Dopamine Modulation of Calcium Currents in Pyloric Neurons of the Lobster Stomatogastric Ganglion

Bruce R. Johnson, Peter Kloppenburg, and Ronald M. Harris-Warrick
Department of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853

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INTRODUCTION

The pyloric network in the crustacean stomatogastric ganglion (STG) is a well-characterized central-pattern-generating circuit that controls rhythmic foregut movements to filter food particles and to circulate gastric fluid (Johnson and Hooper 1992; Marder and Bucher 2001; Marder and Calabrese 1996; Selverston et al. 1998). The output of this network is very plastic: the network can be reconfigured to produce multiple variants of a single motor pattern, and its component neurons can even be recruited into other motor networks (Dickinson and Moulins 1992; Nusbaum and Beenhakker 2002). This plasticity has made the pyloric network a model system in which to examine the cellular and synaptic mechanisms underlying behavioral flexibility (Harris-Warrick et al. 1992). In the spiny lobster, Panulirus interruptus, the pyloric network contains 14 identified neurons in six major classes: the anterior burster (AB), two pyloric dilators (PDs), the ventricular dilator (VD), the inferior cardiac (IC), the lateral pyloric (LP), and eight pyloric constrictors (PYs). The synaptic connectivity of the network is completely known, including the identity of the fast synaptic transmitters used by the pyloric neurons (Marder 1987; Mulloney 1987), and many of the intrinsic electrical properties of individual neurons are characterized (Bal et al. 1988; Guckeheimer et al. 1997; Harris-Warrick et al. 1995a,b; Hartline and Graubard 1992; Kloppenburg et al. 1999).

Pyloric network plasticity arises in part from the actions of neuromodulators, delivered via ascending or descending axons or as circulating hormones, which can transiently change the intrinsic electrical properties of network neurons and/or the strength of network synapses (Harris-Warrick and Marder 1991; Marder and Thirumalai 2002; Skiebe 2001). We have been studying the modulatory effects of dopamine (DA). This monoamine can initiate a unique pyloric motor pattern in quiescent preparations (Anderson and Barker 1981; Eisen and Marder 1984; Flamm and Harris-Warrick 1986a,b) and can alter rhythm frequency in actively cycling preparations (Ayali et al. 1999; Harris-Warrick 1999; Harris-Warrick et al. 1995b).

DA modulates the pyloric network through its distributed effects on the ionic currents that shape the neurons’ intrinsic electrical properties and by modulating the strength of chemical and electrical synapses throughout the network (Ayali et al. 1998; Harris-Warrick et al. 1995ab, 1998; Johnson et al. 1995; Kloppenburg et al. 1999, 2001; Nusbaum and Beenhakker 2002). DA evokes rhythmic bursting in the AB neuron, excites the LP, PY, and IC neurons, and inhibits the VD and PD neurons (Flamm and Harris-Warrick 1986b; Marder and Eisen 1984; Turrigiano and Marder 1993). Graded transmitter release between the network neurons organizes the pyloric output (Hartline et al. 1988). DA enhances the strength of graded synaptic transmission at most of the glutamatergic synapses by increasing glutamate release from the AB, LP, and PY neurons (Johnson and Harris-Warrick 1997). At the same time, it weakens graded synapses from the cholinergic PD neuron by decreasing presynaptic transmitter release (Johnson and Harris-Warrick 1990; Johnson et al. 1995). The ionic mechanisms of this synaptic modulation are at present not known. One reasonable target is the voltage-sensitive calcium current (I_{Ca}), which is a common target by which modulators can regulate transmitter release (Dunlap and Ikeda 1998; Fossier et al. 1999; Wu and Saggau 1997) and activate cellular

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processes that shape the intrinsic firing properties of the pyloric and other neurons (Hille 2001; Liu et al. 1998).

The purpose of our study was to characterize \( I_{Ca} \) in the six classes of pyloric neurons in the spiny lobster and to examine whether DA can modify this current in ways that might explain its effects on synaptic transmission and intrinsic firing properties of the neurons. We have found that DA changes \( I_{Ca} \) in all the pyloric neurons. Most of these effects are consistent with our hypothesis that DA modulates synaptic strength at least in part by targeting presynaptic \( I_{Ca} \). In addition, DA modulation of \( I_{Ca} \) may support its alteration of the intrinsic firing properties in most of the pyloric neurons. Finally, we show that DA acts on different \( Ca^{2+} \) channel subtypes in the different classes of pyloric neurons.

METH ODS

Materials

California spiny lobsters (\( P. \) interruptus) were supplied by Don Tomlinson Commercial Fishing (San Diego, CA) and maintained in marine aquaria at 16°C. All chemicals were purchased from Sigma Chemical.

Preparation

Lobsters were anesthetized by cooling in ice. The stomatogastric nervous system was removed as previously described (S elverston et al. 1976), and pinned in a silicone elastomer (Sylgard)-coated petri dish in chilled \( Panulirus \) saline of the following composition (mM): 479 NaCl, 12.8 KCl, 13.7 CaCl\(_2\), 3.9 \( Na_2 SO_4 \), 10.0 MgSO\(_4\), 2 glucose, and 11.1 Tris base, pH 7.4 (Mulloney and Se lverston 1974). The STG was desheathed, enclosed in a 1-ml pool walled with petroleum jelly (Vaseline) and superfused at 5 ml/min with oxygenated \( Panulirus \) saline (17–18°C).

Cell identification and synaptic isolation

Our methods for identifying the six major classes of pyloric neurons and for isolating them pharmacologically from known synaptic input have been previously described in detail (Johnson and Harris-Warrick 1997; Kloppenburg et al. 1999, 2000a; Peck et al. 2001). We determined the subclass of most of the PY neurons by injecting hyperpolarizing current into a PY neuron and noting any change in firing of the LP neuron. Most PY neurons in this study belonged to a subclass not electrically coupled to the LP neuron (Hartline et al. 1987; Levini et al. 1994). Because there is relatively strong electrical coupling between the AB and PD neurons and weaker coupling of these cells with the VD neuron (Johnson et al. 1993), we isolated a neuron from its electrically coupled partners by photoinactivation (Miller and Selverston 1979) in at least one of each series of experiments for each cell type; this made no obvious differences in our voltage-clamp results (see also Swensen and Marder 2000).

Voltage clamp, current isolation, and characterization

Two-electrode voltage-clamp was used to examine \( I_{Ca} \) and outward currents in pyloric neurons. One electrode, filled with 3 M KCl (7- to 11-M\( \Omega \) resistance), was used to inject current, while the second, filled with 2 M TEA and 2 M CsCl (15- to 25-M\( \Omega \) resistance), was used to record voltage and to iontophorese these \( K^+ \) channel blockers internally. Neurons were voltage-clamped using an Axoclamp-2B amplifier and pCLAMP8 software (Axon Instruments). Linear leak and capacitative currents were digitally subtracted using a p/6 protocol (Armstrong and Bezamilla 1974).

We attempted to isolate \( I_{Ca} \) from other voltage-dependent currents by iontophoresing TEA and CsCl into the cell soma to reduce \( K^+ \) currents and by using a lobster blocking saline containing 100 mM tetraethyl-ammonium chloride (TEA) (\( Na^+ \) reduced appropriately to maintain osmotic and charge balance), 4 mM 4-aminopyridine (4-AP), 5 mM CsCl, and 0.1 \( \mu M \) TTX; the pH was adjusted to 7.4 with HCl. We also added picrotxin (5 \( \times 10^{-6} \) M) to block all glutamatergic transmission within the STG. \( Cl^- \) was substituted for \( SO_4^{2-} \) in all blocking saline to avoid precipitation when using divalents other than \( Ca^{2+} \). A depolarizing leak current is initially induced in PD neurons by 4-AP (Kloppenburg et al. 1999) and other structurally related \( I_x \) blockers (Johnson and Kloppenburg, unpublished observations). However, this disappears within 30 min in the continued presence of 4-AP. In some experiments, we substituted \( Ba^{2+} \) or \( Sr^{2+} \) for \( Ca^{2+} \) as the inward charge carrier. In a few experiments, we further isolated \( I_{Ca} \) in the AB neuron from \( Ca^{2+} \)-dependent currents by intracellularly injecting 0.6 M bis-(2-aminophenoxy)-N,N,N’,N’-tetraacetic acid (BAPTA), in 60 mM KCl, using 500-ms duration, -5-nA pulses at 1 Hz for 10 min. Measurements were begun after ≥30 min in blocking saline.

\( I_{Ca} \) characterization and DA modulation

The \( I-V \) relationship for \( I_{Ca} \) was determined in each pyloric neuron type using 5-mV incremental steps of 150- to 200-ms duration from a holding potential of −60 mV. The effects of DA (100 \( \mu M \)) and the \( Ca^{2+} \) channel blockers CdCl\(_2\) (200–600 \( \mu M \)), \( NiCl_2 \) (100 \( \mu M \)), and nifedipine (10–300 \( \mu M \)) were determined by comparing the mean of five peak \( I_{Ca} \) measurements from a holding potential of −50 mV to −15 or −10 mV, whichever produced the maximal fast inward current with no late outward current (this was determined by prior \( I-V \) characterization), in control (after the peak \( I_{Ca} \) had attained a steady amplitude) and after 5 (DA) or 20 (\( Ca^{2+} \) channel blockers) min perfusion. DA effects on blocker resistant outward currents were determined in a similar way.

DA was freshly dissolved in blocking solution before each application. A stock solution of nifedipine in DMSO was prepared daily and was diluted with blocking saline immediately before use. The final concentration of DMSO (≤0.1%) alone had no effect on \( I_{Ca} \). Nifedipine was applied with room lights off.

Data analysis

We measured the peak current amplitudes with Axograph 4 (Axon Instruments). Results were discarded if the DA effect did not reverse or, in the AB neuron, when DA caused uncontrolled voltage oscillations. An ANOVA, followed by protected \( t \)-test, determined the significant differences between individual data groups. Statistical significance was accepted with \( P < 0.05 \) for \( F \) or \( t \) values. Mean current amplitudes and percentages are reported ± SE.

RESULTS

Inward calcium currents in pyloric neurons

Under conditions designed to minimize voltage-gated \( Na^+ \), \( K^+ \), and \( H^+ \) currents, we recorded voltage-dependent inward currents from identified pyloric neurons during depolarizing voltage steps from either −60 or −50 mV. These inward currents had the characteristics of typical \( Ca^{2+} \) currents. First, \( Cd^{2+} \) was an effective blocker of this current. In the PY neuron of Fig. 1A, 200 \( \mu M \) Cd\(_2\)\(^{2+}\) reduced the inward current at −10 mV by 80%. Similar block was seen in PY, PD, and AB neurons. In three ABs, one PD and one IC neuron, increasing the Cd\(_2\)\(^{2+}\) concentration to 600 \( \mu M \) completely blocked the inward current, leaving a slowly rising outward current that
was not eliminated by our standard blocking saline (see AB example in Fig. 6C).

In a few experiments, we compared the inward current in a neuron using Ca$^{2+}$ or Ba$^{2+}$ as charge carriers. We did not routinely use Ba$^{2+}$ saline because the Ba$^{2+}$ current was often unstable and ran down quickly. Figure 1B shows that in a PY neuron, the Ba$^{2+}$ current’s amplitude was larger and it showed much slower inactivation than $I_{Ca}$, as is characteristic of Ca$^{2+}$ channels. Similar results were seen in three PY neurons, two ABs, a PD and an IC neuron. These results indicate that under our conditions of pharmacological blockade, the inward current in pyloric neurons is mediated by Ca$^{2+}$ channels.

We also examined the effects of 100 µM NiCl$_2$ on $I_{Ca}$ in AB, PD, and PY neurons because Ni$^{2+}$ blocks the low-threshold $I_{Ca}$ responsible for graded chemical synaptic transmission in the leech (Angstadt and Calabrese 1991). The pyloric Ca$^{2+}$ current was not affected by Ni$^{2+}$ (Fig. 1C; $n = 3$ for all cell types), even after very long applications (Fig. 1D; this figure also shows the stability of the $I_{Ca}$ in our preparation).

Voltage dependence of $I_{Ca}$ in pyloric neurons

Figure 2 shows typical examples of $I_{Ca}$ for each pyloric neuron in response to a series of depolarizing steps from $–60$ to $+20$ mV. Small voltage steps evoked small, slowly activating currents (see clearest examples in Fig. 2, C and D). Above a critical threshold, most cells showed a sudden jump to a much larger current, but with a variable latency to initiate the current after the voltage step began. With greater depolarizing steps, this large current activated with a shorter latency. In some cells, maximal current amplitude could be reached with the first large current jump (Fig. 2, A, C, and E). In others, the current increased in a more graded manner until a peak was reached (Fig. 2, B, D, and F). With further depolarization, the peak current declined, as expected for a reduction in driving force. Despite the presence of external and internal K$^+$ channel blockers, almost all PD and AB cells, most IC cells and half of the PY cells showed small outward currents at voltage steps above $+10$ mV (Fig. 2, A, B, E, and F). However, in VD and LP cells, this outward current was not seen (Fig. 2, C and D). In all cell types, a slowly deactivating inward current remained after the end of the voltage step. Repolarization was often accompanied by an active and uncontrolled inward current, especially in AB, LP, and IC cells (for example, Fig. 2C). This uncontrolled inward current was amplified in Ba$^{2+}$ saline (Fig. 1B), suggesting that it was carried through Ca$^{2+}$ channels rather than a Ca$^{2+}$-activated inward current.

The characteristics of these inward currents suggest that they did not arise in the cell body, where our electrodes were placed. Instead, they probably arose from the neuropil that is electrotonically distant from the soma and that was under incomplete voltage control (Graubard and Hartline 1991). This poor space clamp of a distal current causes the delayed onset of rapidly activating currents, the sudden jump to near peak current with small step increases, and the long-lasting inward currents after repolarization.

This imperfect voltage control and the apparent contamination of $I_{Ca}$ by residual outward currents prevented a detailed biophysical analysis of the current parameters in each pyloric neuron. Nonetheless, average $I$-$V$ plots for the inward currents can still give some insight into the approximate voltage dependence of this current and its function in pyloric neurons. Figure 3 shows mean $I$-$V$ plots for the AB ($n = 9$), PD ($n = 12$), LP ($n = 9$), VD ($n = 8$), IC ($n = 8$), and PY ($n = 11$) neurons. The AB and LP neurons appear to have slightly more hyperpolarized average voltage thresholds (around $–45$ mV: Fig. 3, A and C) than the other neurons (around $–40$ mV). The sudden jump in current amplitude (Fig. 3, A, C, and E) reflects the activation of currents in the poorly clamped region of the cells (Fig. 2). The LP neuron appears to have the largest mean peak amplitude of the current, whereas the AB has the smallest. Due to the
FIG. 2. Voltage dependence of $I_{Ca}$ in the different pyloric neuron types. Sets of current traces in response to 200-ms depolarizing voltage steps from $-60$ to $+20$ mV in 5-mV increments are shown for A, AB; B, PD; C, lateral pyloric (LP); D, ventricular dilator (VD); E, inferior cardiac (IC); and F, PY neurons. Time bar applies to all traces.

FIG. 3. Current-voltage relationships for $I_{Ca}$ from the different pyloric neuron types in control blocking saline. The mean peak current ($\pm$ SE, calculated for the variability across the $n$ values for a cell type at a specific voltage), is shown in response to each 5-mV incremental voltage step to $+20$ or $+25$ mV from a holding potential of $-60$ mV for A, AB ($n = 9$); B, PD ($n = 12$); C, LP ($n = 9$); D, VD ($n = 8$); E, IC ($n = 8$); and F, PY ($n = 11$).
space-clamp problems described in the preceding text, it is difficult to determine the voltage of half-activation or the voltage for the peak inward current in these neurons. Finally, the extrapolated reversal potential in all cell types was around +40 mV.

Poor voltage control of a current arising at a significant electrotonic distance from the recording site will, of course, skew the I-V parameters described in the preceding text. However, in some neurons, much better voltage control was achieved, and in these cases, the I-V characteristics were also within the range of values seen in Fig. 3. For example, the PY neuron in Fig. 4A shows inward Ba\(^{2+}\) currents under much better voltage control as seen by the graded increase in inward current with step depolarizations, the lack of an initial delay in current activation, and good voltage control on repolarization. The I-V plot from this cell (Fig. 4B) shows an activation threshold around −45 to −50 mV, and a peak current of 11 nA at −10 mV. The activation threshold is at the hyperpolarized end of the range for PY cells, perhaps due to better space clamp. However, the values for peak current amplitude and peak voltage are well within the range seen for PY currents recorded under poorer voltage control (Fig. 3). This suggests that the voltage properties in Fig. 3 may roughly approximate the properties of \(I_{Ca}\) in the different pyloric neurons.

**DA modulation of pyloric Ca\(^{2+}\) currents**

We limited our analysis of the effects of DA to the peak \(I_{Ca}\) amplitude because it appeared to be less affected by imperfect space clamp than other current parameters. An ANOVA test showed that DA had overall statistically significant effects \((P < 0.05)\) on the peak amplitude of \(I_{Ca}\) across the different pyloric neuron types.

DA enhanced \(I_{Ca}\) in most PY and LP neurons and in all IC neurons (Fig. 5A). In seven of eight PY neurons, DA significantly increased the mean peak \(I_{Ca}\) by 15 ± 4%. In the eighth PY neuron, DA reversibly decreased \(I_{Ca}\) by 20%. We did not include this cell in our statistical analysis because it was more than 4 SD from the mean DA effect (but see DISCUSSION). Six PY neurons (including the outlier) belonged to the subclass of PY neurons not electrically coupled to the LP; one other PY was electrically coupled to the LP, whereas one PY neuron was not categorized. Thus the effects of DA on \(I_{Ca}\) in PY neurons did not appear to correlate with PY subclass type. Figure 5B shows an example of the time course of the DA enhancement of \(I_{Ca}\) in one PY neuron.

In seven LP neurons, DA significantly increased the mean peak \(I_{Ca}\) by 8 ± 4%. DA weakly, but consistently, and thus significantly, increased the peak \(I_{Ca}\) in IC neurons by 4 ± 1% \((n = 5;\) Fig. 5A). We conclude that the main effect of DA is to enhance \(I_{Ca}\) in PY, LP, and IC neurons.

In contrast, DA weakly decreased the peak \(I_{Ca}\) in VD neurons and more strongly decreased \(I_{Ca}\) in PD and AB neurons (Fig. 5A). Peak \(I_{Ca}\) in VD neurons was weakly but consistently decreased by an average of 4 ± 1% \((n = 5)\). DA evoked a more robust and statistically significant 23 ± 4% decrease of mean peak \(I_{Ca}\) in PD neurons \((n = 8;\) example in Fig. 5C). Finally, DA caused a large and significant 50 ± 9% decrease of mean peak \(I_{Ca}\) in AB neurons \((n = 12;\) example in Fig. 5D).

**Effects of DA on blocker resistant outward currents in the AB neuron**

We were surprised that DA appeared to reduce \(I_{Ca}\) in AB neurons because DA strongly enhances release from AB synapses (Johnson and Harris-Warrick 1997; Johnson et al. 1995). Therefore we examined whether DA enhancement of an outward current that persists in our blocker saline (Fig. 2A) might mask a direct DA effect on \(I_{Ca}\). Under control conditions in blocker saline, this outward current first appeared around −15 mV at the end of the current trace, and then progressively dominated the current above +10 mV (Fig. 6A, control). The current at the higher voltage steps rose relatively slowly (in part due to the counter inward \(I_{Ca}\)), and did not inactivate during the voltage step (Fig. 6A, control). DA increased the peak amplitude of the outward current at the end of the voltage step while reducing the peak amplitude of \(I_{Ca}\) at the beginning of the step (Fig. 6A, DA). During DA application, the outward current became apparent at more hyperpolarized voltage steps (around −25 mV); it dominated the current trace at steps above −10 mV and did not reach its peak during the 200-ms voltage step (Fig. 6A, DA). In four AB experiments where we compared DA’s effects on \(I_{Ca}\) and the outward current, DA significantly and reversibly reduced the mean peak \(I_{Ca}\) by 2.7 ± 1.3 nA and significantly and reversibly enhanced the peak outward current at +20 mV by 3.5 ± 1.2 nA (Fig. 6B). In a separate

**FIG. 4.** Voltage dependence of a Ba\(^{2+}\) current in a PY neuron. A: inward currents in control blocking saline in response to 5-mV incremental voltage steps from a holding potential of −70 mV. Better voltage control than in Fig. 2 is indicated by the graded increase in inward current with step depolarizations, the lack of an initial delay in current activation, and good voltage control on repolarization. B: I-V relationship for the currents in A.
The apparent inactivation of the experiment shown in Fig. 7, intracellular BAPTA abolished (not shown). Intracellular injection of the calcium chelator, BAPTA, however, did separate (Fig. 6C). This remaining, Cd\(^{2+}\)-resistant outward current was not significantly modified by DA (Fig. 6D: \(n = 3\). Thus DA enhancement of a Ca\(^{2+}\)-dependent outward current in AB neurons could possibly mask a direct effect of DA on I\(_{\text{Ca}}\).

We then set out to separate I\(_{\text{Ca}}\) from the blocker resistant, Ca\(^{2+}\)-dependent outward current. Preliminary experiments using Ba\(^{2+}\) as the inward charge carrier were not successful, because the Ba\(^{2+}\) currents were very unstable and rapidly ran down in the AB neuron. Experiments using Sr\(^{2+}\), reduced external Ca\(^{2+}\) (25–50% of normal) or lower concentrations of Cd\(^{2+}\) (200 \(\mu\)M) failed to separate I\(_{\text{Ca}}\) from the outward current and still allowed enhancement of the outward current by DA (not shown). Intracellular injection of the calcium chelator, BAPTA, however, did separate I\(_{\text{Ca}}\) from the outward current. In three experiments, intracellular BAPTA reduced the outward current at +20 mV to 84 ± 8% of preinjection values. In the experiment shown in Fig. 7, intracellular BAPTA abolished the apparent inactivation of I\(_{\text{Ca}}\) during the voltage step to −20 mV (where the outward current was not yet activated), and enhanced the uncontrolled inward current following the voltage step (Fig. 7A). Under these conditions, DA still reversibly reduced I\(_{\text{Ca}}\) by 47% (Fig. 7B), but this was no longer linked to I\(_{\text{out}}\), which was detectable at higher voltages. Similar results were seen in two other AB neurons. These experiments showed a statistically significant reduction in I\(_{\text{Ca}}\) of 56 ± 11% (measured at −20 mV), and a much more variable and not statistically significant increase in the outward current (57 ± 25%, measured at +20 mV: \(P = 0.15\); Fig. 7C). We conclude that the DA-induced reduction of I\(_{\text{Ca}}\) in the AB neuron is a direct effect and is not caused by a consistently enhanced outward current.

**Nifedipine blocks the effect of DA on I\(_{\text{Ca}}\) in PD and PY neurons but not in AB neurons**

Hurley and Graubard (1998) demonstrated that the dihydropyridine nifedipine, a selective L-type channel blocker in vertebrates, blocks I\(_{\text{Ca}}\) in unidentified, cultured crab STG neurons. We examined the effect of nifedipine on DA-induced modulation of I\(_{\text{Ca}}\) from Panulirus AB, PD and PY neurons in situ because DA had the largest effects on I\(_{\text{Ca}}\) in these cells.

Nifedipine was an effective blocker of I\(_{\text{Ca}}\) in PY neurons (Fig. 8A). Detectable block was obtained with 10 \(\mu\)M nifedipine; 100 \(\mu\)M nifedipine significantly reduced mean peak I\(_{\text{Ca}}\) by 38 ± 8% (\(n = 5\)) whereas 200 \(\mu\)M nifedipine had only a slightly greater effect (56 ± 9% reduction, \(n = 4\)). Similar effects were seen in PD neurons (Fig. 8B), with detectable reductions at 10 and 50 \(\mu\)M, 55 ± 11% (\(n = 6\)) reductions at

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** Dopamine (DA) effects on I\(_{\text{Ca}}\) in pyloric neurons. A: summary of the effects of DA on I\(_{\text{Ca}}\) in pyloric neurons. The mean percent change from control peak I\(_{\text{Ca}}\) (± SE) caused by DA is shown for each cell type. *, statistically significant change from the mean control value (\(P < 0.05\)). \(n\), number of preparations. B: DA enhancement of peak I\(_{\text{Ca}}\) in a PY neuron. C: DA reduction of peak I\(_{\text{Ca}}\) in a PD neuron. D: DA reduction of I\(_{\text{Ca}}\) in an AB neuron. Single depolarizing voltage steps from −50 mV to a level (−15 or −10 mV) that produced the maximal fast rising peak inward current were repeated every minute and DA applied during the time of the filled bar. Insets: examples of currents in control blocking saline and in DA. Time bar in D applies to all cells.
L<sub>Ca</sub> in the AB neuron (n = 3). Dopamine significantly reduced the peak I<sub>Ca</sub> by 75 ± 13% before nifedipine treatment; after recovery of I<sub>Ca</sub>, 200 µM nifedipine reduced mean peak I<sub>Ca</sub> by 33 ± 14% (P = 0.16; Fig. 8F). Addition of DA in the presence of nifedipine completely abolished the remaining I<sub>Ca</sub> (Fig. 8F; example in Fig. 8I). Thus DA appears to act on nifedipine-sensitive Ca<sup>2+</sup> channels in PY and PD neurons but at least in part on nifedipine-resistant channels in the AB neuron.

**DISCUSSION**

**Properties of Ca<sup>2+</sup> currents in pyloric neurons**

Our results provide an initial characterization of I<sub>Ca</sub> in pyloric neurons of the spiny lobster. From a holding potential of ~60 mV, I<sub>Ca</sub> appeared between ~50 and ~40 mV in the different neurons. As discussed in RESULTS, these values are not very accurate due to poor space clamp of currents arising in the distal neuropil (Kloppenburg et al. 2000a). It is likely that we could not detect small, distant Ca<sup>2+</sup> currents, and thus the real thresholds for activation are probably more hyperpolarized than our values (see also Charlton and Augustine 1990). In the few cases where we had better voltage control, however, the I-V parameters of I<sub>Ca</sub> were similar to the population values, suggesting that our characterization at least approximated the real values.

In the vertebrates, Ca<sup>2+</sup> currents are usually classified into low-voltage-activated (LVA or T-type, with activation positive to ~70 mV) and high-voltage-activated (HVA, activation positive to around ~30 mV) classes; subtypes of the HVA currents (L-, P-, Q-, N-, and R-type) are defined by biophysical and pharmacological properties (Ertel et al. 2000; Hille 2001; Triggle 1999). The I<sub>Ca</sub> in pyloric neurons does exhibit the general characteristics of some HVA channel types: Ba<sup>2+</sup> is a better charge carrier than Ca<sup>2+</sup>, inactivation is Ca<sup>2+</sup>-dependent and relatively slow compared with LVA channels, the currents are insensitive to Ni<sup>2+</sup>, and a majority of the current in some cells is blocked by the L-type blocker nifedipine. However, the activation range of our currents is more hyperpolarized than traditional HVA...
channels and thus resemble currents with L-type properties that have more mid-voltage activated ranges. Such currents in vertebrates are seen in hypothalamic neurons (Akaike et al. 1989), retinal bipolar neurons (Heidelberger and Matthews 1992), hippocampal neurons (Avery and Johnston 1996), hair cells (Fuchs 1996), and the L-type subunit CaV 1.3/H9251 expressed in Xenopus oocytes (Xu and Lipscombe 2001). The calcium currents in the DG gastric motor neuron in the STG of the crab Cancer borealis (Zhang and Harris-Warrick 1995), in unidentified cultured STG neurons from the spiny lobster (Turrigiano et al. 1995), and in crayfish motor neurons (Hong and Lnenicka 1995; Wright et al. 1996) also have mid-voltage activation ranges as do other invertebrate Ca2+/H11001 currents, such as those in squid giant presynaptic terminals (Charlton and Augustine 1990), insect neurons (Wicher and Penzlin 1997), and snail neurons (Pin et al. 1990). Calcium currents recorded from the LP neuron in the crab, C. borealis (Golowasch and Marder 1992), and from unidentified cultured STG neurons from the crab C. productus (Hurley and Graubard 1998) appear to have more depolarized I-V characteristics and thus, in this way, resemble more classical HVA currents.

Our limited experiments with nifedipine suggest that the total I_{Ca} we recorded in pyloric neurons is composed of multiple subtypes and that these subtypes can differ between pyloric neurons. Hurley and Graubard (1998) first provided evidence that multiple Ca2+/H11001 channel types contribute to the total I_{Ca} and the control of synaptic transmission in crab STG neurons as is the case in many other neurons (for example, Fossier et al. 1999; Lnenicka and Hong 1997). Different STG Ca2+/H11001 channel types could be targeted to different cellular sites (Hartline and Graubard 1992; see also French et al. 2002). In our experiments, nifedipine, even at high concentrations, only blocked part of I_{Ca} in PD and PY neurons. The much weaker block by nifedipine of I_{Ca} in the AB neuron suggests that the different pyloric neuron types may express different ratios of Ca2+/H11001 channel types.

In addition, it appears that DA modulates different Ca2+/H11001 currents in pyloric neurons. The mean percent of control peak I_{Ca} (±SE) during nifedipine application (100 and 200 μM) is shown for A, PY (n = 5 at 100 μM; 4 at 200 μM); B, PD (n = 6 at 100 μM; 5 at 200 μM). C, AB (n = 4 at 100 μM; 6 at 200 μM). *, statistically significant change from the mean control value (P < 0.05). A summary of the nifedipine-DA interaction is shown for D, PY; E, PD; and F, AB neurons. Each graph shows the mean peak I_{Ca} (±SE; n = 3 for all cells) in control (C), DA (DA), nifedipine (Nif, 200 μM), and DA applied during nifedipine perfusion (DA/Nif). *, statistically significant change from the mean control value (P < 0.05). In D and E, the mean peak I_{Ca} for the Nif and DA/Nif were not significantly different. In F, no I_{Ca} was observable with DA application during nifedipine perfusion in any experiment. Example experiments are shown for G, PY; H, PD; and I, AB neurons. The peak I_{Ca} is plotted every minute in control blocking solution (○) with DA application (DA, □), and in nifedipine solution (●) with DA application (Nif + DA, ■).
channel types in different pyloric neurons because nifedipine completely blocked the DA effect on $I_{Ca}$ in PY and PD neurons but not in AB neurons. Differing complements of $Ca^{2+}$ channel types in the different pyloric neurons, especially the AB neuron, should not be surprising, considering the differing roles the neuron types play in pyloric rhythm generation.

Dopamine configuration of the pyloric network

Dopamine reconfigures the Panulirus pyloric network by modifying the intrinsic properties of the pyloric neurons and altering the strength of all of the network’s synaptic connections (Harris-Warrick et al. 1998). DA can initiate a motor pattern from a quiescent preparation, with descending input blocked, and can alter the pattern in an actively cycling preparation. It does so by enhancing bursting pacemaker properties in the AB neuron, exciting the LP, PY, and IC neurons, but inhibiting and reducing activity in the PD and VD neurons (Eisen and Marder 1984; Flamm and Harris-Warrick 1986a,b; Makino et al. 2000). In addition, it strengthens or weakens virtually every synapse in the network (Johnson et al. 1995).

Dopamine modulation of intrinsic firing properties of pyloric neurons

We are beginning to understand some of DA’s actions on the intrinsic electrical properties of pyloric neurons and their underlying currents, which contribute to pyloric network configuration. DA inhibits the transient $K^+$ current ($I_K$) in the AB, LP, PY, and IC neurons, contributing to their enhanced activity and phase advances in the pyloric motor pattern (Harris-Warrick et al. 1995a,b; Peck et al. 2001; Tierney and Harris-Warrick 1992). At the same time DA enhances $I_K$ and a calcium-dependent outward current ($I_{K(Ca)}$) in the PD cell, contributing to its reduced activity (Kloppenburg et al. 1999). DA also enhances $I_V$ in the LP, further enhancing its postinhibitory rebound properties (Harris-Warrick et al. 1995b).

Dopamine’s effects on $I_{Ca}$ could contribute to its modulation of the firing properties of the pyloric neurons. DA increases peak $I_{Ca}$ in the LP, PY, and IC neurons, consistent with its enhancement of firing in these cells, and reduces peak $I_{Ca}$ in PD and VD neurons, consistent with its inhibition of these cells (Flamm and Harris-Warrick 1986b; Turrigiano and Marder 1993). These effects could reflect modulation of the direct depolarizing effects of $I_{Ca}$ which in our measurements had a rather slow rate of inactivation (see Figs. 1 and 2). Thus in neurons that are excited, the enhanced $I_{Ca}$ which is activated at subthreshold voltages (Fig. 3), could help initiate bursting and bistability and prolong them for a longer time, as seen, for example, in spinal motor neurons (Alaburda et al. 2002; Carlin et al. 2000). Alternatively, increases in intracellular $Ca^{2+}$ could activate calcium-activated nonselective currents ($I_{Ca(Na)}$); this would boost the effects of calcium entry (Partridge et al. 1994; Zhang et al. 1995), and/or initiate second-messenger cascades that could enhance firing (Hille 2001). The loss of such inward current might also contribute to the hyperpolarization and reduction in firing of the PD and VD neurons.

Dopamine initiates bursting in a quiescent AB neuron (Flamm and Harris-Warrick 1986b), and enhances burst amplitude and cycle frequency in an isolated oscillating AB neuron (Ayali and Harris-Warrick 1999). We show here that DA significantly decreases peak $I_{Ca}$ by 50% in the isolated AB neuron, suggesting that $Ca^{2+}$ is not responsible for the main depolarizing drive underlying DA-evoked bursting in AB neurons as it is for other invertebrate bursting neurons (Kits and Mansvelder 1996). In a previous study (Harris-Warrick and Johnson 1987; see also Gola and Selverston 1981), we showed that reduction of extracellular $Ca^{2+}$ and partial blockade of $Ca^{2+}$ currents can also initiate rhythmic bursting in a quiescent AB neuron. A sufficient reduction in $[Ca^{2+}]_{out}$ during DA-induced bursting depolarizes the neuron, and bursting stops at the peak level of the voltage oscillation (Harris-Warrick and Flamm 1987; Johnson et al. 1992). These results together suggest that in the AB neuron, $I_{Ca}$ may actually be inhibiting rhythmic oscillatory capability and a reduction in $I_{Ca}$ will paradoxically enhance bursting. This could occur by a block of $I_{K(Ca)}$, which might be actively preventing bursting from occurring. Indeed, TEA, which blocks $I_{K(Ca)}$ as well as the very small $I_{K(V)}$ in these neurons, also initiates rhythmic bursting in a quiescent AB neuron (Harris-Warrick and Johnson 1987). However, our results here suggest that DA enhances a calcium-dependent outward current, possibly $I_{K(Ca)}$ in the AB neuron (Fig. 6). It is possible that DA has additional or different effects on currents that are not easily detected from the soma, or that additional, unidentified $Ca^{2+}$-dependent outward currents also inhibit and structure AB bursting activity. Further work will be needed to understand the dynamics of bursting in this pacemaker cell.

Dopamine modulation of synaptic interactions in the pyloric network

The mechanisms of DA modulation of the pyloric graded chemical synapses are less clear. All synapses in the pyloric network are targets of DA: the AB, LP, and PY chemical graded synapses are enhanced by DA, whereas the PD, VD, and (to a lesser extent) IC chemical synapses are inhibited (Johnson et al. 1995). We know little of the synaptic mechanisms or ionic currents targeted by DA to alter synaptic strength. However, at many of these synapses, DA appears to act at least in part presynaptically to enhance or decrease release of transmitter (Johnson and Harris-Warrick 1997). The simplest way for DA to influence transmitter release is through modulation of $I_{Ca}$, and we have tested this in our study.

Dopamine modulation of presynaptic $I_{Ca}$

Many previous studies have documented modulation, by DA and other neuromodulators, of $Ca^{2+}$ entry into presynaptic terminals that may alter transmitter or hormone release (for example, see Dunlap and Ikeda 1998; Hernandez-Lopez et al. 1997; Koga and Momiyama 2000; Nussinovitch and Kleinhaus 1992). We limited our study to the effects of DA on the peak $I_{Ca}$ because this appeared less affected by space clamp limitations and incomplete $K^+$ channel block.

DA affected the peak $I_{Ca}$ in all the pyloric neurons. The DA enhancement of $I_{Ca}$ in LP and PY neurons is consistent with our hypothesis that DA enhances transmitter release from these neurons (Johnson and Harris-Warrick 1997). In one outlier experiment, DA reversibly decreased peak $I_{Ca}$ in a PY neuron. Preliminary multiphoton microscopy $Ca^{2+}$ imaging results have suggested that in a single PY neuron, DA can enhance
Ca\(^{2+}\) entry into some presynaptic varicosities, while reducing Ca\(^{2+}\) entry into others (Kloppenburg et al. 2000b). Because pyloric neurons have multiple sites of synaptic contact onto their postsynaptic targets (King 1976), DA-enhancement of \(I_{Ca}\) and transmitter release from PY neurons may arise from a net synaptic response dominated by enhanced Ca\(^{2+}\) entry into the majority of terminals. Occasionally, the balance may shift, leaving a net reduction in Ca\(^{2+}\) entry and creating some variability in modulatory effects on synaptic transmission (Johnson et al. 1994).

The DA reduction of \(I_{Ca}\) in PD neurons is also consistent with DA targeting \(I_{Ca}\) to reduce, and sometimes completely abolish, transmitter release from these neurons (Johnson and Harris-Warrick 1990, 1997). These voltage-clamp results are consistent with our recent Ca\(^{2+}\)-imaging studies (Kloppenburg et al. 2000a), which showed that DA reduces depolarization-activated Ca\(^{2+}\) entry into fine varicosities of PD neurons. DA also markedly weakened PD synapses (Johnson et al. 1995) while evoking only a very small (though significant) reduction in \(I_{Ca}\). These pronounced inhibitory synaptic effects are very dramatic, given the rather modest reductions in \(I_{Ca}\); perhaps DA has additional effects at these synapses, for example to affect the release machinery directly in addition to modulating \(I_{Ca}\) in PD and VP terminals (see following text).

DA has only modest effects on the IC neuron, but they appear to be contradictory: DA weakly enhances \(I_{Ca}\) while the IC neuron’s single synapse, onto the VP neuron, is slightly weakened. However, the postsynaptic response of the VP neuron to the IC’s inhibitory transmitter, glutamate, is significantly reduced by DA (Johnson and Harris-Warrick 1997). Thus at this synapse, the postsynaptic reduction in glutamate responsiveness appears to outweigh the small expected increase in transmitter release due to DA’s weak enhancement of \(I_{Ca}\).

The major contradiction between DA’s effects on synaptic strength and on \(I_{Ca}\) is with the AB neuron. DA markedly increases AB synaptic strength (enough to even activate a silent synapse) (Johnson et al. 1995), but we show here that DA reduces \(I_{Ca}\) by \(\geq 50\%\) in this cell. We initially suspected that a Ca\(^{2+}\)-dependent outward current that persisted in our blocker saline and was enhanced by DA might mask a direct DA enhancement of \(I_{Ca}\). However, intracellular injection of BAPTA allowed us to separate the Ca\(^{2+}\)-dependent outward current from \(I_{Ca}\), and indicated that DA was directly reducing \(I_{Ca}\) in the AB neuron. Thus enhancement of AB transmitter release appears to occur concurrently with a reduction in presynaptic \(I_{Ca}\). Mismatches between DA’s effects on \(I_{Ca}\) and on transmitter/hormone release have been reported in other preparations. For example, in the retina, DA suppresses rod inputs and enhances cone inputs to horizontal cells (Witkovsky and Dearry 1991), while enhancing \(I_{Ca}\) in rods and suppressing it in a large fraction of cone cells (Stella and Thoreson 2000). DA can also inhibit Ca\(^{2+}\) currents in melanotropes (Keja et al. 1992), without altering hormone secretion (Mansvelder et al. 2002). This mismatch between release and \(I_{Ca}\), of course, occurs with other neuromodulators; for example, serotonin enhances neurotransmitter release at the crayfish neuromuscular junction by modulating \(I_{Na}\) (Beaumont and Zucker 2000; see also Saitow and Konishi 2000) without increasing intracellular Ca\(^{2+}\) levels (Delaney et al. 1991). Modulation of transmitter release could occur by a number of mechanisms, including modulation of presynaptic K\(^{+}\), Cl\(^{-}\), and H currents, transmitter transporters, Ca\(^{2+}\)-induced Ca\(^{2+}\) release from internal stores, and direct actions to change the activity of proteins driving release (Beaumont and Zucker 2000; Bouron 2001; Congar et al. 2002; Haydon and Trudeau 1998). For example, the DA enhancement of presynaptic \(I_{Ca}\) in rods decreases transmitter release through activation of a Ca\(^{2+}\)-activated Cl\(^{-}\) current (Thoreson et al. 2002). DA also reduces \(I_{Ca}\) in the AB (Peck et al. 2001), which is present in pyloric neuron synaptic terminals (Baro et al. 2000) and in general, increases the input resistance of the AB neuron (Johnson et al. 1993). Perhaps these factors contribute to the DA-induced enhancement of AB graded transmitter release. It is also possible that a subset of Ca\(^{2+}\) channels with different properties controls release from AB terminals, and these may not be inhibited by DA.

**Functional significance of DA-sensitive Ca\(^{2+}\) currents recorded from pyloric neuron cell somata**

Our analysis of DA’s effects on synaptic transmission assumed that the Ca\(^{2+}\) current we recorded is representative of the presynaptic \(I_{Ca}\) controlling graded chemical transmitter release, but it also could help to shape bistability and bursting in pyloric neurons. As discussed in the preceding text, Ca\(^{2+}\) entry is probably not the main depolarizing force for AB bursting in the presence of DA. In other pyloric neurons though, active responses and plateau potentials can be triggered by small depolarizations or hyperpolarizations that bring the membrane potential within the activation range of the currents we recorded (Bal et al. 1988; Russell and Hartline 1982). Plateau potentials in the gastric DG neuron of the STG are supported by Ca\(^{2+}\) currents with a similar activation range as the ones measured here (Zhang and Harris-Warrick 1995). The threshold for graded synaptic transmission between pyloric neurons can vary depending on the synaptic pair and the modulatory conditions, but it is usually around the resting potential of the presynaptic neuron (Hartline and Graubard 1992; Johnson and Harris-Warrick 1990). When pyloric neurons are held at \(-50\) mV, graded transmitter release begins with depolarizations to \(-45\) mV (Manor et al. 1997). Thus our \(I_{Ca}\) could participate in controlling graded chemical transmission. The congruence of DA’s effects on \(I_{Ca}\) in the LP, PY, VP, and PD neurons with its effects on release from those neurons also supports this \(I_{Ca}\) as contributing to graded transmitter release. The Ca\(^{2+}\) currents underlying graded chemical transmission in invertebrates have been most extensively studied in leech heart interneurons (Angstadt and Calabrese 1991; Ivanov and Calabrese 2000; Lu et al. 1997). These currents resemble LVA-type currents: they have more hyperpolarized activation ranges than our \(I_{Ca}\) and Ni\(^{2+}\) blocks them. The lack of sensitivity of pyloric \(I_{Ca}\) to Ni\(^{2+}\) as well as a report of enhancement of pyloric graded chemical transmission by Ni\(^{2+}\) (Zirpel et al. 1993) suggest that the currents underlying leech graded transmission differ from the \(I_{Ca}\) controlling pyloric graded chemical transmission. In its activation range and relatively low sensitivity to nifedipine, the \(I_{Ca}\) in pyloric neurons more closely resembles the L-type current mediating graded transmitter release from cochlear hair cells (Spassova et al. 2001) and vertebrate retinal neurons (Bernston et al. 2003; Taylor and Morgans 1998). Further work will be needed to clarify the functional role of the \(I_{Ca}\) recorded from pyloric neuronal somata.
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Present address of P. Kloppenburg: Universität zu Köln, Institut für Zoologie/Physiologie, AG Zelluläre Neurophysiologie, Weyertal 119, D-50923 Köln, Germany.

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