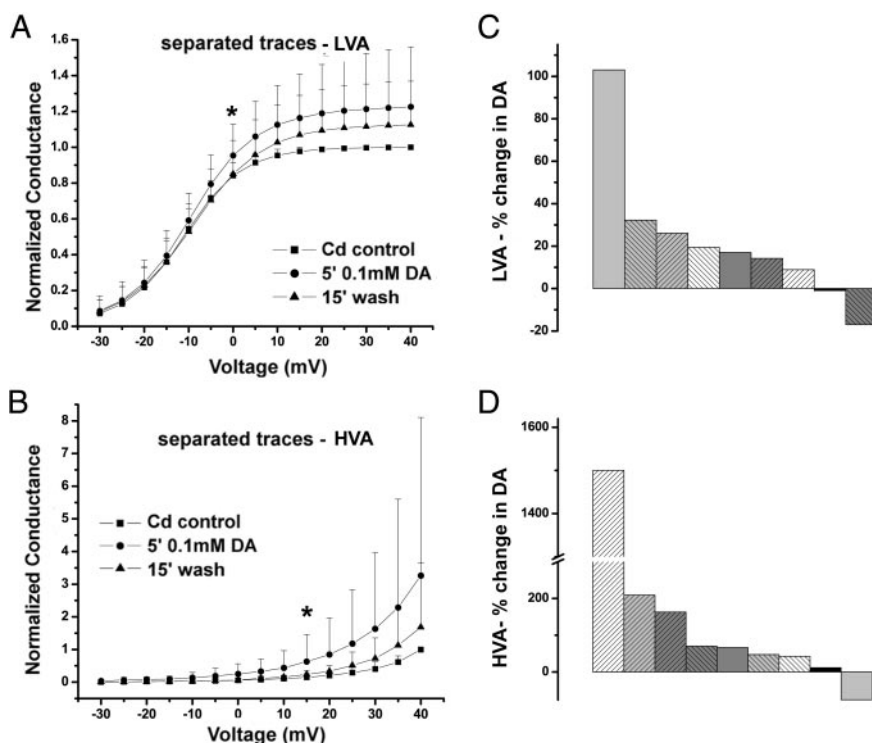


FIG. 8. DA effect on separated LVA and HVA components of $I_{K(V)}$ in PY neurons. g/V relationships of the LVA component (*A*), and HVA component (*B*) under control (■), after 5-min perfusion with 0.1 mM DA (●) and after 15-min wash (▲). Asterisk marks the voltages in DA above which the increase becomes 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

	LVA Component Values Alone After Separation and Refit		
	$g_{\max}$ μS	$V_{1/2}$ mV	Slope
PY control	0.22 ± 0.08	-11.5 ± 3.2	-6.8 ± 2.5
DA	$0.26 \pm 0.08^*$	-9.7 ± 3.4	-7.5 ± 2.9
Wash	0.24 ± 0.07	-9.1 ± 2.8	-8.2 ± 3.2

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

DISCUSSION

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

After blocking I_{Na} , I_{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; Martinez-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 ($g_{\max}$) 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 $g_{\max}$, but the second largest HVA $g_{(+40)}$. 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 neuropil 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; Kloppenburg 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 $100 \mu M$ 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* Shaw 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_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). 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_{Ca} , and I_h (Harris-Warrick et al. 1995a,b; Johnson et al. 2003; Peck et al. 2001; JH Peck, ST Nakanishi, R Yaple, 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_A , 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.

ACKNOWLEDGMENTS

We thank Drs. Bruce Johnson and Jack Peck, as well as M. Goeritz for valuable comments and discussions. Thanks to Dr. Peck also for help with the statistical analysis.

GRANTS

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-17323 to R. M. Harris-Warrick.

REFERENCES

- Armstrong CM and Bezanilla F. Charge movement associated with the opening and closing of the activation gates of the Na channels. *J Gen Physiol* 63: 533–552, 1974.
- Ayali A and Harris-Warrick RM. Monoamine control of the pacemaker kernel and cycle frequency in the lobster pyloric network. *J Neurosci* 19: 6712–6722, 1999.
- Ayali A, Johnson BR, and Harris-Warrick RM. Dopamine modulates graded and spike-evoked synaptic inhibition independently at single synapses in pyloric network of lobster. *J Neurophysiol* 79: 2063–2069, 1998.
- Baranauskas G, Tkatch T, and Surmeier DJ. Delayed rectifier currents in rat globus pallidus neurons are attributable to Kv2.1 and Kv3.1/3.2 K^+ channels. *J Neurosci* 19: 6394–6404, 1999.
- Baro DJ, Ayali A, French L, Scholz NL, Labenia J, Lanning CC, Graubard K, and Harris-Warrick RM. Molecular underpinnings of motor pattern generation: differential targeting of shal and shaker in the pyloric motor system. *J Neurosci* 20: 6619–6630, 2000.
- Baro DJ, Cole CL, and Harris-Warrick RM. RT-PCR analysis of *shaker*, *shab*, *shaw*, and *shal* gene expression in single neurons and glial cells. *Receptors Channels* 4: 149–159, 1996a.
- Baro DJ, Coniglio LM, Cole CL, Rodriguez HE, Lubell JK, Kim MT, and Harris-Warrick RM. Lobster *shal*: comparison with *Drosophila shal* and native potassium currents in identified neurons. *J Neurosci* 16: 1689–1701, 1996b.

- Baro DJ and Harris-Warrick RM. Differential expression and targeting of K^+ channel genes in the lobster pyloric central pattern generator. *Ann NY Acad Sci* 860: 281–295, 1998.
- Baro DJ, Levini RM, Kim MT, Willms AR, Lanning CC, Rodriguez HE, and Harris-Warrick RM. Quantitative single-cell-reverse transcription-PCR demonstrates that A-current magnitude varies as a linear function of *shal* gene expression in identified stomatogastric neurons. *J Neurosci* 17: 6597–6610, 1997.
- Calabrese RL. Neuronal networks: dissection one channel at a time. *Curr Biol* 14: R154–R155, 2004.
- Coetzee WA, Amarillo Y, Chiu J, Chow A, Lau D, McCormack TOM, Morena H, Nadal MS, Ozaita A, Pountney D, Saganich M, De Miera EV-S, and Rudy B. Molecular diversity of K^+ channels. *Ann NY Acad Sci* 868: 233–285, 1999.
- Covarrubias M, Wei AA, and Salkoff L. *Shaker*, *Shal*, *Shab*, and *Shaw* express independent K^+ current systems. *Neuron* 7: 763–773, 1991.
- Dodson PD and Forsythe ID. Presynaptic K^+ channels: electrifying regulators of synaptic terminal excitability. *Trends Neurosci* 27: 210–217, 2004.
- Elkes DA, Cardozo DL, Madison J, and Kaplan JM. EGL-36 *Shaw* channels regulate *C. elegans* egg-laying muscle activity. *Neuron* 19: 165–174, 1997.
- Flamm RE and Harris-Warrick RM. Aminergic modulation in lobster stomatogastric ganglion. I. Effects on motor pattern and activity of neurons within the pyloric circuit. *J Neurophysiol* 55: 847–865, 1986a.
- Flamm RE and Harris-Warrick RM. Aminergic modulation in lobster stomatogastric ganglion. II. Target neurons of dopamine, octopamine, and serotonin within the pyloric circuit. *J Neurophysiol* 55: 866–881, 1986b.
- French LB, Lanning CC, Matly M, and Harris-Warrick RM. Cellular localization of *Shab* and *Shaw* potassium channels in the lobster stomatogastric ganglion. *Neuroscience* 123: 919–930, 2004.
- Golowasch J, Buchholtz F, Epstein IR, and Marder E. Contribution of individual ionic currents to activity of a model stomatogastric ganglion neuron. *J Neurophysiol* 67: 341–349, 1992.
- Golowasch J and Marder E. Ionic currents of the lateral pyloric neuron of the stomatogastric ganglion of the crab. *J Neurophysiol* 67: 318–331, 1992.
- Graubard K and Hartline DK. Voltage clamp analysis of intact stomatogastric neurons. *Brain Res* 557: 241–254, 1991.
- Gruhn M, Guckenheimer J, Land B, and Harris-Warrick RM. Dopamine modulation of delayed rectifier-type potassium currents in the pyloric circuit of the lobster stomatogastric ganglion. *Proc Ann Meeting Soc Neurosci San Diego, CA*, 2004.
- Gruhn M and Harris-Warrick RM. Characterization of delayed rectifier-type potassium currents in cells in the pyloric circuit of the stomatogastric ganglion in the spiny lobster. *Proc Ann Meeting Soc Neurosci New Orleans, LA*, 2003.
- Harris-Warrick RM. Voltage-sensitive ion channels in rhythmic motor systems. *Curr Opin Neurobiol* 12: 646–651, 2002.
- Harris-Warrick RM, Coniglio LM, Barazangi N, Guckenheimer J, and Gueron S. Dopamine modulation of transient potassium current evokes phase shifts in a central pattern generator network. *J Neurosci* 15: 342–358, 1995a.
- Harris-Warrick RM, Coniglio LM, Levini RM, Gueron S, and Guckenheimer J. Dopamine modulation of two subthreshold currents produces phase shifts in activity of an identified motoneuron. *J Neurophysiol* 74: 1404–1420, 1995b.
- Harris-Warrick RM, Johnson BR, Peck JH, Kloppenburg P, Ayali A, and Skarbinski J. Distributed effects of dopamine modulation in the crustacean pyloric network. *Ann NY Acad Sci* 860: 155–167, 1998.
- Harris-Warrick R, Marder E, Selverston A, and Moulins M. *Dynamic Biological Networks: The Stomatogastric Nervous System*. Cambridge, MA: MIT Press, 1992.
- Hartline DK. Pattern generation in the lobster (*Panulirus*) stomatogastric ganglion. II. Pyloric network simulation. *Biol Cybern* 33: 223–236, 1979.
- Hartline DK, Gassie DV, and Sirchia CD. PY cell types in the stomatogastric ganglion of *Panulirus*. In: *The Crustacean Stomatogastric System*, edited by Selverston AI and Moulins M. Berlin: Springer-Verlag, 1987, p. 75–77.
- Hartline DK and Graubard K. Cellular and synaptic properties in the stomatogastric nervous system. In: *Dynamic Biological Networks: The Stomatogastric Nervous System*, edited by Harris-Warrick RM, Marder E, Selverston AI, and Moulins M. Cambridge, MA: MIT Press, 1992, p. 1–30.
- Johnson BR and Harris-Warrick RM. Aminergic modulation of graded synaptic transmission in the lobster stomatogastric ganglion. *J Neurosci* 10: 2066–2076, 1990.
- Johnson BR, Kloppenburg P, and Harris-Warrick RM. Dopamine modulation of calcium currents in pyloric neurons of the lobster stomatogastric ganglion. *J Neurophysiol* 90: 631–643, 2003.
- Johnson BR, Nadim F, Schneider L, and Harris-Warrick RM. Dopamine modifies firing phase in a rhythmic network through synaptic sign reversal and activation of silent synapses. *Proc Ann Meeting Soc Neurosci San Diego, CA*, 2004.
- Johnson BR, Peck JH, and Harris-Warrick RM. Distributed amine modulation of graded chemical transmission in the pyloric network of the lobster stomatogastric ganglion. *J Neurophysiol* 74: 437–452, 1995.
- Johnstone DB, Wei A, Butler A, Salkoff L, and Thomas JH. Behavioral defects in *C. elegans* egl-36 mutants result from potassium channels shifted in voltage-dependence of activation. *Neuron* 19: 151–164, 1997.
- Kim M, Baro DJ, Lanning CC, Doshi M, Farnham J, Moskowitz HS, Peck JH, Olivera BM, and Harris-Warrick RM. Alternative splicing in the pore-forming region of shaker potassium channels. *J Neurosci* 17: 8213–8224, 1997.
- Kim M, Baro DJ, Lanning CC, Doshi M, Moskowitz HS, Farnham J, and Harris-Warrick RM. Expression of *Panulirus* shaker potassium channel splice variants. *Receptors Channels* 5: 291–304, 1998.
- Kloppenburg P, Levini RM, and Harris-Warrick RM. Dopamine modulates two potassium currents and inhibits the intrinsic firing properties of an identified motor neuron in a central pattern generator network. *J Neurophysiol* 81: 29–38, 1999.
- Macica CM, von Hehn CAA, Wang L-Y, Ho C-S, Yokoyama S, Joho RH, and Kaczmarek LK. Modulation of the Kv3.1b potassium channel isoform adjusts the fidelity of the firing pattern of auditory neurons. *J Neurosci* 23: 1133–1141, 2003.
- Marder E and Thirumalai V. Cellular, synaptic and network effects of neuromodulation. *Neural Networks* 15: 479, 2002.
- Martinez-Padron M and Ferrus A. Presynaptic recordings from *Drosophila*: correlation of macroscopic and single-channel K^+ currents. *J Neurosci* 17: 3412–3424, 1997.
- Mulloney B and Selverston AI. Organization of the stomatogastric ganglion of the spiny lobster. III. Coordination of the two subsets of the gastric system. *J Comp Physiol* 91: 53–78, 1974.
- Ono F, Katsuyama Y, Nakajo K, and Okamura Y. Subfamily-specific posttranscriptional mechanism underlies K^+ channel expression in a developing neuronal blastomere. *J Neurosci* 19: 6874–6886, 1999.
- Ozaita A, Petit-Jacques J, Volgyi B, Ho CS, Joho RH, Bloomfield SA, and Rudy B. A unique role for Kv3 voltage-gated potassium channels in starburst amacrine cell signaling in mouse retina. *J Neurosci* 24: 7335–7343, 2004.
- Pak MD, Covarrubias M, Ratcliffe A, and Salkoff L. A mouse brain homolog of the *Drosophila* *Shab* K^+ channel with conserved delayed-rectifier properties. *J Neurosci* 11: 869–880, 1991.
- Pal S, Hartnett KA, Nerbonne JM, Levitan ES, and Aizenman E. Mediation of neuronal apoptosis by Kv2.1-encoded potassium channels. *J Neurosci* 23: 4798–4802, 2003.
- Panofen F, Rabe H, Henne J, and Jeserich G. Molecular cloning and functional characterization of *Shaw*-related potassium channels of trout CNS. *Brain Res Mol Brain Res* 83: 9–19, 2000.
- Peck JH, Nakanishi ST, Yapple R, and Harris-Warrick RM. Amine modulation of the transient potassium current in identified cells of the lobster stomatogastric ganglion. *J Neurophysiol* 86: 2957–2965, 2001.
- Peck JH, Repicky S, and Harris-Warrick RM. The effect of blocking the hyperpolarization-activated inward current (I_h) on the pyloric motor rhythm of the stomatogastric ganglion (STG) of the lobster, *Panulirus interruptus*. *Proc Ann Meeting Soc Neurosci San Diego, CA*, 2004.
- Rashid AJ, Morales E, Turner RW, and Dunn RJ. The contribution of dendritic Kv3 K^+ channels to burst threshold in a sensory neuron. *J Neurosci* 21: 125–135, 2001.
- Rothman JS and Manis PB. Differential expression of three distinct potassium currents in the ventral cochlear nucleus. *J Neurophysiol* 89: 3070–3082, 2003.
- Rudy B, Lau D, Amarillo Y, Ozaita A, Saganich M, Moreno H, Nadal MS, Hernandez-Pineda R, Hernandez-Cruz A, Erisir A, Leonard C, and Vega-Saenz de Miera E. Contributions of Kv3 channels to neuronal excitability. *Ann NY Acad Sci* 868: 304–343, 1999.
- Rudy B and McBain CJ. Kv3 channels: voltage-gated K^+ channels designed for high-frequency repetitive firing. *Trends Neurosci* 24: 517–526, 2001.

- Salkoff L, Baker K, Butler A, Covarrubias M, Pak MD, and Wei A.** An essential "set" of K^+ channels conserved in flies, mice and humans. *Trends Neurosci* 15: 161–166, 1992.
- Silverston AI, Russel DF, Miller JP, and King DG.** The stomatogastric nervous system: structure and function of a small neural network. *Prog Neurobiol* 7: 215–290, 1976.
- Shevchenko T, Ryoichi T, and Armstrong WE.** High threshold, Kv3-like potassium currents in magnocellular neurosecretory neurons and their role in spike repolarization. *J Neurophysiol* 92: 3043–3053, 2004.
- Singh A and Singh S.** Unmasking of a novel potassium current in *Drosophila* by a mutation and drugs. *J Neurosci* 19: 6838–6843, 1999.
- Tierney AJ and Harris-Warrick RM.** Physiological role of the transient potassium current in the pyloric circuit of the lobster stomatogastric ganglion. *J Neurophysiol* 67: 599–609, 1992.
- Tsunoda S and Salkoff L.** Genetic analysis of *Drosophila* neurons: *Shal*, *Shaw*, and *Shab* encode most embryonic potassium currents. *J Neurosci* 15: 1741–1754, 1995a.
- Tsunoda S and Salkoff L.** The major delayed rectifier in both *Drosophila* neurons and muscle is encoded by *Shab*. *J Neurosci* 15: 5209–5221, 1995b.
- Wicher D, Walther C, and Wicher C.** Non-synaptic ion channels in insects—basic properties of currents and their modulation in neurons and skeletal muscles. *Prog Neurobiol* 64: 431–525, 2001.