Amine Modulation of $I_h$ in a Small Neural Network

Jack H. Peck, Eric Gaier, Erin Stevens, Sarah Repicky, and Ronald M. Harris-Warrick.

Amine modulation of $I_h$ in a small neural network. J Neurophysiol 96: 2931–2940, 2006. First published August 30, 2006; doi:10.1152/jn.00423.2005. We studied the functional role and modulation of the hyperpolarization-activated inward current ($I_h$) in the pyloric network of the lobster stomatogastric ganglion. In isolated neurons, $I_h$ is a small current with a hyperpolarized voltage of half-activation ($V_{1/2}$) and a slow time constant of activation ($\tau_{act}$). Bath application of dopamine (DA), octopamine (OCT), or serotonin (5HT) modified $I_h$ in selected synaptically isolated pyloric neurons. DA significantly enhanced $I_h$ in the anterior burster (AB) neuron by depolarizing its $V_{act}$, accelerating its $\tau_{act}$, and enhancing its maximal conductance ($g_{max}$). DA more weakly enhanced $I_h$ in the pyloric constrictor (PY) and ventricular dilator (VD) neurons. OCT weakly depolarized $V_{act}$ and accelerated $\tau_{act}$ in the VD and inferior cardiac (IC) neurons. 5HT depolarized $V_{act}$ in the IC neuron. Under control conditions with intact modulatory inputs from other ganglia, the pyloric rhythm cycles strongly at about 1–2 Hz. Bath application of the $I_h$ blocker cesium ($Cs^+$) caused a mean increase in the period of 8%, although this effect was highly variable. When $Cs^+$ was applied to an isolated ganglion where the pyloric rhythm had been activated only by DA, the cycle period was consistently increased by 13.5%, with no other strong changes in rhythm parameters. These results suggest that $I_h$ regulates the pyloric rhythm by accelerating AB pacemaker frequency, but that this effect can vary with the modulatory conditions.

INTRODUCTION

The hyperpolarization-activated inward current ($I_h$) is a mixed Na$^+$–K$^+$ current with very slow kinetics of activation and deactivation. It was first discovered in heart sinoatrial node (Noma and Irisawa 1976) and, over the years, has had different designations such as $I_{ch}$ (Noma and Irisawa 1976) and, over the years, has had different and deactivation. It was first discovered in heart sinoatrial node (Noma and Irisawa 1976) and, over the years, has had different designations such as $I_{ch}$ (Noma and Irisawa 1976) and, over the years, has had different and deactivation. It was first discovered in heart sinoatrial node (Noma and Irisawa 1976) and, over the years, has had different

In this communication, we continue our investigation of the roles of amines in modulating the intrinsic properties of neurons (Harris-Warrick et al. 1995a,b) by further analyzing the role of $I_h$ in the pyloric network of the spiny lobster, Panulirus interruptus. We complete our analysis of $I_h$ in the pyloric network by determining its properties in five pyloric cell types [anterior burster (AB), pyloric dilator (PD), pyloric constrictor (PY), ventricular dilator (VD), and inferior cardiac (IC)] and its modulation in these cells by three amines: dopamine (DA), octopamine (OCT), and serotonin (5HT). We find that in pyloric neurons, $I_h$ is a relatively small and slowly activating current with activation voltages that are hyperpolarized com-

Address for reprint requests and other correspondence: J. H. Peck, Department of Psychology, Ithaca College, 1119 Williams Hall, Ithaca, NY 14850 (E-mail: peck@ithaca.edu).

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pared with the normal operating voltage ranges of pyloric neurons. Our results also show that monoamines can modulate \( I_h \) in selected subsets of pyloric neurons, by depolarizing its voltage of activation and accelerating its activation rate constant. Our results suggest that the relative importance of \( I_h \) in shaping the pyloric motor pattern varies depending on the modulatory milieu.

**METHODS**

**Materials**

California spiny lobsters, *Panulirus interruptus*, were obtained from Don Tomlinson Commercial Fishing (San Diego, CA) and maintained for \( \leq 4 \) wk in artificial seawater at 16°C. Chemicals were obtained from Sigma Chemical (St. Louis, MO), except for ZD7288, which was obtained from Tocris Cookson (Ellisville, MO).

**Dissection**

Lobsters were anesthetized by packing in ice for 30 min before dissection. The foregut was removed and the stomatogastric nervous system (STNS) was pinned out in a Sylgard-lined petri dish as described previously (Selverston et al. 1976). The STG was desheathed and a Vaseline well was placed around it. The STNS was superfused with *Panulirus* saline (composition in mM: 479 NaCl, 12.8 KCl, 13.7 CaCl₂, 3.9 Na₂SO₄, 10.0 MgSO₄, 2.0 glucose, 11.1 Tris base, 5.1 maleic acid, pH 7.35) (Mulloney and Selverston 1974) at 20°C, 3 ml/min.

**Cell and current isolation**

Using standard intracellular techniques (3 M KCl-filled microelectrodes, 10–25 MΩ), cells were identified by matching the intracellular firing patterns to extracellular recordings of nerves innervating identified muscles and by the timing and pattern of intracellular spikes. We isolated pyloric neurons from all detectable synaptic input by three steps. First, we photoinactivated cholinergic cells or neurons electrically coupled to the cell being studied (Miller and Selverston 1979) by injection of 6-carboxyfluorescein into the cell and illumination with blue light until its resting potential was reduced to 0 mV. Second, picrotoxin (PTX, 5 × 10⁻⁶ M) was added to block glutamatergic synapses (Marder 1987). Finally, inputs from other ganglia were blocked by bath application of tetrodotoxin (TTX, 10⁻⁷ M), to block voltage-gated Na⁺ currents. In addition, currents other than \( I_h \) were substantially reduced by the addition of cadmium (CdCl₂, 0.2 mM, to block Ca²⁺ and Ca²⁺-activated currents), 4-aminopyridine (4-AP, 4 mM, to block the transient K⁺ current, \( I_A \)), and tetraethyl ammonium (TEA chloride, 20 mM, to block rectifying voltage-gated K⁺ currents). Drugs and amines were applied to a Vaseline-lined pool surrounding the STG.

**Voltage clamp**

We carried out voltage-clamp protocols using an Axoclamp 2B amplifier controlled by pClamp software (Molecular Devices) running on a PC. For the \( I_h \) voltage activation protocol, all cell types but the AB were held at −50 mV and hyperpolarized for 7-s in −5-mV steps from −60 to −120 mV. Hyperpolarization < −100 mV killed the AB cell in several preparations, so we hyperpolarized the AB only to −100 mV. A grounded metal plate was inserted between the current and voltage electrodes to reduce capacitative coupling.

Because the time constant of activation (\( \tau_{Act} \)) is so slow, we did not use leak subtraction for our measurements. As a consequence, H-currents were calculated by measuring the difference between the initial value of the current after the jump resulting from the leak current and the trough of the slowly activating inward current at each voltage step, using Clampfit protocols (Molecular Devices; see Fig. 1A). Currents were converted to conductance using an estimated \( V_{max} \) of −35 mV (Golowasch and Marder 1992; Kiehn and Harris-Warrick 1992; Zhang et al. 2003; J. Peck, unpublished data) and fit to a first-order Boltzmann equation of the form

\[
g = \frac{g_{max}}{1 + e^{(V_{Act} - V)/\Delta V} + C}
\]

where \( g \) is the calculated conductance, \( g_{max} \) is the maximal conductance, \( V_{Act} \) is the voltage of half-activation, \( V \) is the voltage step, and \( \Delta V \) is the slope factor (Clampfit software, Molecular Devices). \( \tau_{Act} \) was analyzed by fitting a single exponential to the current trace during 7-s voltage steps to −100 mV (Clampfit).

**Amine application**

DA (10⁻⁴ M), 5-HT (10⁻⁵ M), and OCT (10⁻⁵ M) were dissolved in saline immediately before use and individually bath applied for 5

![FIG. 1. Parameter measurements of hyperpolarization-activated inward current (\( I_h \)) in pyloric neurons. A: measurement of \( I_h \) by voltage clamp of a ventricular dilator (VD) neuron from a holding potential of −50 mV showing 7-s voltage steps from −60 to −120 mV in 5-mV increments (top) and current responses (bottom). Measurement of \( I_h \) is indicated for the largest current trace. B: example current traces during a step to −120 mV showing blockade of \( I_h \) by 5-min bath application of 5 mM Cs⁺ or 100 \( \mu \)M ZD7288 to 2 different pyloric dilator (PD) neurons. C: example current traces at −120 mV, of temperature dependency of \( I_h \) at 15 and 20°C in a PD neuron.](Image 336x138 to 541x524)
min. $I_h$ was measured before the amine was applied, after 5 min of application and after a minimum 30-min washout, before the next amine was applied. For any given experiment, we varied the order of amine application, and not every amine was applied to every preparation (because of cell death or damage). Data were used only if the amine effect was fully reversed by washout.

### Pyloric rhythm

We measured the pyloric rhythm by recording intracellularly from two to four cells in a preparation. We studied the effects of blocking $I_h$ in intact preparations by comparing recordings before and after application of 5 mM Cs$^+$ for 10 min. For measurements of the DA-induced rhythm, descending modulatory inputs from ganglia were blocked by a Vaseline pool filled with TTX on the input stomatogastric nerve ($\text{sln}$). Under these conditions, the ongoing rhythm ceased. DA (10$^{-7}$ M) was bath applied to the STG for 30 min, followed by 10 min of 5 mM Cs$^+$ plus DA, and another 30-min wash in DA.

Ten-second recordings of data were analyzed for control, Cs$^+$, DA, and DA plus Cs$^+$ applications. The cycle period was measured using either the first action potential in successive cycles of the PD cell during intact preparation applications or the peak of the PD cell oscillations in successive cycles during DA (isolated STG preparation) applications; the PD usually does not fire action potentials during DA application. Trough voltages were averages of the lowest voltage each cell reached during the cycle. Action potentials per cycle were counted and averaged for each cell. Phase values were measured as the time from the first PD action potential (or the peak of the PD cell oscillation if there were no action potentials) to the first action potential of the follower cells divided by the cycle period. Measurements were compared during control and Cs$^+$ application for the intact preparation and during application of DA and DA plus Cs$^+$ for the isolated STG preparation. Data were accepted only if the effect of Cs$^+$ reversed on washout.

### Statistics

Voltage-clamp data were analyzed using a 2 (control–treatment; repeated measures) × 3 (amine: DA, OCT, 5HT) × 5 (cell type: AB, PD, PY, VD, IC) mixed factorial design for each of the four dependent measures ($g_{\text{max}}, V_{\text{Act}}, \text{Act}, \text{slope}, \tau_{\text{Act}}$). Tests for statistical significance were carried out using ANOVA and subsequent protected t-test using SPSS software. Pyloric rhythm data were analyzed using a one-way ANOVA and subsequent protected t-test. Statistical significance was determined by using t-test (SPSS software). Data with a $P < 0.05$ were accepted as statistically significant. Means are presented ± SD for tables, Boltzmann curves, and graphs.

### Results

#### Characterization of $I_h$ in pyloric neurons

Figure 1A shows an example of voltage steps and current traces for the PD neuron, which had the largest average $I_h$ of the pyloric neurons. The full range of traces is shown for 7-s steps from a holding potential of −50 mV in 5-mV increments between −60 and −120 mV. The current traces are not leak-subtracted, so the initial “instantaneous” drop in current reflects the leak current of the neuron. As indicated for the most hyperpolarized step (Fig. 1A), $I_h$ was measured from the initial level of the leak current after the capacitive currents had dissipated, to the value at the end of the step. As seen previously with the LP neuron (Harris-Warrick et al. 1995b), in the pyloric neurons $I_h$ is an inward current that very slowly activates during hyperpolarizing steps, with a typical threshold for activation 10 to 20 mV below the normal resting potential.

To verify that the current we were measuring was indeed $I_h$, we applied 5 mM Cs$^+$ or 100 μM ZD7288, both of which block $I_h$ in other preparations (Gasparini and DiFrancesco 1997; Robinson and Siegelbaum 2003; Thoby-Brisson et al. 2000). Both drugs rapidly reduced the current with an almost complete block after 5 min (Fig. 1B). The effects of Cs$^+$ reversed rapidly on washout, but those of ZD7288 reversed slowly and incompletely. Because extracellular Cs$^+$ does not block other currents in pyloric neurons at these concentrations (Harris-Warrick et al. 1995b), it was the preferred antagonist for this study.

Although our previous experiments on the modulation of other ion channels were done at 16°C (Harris-Warrick et al. 1995b; Peck et al. 1999), $I_h$ was very small at that temperature. At 20°C $I_h$ almost doubled (from means of 6.5 to 12 nA at −120 mV in the PD neuron, $n = 2$; Fig. 1C), so our measurements in this study were all made at that temperature. This is within the normal annual temperature range these lobsters experience in the coastal waters off southern California.

#### $I_h$ under control conditions

Table 1 shows the calculated Boltzmann values for $g_{\text{max}}, V_{\text{Act}}, \text{Act}$, and slope, as well as the $\tau_{\text{Act}}$ (measured at −100 mV), for $I_h$ for each of the cell types under control conditions. For convenience, data for the LP neuron from our previous study (Harris-Warrick et al. 1995b) are also shown. For $g_{\text{max}}$, there was a significant effect of cell type [$F(4,26) = 12.6, P < 0.001$]. The PD cell had a significantly higher $g_{\text{max}}$ than that of any of the other cell types. The PD had an intermediate $g_{\text{max}}$, but it was not significantly different from the AB, IC, and PY cells that have $g_{\text{max}}$ values less than half that of the PD neuron. The voltage for half-activation, $V_{\text{Act}}$, showed significant variability between the neurons [$F(4,26) = 5.9, P = 0.002$], with almost 20-mV differences between the cell types. The IC cell had a significantly more hyperpolarized $V_{\text{Act}}$ than that of the PD and AB cells, whereas the AB had the most depolarized $V_{\text{Act}}$ and was significantly different from the PD, PY, and IC cells. The slope factor for $I_h$ varied between 7 and 11 mV and showed no significant effect of cell type. Finally, the $\tau_{\text{Act}}$ also showed significant variation between cell types [$F(4,26) = 3.9, P = 0.017$]. The AB had a slower $\tau_{\text{Act}}$ than that of all the other cell types, which were very similar to one another.

#### Amine modulation of $I_h$

DA, OCT, and 5HT can all evoke unique patterns of the pyloric rhythm (Flamm and Harris-Warrick 1986a,b), by their different sets of actions on ionic currents in the individual neurons. To study how modulation of $I_h$ might contribute to these changes, we examined the effects of DA, OCT, and 5HT on the voltage and kinetic parameters of $I_h$ in the AB, PD, PY, VD, and IC neurons; this analysis was reported earlier for the LP neuron (Harris-Warrick et al. 1995b). Significance was determined by comparing each amine to its immediately preceding control.

Our ANOVA analysis showed that there was a significant interaction of control–treatment by amine [$F(2,27) = 3.0, P = 0.05$] for $g_{\text{max}}$, which resulted from DA and 5HT producing...
overall larger increases in $g_{\text{max}}$ than did OCT. There was a similar effect on $V_{\text{Act}}$ that was both reliable and large [$F_{(2,77)} = 12.1, P < 0.001$]. In this case, DA produced larger depolarizing shifts than did OCT and 5HT. Because these ANOVAs were statistically significant, we were then able to analyze the effects of each amine on each cell type.

**DOPAMINE.** We previously studied the effects of DA on the pyloric network in detail (Ayali and Harris-Warrick 1999; Flamm and Harris-Warrick 1986a,b; Harris-Warrick et al. 1995a,b; Kloppenburg et al. 1999). DA excites and enhances firing of the AB, LP, PD, PY, and IC neurons while inhibiting and hyperpolarizing the PD and VD neurons (Flamm and Harris-Warrick 1986b). We previously showed that DA enhances $I_h$ in the LP neuron by significantly shifting its $V_{\text{Act}}$ in the depolarizing direction (Harris-Warrick et al. 1995b). Here we monitored the effect of DA on $I_h$ in the other five pyloric cell types. The most dramatic effect of DA was on AB, the primary pacemaker neuron of the network. DA caused a significant shift in AB’s $V_{\text{Act}}$ by 20 mV in the depolarizing direction (from $-85 \pm 4$ to $-65 \pm 3$ mV, $n = 7; t = 5.5, P = 0.002$) and a significant (33%) decrease in $\tau_{\text{Act}}$ (from $4.15 \pm 0.7$ to $2.78 \pm 0.2$ s, $n = 4; t = 6.0, P = 0.027$; Fig. 2, A and D), with no change in the slope of the activation curve. In addition, $g_{\text{max}}$ was increased by about 50%, although this was not significant (from $0.06 \pm 0.02$ to $0.09 \pm 0.02$ $\mu$S, $n = 7; t = 2.6, P = 0.057$). As a consequence, in the presence of DA, there is a significant amount of active $I_h$ within the normal voltage range of the AB cell ($-55$ to $-35$ mV), whereas without DA the activation curve is much more hyperpolarized. Figure 2 also shows that DA reduced the leak current by roughly 10%, seen as a reduction in the instantaneous drop at the beginning of the voltage step (this reduction was present in five of the seven AB cells tested with DA). This decrease is consistent with the increase in input resistance that we reported earlier under DA conditions (Johnson et al. 1993).

In the PY neurons, DA also evoked a significant 17-mV depolarizing shift in the $V_{\text{Act}}$ (from $-99 \pm 3$ to $-82 \pm 4$ mV, $n = 8; t = 7.1, P < 0.001$) and a significant (30%) decrease in $\tau_{\text{Act}}$ (from $2.23 \pm 0.1$ to $1.55 \pm 0.1$ s, $n = 4; t = 4.7, P = 0.018$), with no change in the $g_{\text{max}}$ or slope of the Boltzmann relation (Fig. 2, B and E). However, because the $V_{\text{Act}}$ in the PY neurons under control conditions is significantly more hyperpolarized than that of the AB neuron, this DA-evoked shift causes only a marginal change in the active $I_h$ in the physiologically relevant voltage range. As can be seen in Fig. 2B, DA also decreased the leak current in PY neurons, as previously reported (Johnson et al. 1993). DA produced a significant, although smaller, depolarizing shift in $V_{\text{Act}}$ in the PD neuron (from $-94 \pm 2$ to $-86 \pm 2$ mV, $n = 6; t = 16.9, P < 0.001$) along with a significant (26%) decrease in $\tau_{\text{Act}}$ (from $2.16 \pm 0.2$ to $1.6 \pm 0.3$ s, $n = 6; t = 8.3, P = 0.001$), with no change in $g_{\text{max}}$ or slope (Fig. 2, C and F). This result was unexpected because DA hyperpolarizes and inhibits the PD neuron but depolarizes and excites the AB and many of the PY neurons in measurements from either isolated neurons or from the intact circuit (Flamm and Harris-Warrick 1986b). DA had no detectable effect on $I_h$ in the PD and IC neurons, which are inhibited and excited by DA, respectively (data not shown).

**OCTOPAMINE.** In our earlier work, we showed that OCT excites, at least weakly, all the neurons of the pyloric network in both isolated and intact preparations (Flamm and Harris-Warrick 1986a,b). Supporting this excitation, OCT weakly enhanced $I_h$ in two types of pyloric neurons. Like DA, OCT shifted the $V_{\text{Act}}$ of the PD neuron in the depolarizing direction (from $-95 \pm 2$ to $-88 \pm 3$ mV, $n = 6; t = 4.9, P = 0.004$), along with a significant decrease in $\tau_{\text{Act}}$ (from $2.16 \pm 0.2$ to $1.82 \pm 0.2$ s, $n = 6; t = 3.7, P = 0.02$), with no significant effect on the slope or $g_{\text{max}}$. In addition, OCT evoked a similar 6-mV depolarizing shift in $V_{\text{Act}}$ in the IC neuron (from $-103 \pm 4$ to $-98 \pm 4.7$ mV, $n = 6; t = 3.0, P = 0.029$), with no effect on other parameters (Fig. 3, A and B). OCT had no detectable effects on the AB, PD, or PY neurons.

**SEROTONIN.** The only neuron affected by 5HT was IC, which showed a 17-mV depolarizing shift in $V_{\text{Act}}$ during 5HT administration (from $-105 \pm 4$ to $-88 \pm 9$ mV, $n = 6; t = 6, P = 0.002$). There was also a decrease in $\tau_{\text{Act}}$ (from $3.2 \pm 1.1$ to $2.2 \pm 1.4$, $n = 6; t = 2.4, P = 0.059$), although this difference did not reach statistical significance. There was no change in the $g_{\text{max}}$ or slope in the IC neuron (Fig. 4) and 5HT had no effect on $I_h$ in the remaining pyloric neurons.

**Role of $I_h$ during the control pyloric rhythm**

Under our control conditions, the commissural and esophageal ganglia provide neuromodulatory inputs to the STG (Marder 1987). We do not control these modulatory inputs and their activity varies somewhat from preparation to preparation; as a result, the frequency of the pyloric rhythm also varies somewhat from day to day, for example, cycling at frequencies from 1 to 2 Hz in our preparations. To assess the role of $I_h$ in this control ongoing pyloric rhythm, we bath-applied 5 mM Cs+ (which reversibly eliminates $I_h$ within 5 min; Fig. 1B)

**TABLE 1.** Boltzmann and tau control parameters for each cell type

<table>
<thead>
<tr>
<th>Control Parameter</th>
<th>AB</th>
<th>PD</th>
<th>PY</th>
<th>VD</th>
<th>IC</th>
<th>LP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{\text{max}}, \mu$S</td>
<td>$0.06 \pm 0.04 (7)$</td>
<td>$0.09 \pm 0.02 (6)$</td>
<td>$0.05 \pm 0.02 (8)$</td>
<td>$0.15 \pm 0.02 (6)$</td>
<td>$0.06 \pm 0.03 (6)$</td>
<td>$0.09 \pm 0.01 (5)$</td>
</tr>
<tr>
<td>$V_{\text{Act}}, \text{mV}$</td>
<td>$-85 \pm 9$</td>
<td>$-96 \pm 4$</td>
<td>$-99 \pm 7.2$</td>
<td>$-94 \pm 4.7$</td>
<td>$-103 \pm 4$</td>
<td>$-113 \pm 1.5$</td>
</tr>
<tr>
<td>Slope, $\text{mV}$</td>
<td>$11 \pm 5.7$</td>
<td>$8.2 \pm 1.6$</td>
<td>$9 \pm 3.2$</td>
<td>$9.9 \pm 1.5$</td>
<td>$7.0 \pm 0.8$</td>
<td>$-113 \pm 1.5$</td>
</tr>
<tr>
<td>$\tau_{\text{Act}}, \text{s}$</td>
<td>$4.2 \pm 1.5 (4)$</td>
<td>$2.6 \pm 0.5 (6)$</td>
<td>$2.2 \pm 0.3 (4)$</td>
<td>$2.16 \pm 0.4 (5)$</td>
<td>$2.8 \pm 0.10 (6)$</td>
<td>$2.4 \pm 0.4 (5)$</td>
</tr>
</tbody>
</table>

Values are means ± SD; values of $n$ are in parentheses and apply to the rest of the column for each cell type; $\tau_{\text{ Act}}$ for each cell type is listed separately. $\tau_{\text{Act}}$ measured at $-100$ mV. Smaller $N$ values result from an inability to fit the very small $-100$-mV current trace in some AB and PY neurons. *LP values taken from a previous experiment (Harris-Warrick et al. 1995b); slope not reported.
selectively to the STG. We analyzed changes in five parameters of the pyloric rhythm: period, trough voltages, phasing, burst duration, and spike frequency. Figure 5A shows an experiment (chosen because the effect of Cs⁺/H₁₁₀₀₁ was close to the mean response) where Cs⁺/H₁₁₀₀₁ slowed down the pyloric rhythm, increasing the period by about 6% over the control. The first and third traces are from the PD neuron, a member of the pacemaker group. The second and fourth traces are from the LP neuron, one of the follower neurons. Although the cycle frequency decreased, there were no obvious changes in the other firing properties of the neurons. Under control conditions with variable modulatory inputs from the higher ganglia, there was significant variability in the effects of Cs⁺/H₁₁₀₀₁ on the pyloric cycle frequency between preparations; in three of 12 preparations there was no effect, whereas in others there was a quite strong reduction in cycle frequency. Figure 5C shows the mean percentage change in period from control to Cs⁺/H₁₁₀₀₁ conditions. The average period changed from 0.757 ± 0.290 s in control to 0.836 ± 0.361 s in Cs⁺, representing a significant increase of 8.2% over control ($n = 12; t = 2.7, P = 0.021$). Analysis of the other rhythm parameters revealed only one significant difference: the PD neuron’s burst duration modestly increased by 15 ms (8%) from 177 ± 86 ms in control to 192 ± 79 ms in Cs⁺ conditions ($n = 10; t = 2.6, P = 0.049$). There were no statistically significant changes in trough values, phasing, burst duration, or number of action potentials in any of the other neurons.

**Role of $I_h$ in the DA-induced pyloric rhythm**

Although Cs⁺ increased the period of the control ongoing pyloric rhythm, there was a large amount of variability in its effect (Fig. 5C). We hypothesized that this large variability might be attributable to the inherent variability in modulatory inputs during the control pyloric rhythm, and thus to the variable amount of $I_h$ active in the physiological voltage range. To reduce this variability, we eliminated all descending modulatory inputs by blocking action potential conduction on the stn (the input nerve to the STG) with TTX, and then added back a single modulator at a known concentration. As described above, DA had the strongest effects of any of the amines on the pyloric neurons, including a strong enhancement of $I_h$ in the primary pacemaker neuron AB. Accordingly, we examined the role of $I_h$ in the pyloric rhythm evoked only by

![FIG. 2. Dopamine (DA) increases $I_h$ in anterior burster (AB), pyloric constrictor (PY), and VD neurons. A–C: typical experiments showing current traces of the AB, PY, and VD neurons, respectively, under control conditions and during application of $10^{-4}$ M DA. Voltage steps are at 5-mV intervals over the ranges of: AB from −50 to −110 mV; PY from −60 to −115 mV; VD from −60 to −120 mV. D–F: averaged Boltzmann fits to the voltage activation curves for $I_h$ in the AB ($n = 7$), PY ($n = 8$), and VD ($n = 6$) neurons, respectively, under control and $10^{-4}$ M DA conditions.](http://jn.physiology.org/content/96/6/2935.f2)
DA. After blocking descending modulatory inputs, the rhythm fell silent and only a few cells fired tonically at low frequencies (Flamm and Harris-Warrick 1986a). We then bath-applied DA, activating the specific “DA” rhythm, where the PD and VD neurons were inhibited and fired weakly or fell silent, whereas the remaining four neuron types were highly excited and fired strong bursts of action potentials.

Figure 5B shows an example of the pyloric rhythm in the PD and LP neurons after blocking modulatory inputs from the stn and adding DA, and then blocking \( I_h \) with Cs. In DA, the period was longer than that under control conditions (Ayali and Harris-Warrick 1999). The PD neuron was hyperpolarized (trough voltages were \(-60 \text{ mV}\), compared with \(-55 \text{ mV} \) in control conditions) and less active, whereas the LP neuron was depolarized (trough voltages were \(-65 \text{ mV}\), compared with \(-70 \text{ mV} \) in control conditions) and fired at higher frequencies. Addition of Cs significantly increased the DA-induced cycle period in this preparation (Fig. 5B) and in every preparation we tested (\( n = 10 \)). The cycle period changed on average from 0.733 to 0.829 s. Because of the variability in control cycle period, the changes were expressed as percentage change for each experiment (Fig. 5C). On average when Cs was added to the DA-induced rhythm, the period was significantly increased by 13.5% (\( t = 10.4, P < 0.001 \); Fig. 5C). This was not significantly different from the Cs effect under control conditions (\( t = 1.5, P = 0.149 \)), but the SD was smaller (from 10.4 to 4.1%; \( F = 3.7, P = 0.069 \)). Although its effect on cycle period was robust, Cs produced only two significant differences in the other parameters of the DA-induced motor pattern: The LP burst duration was increased by 74 ms (34%) from 219 ± 160 to 293 ± 173 ms (\( n = 8 \); \( t = 4.1, P = 0.005 \)) and the number of LP action potentials per burst was increased by two (21%) from 9.6 ± 5.6 to 11.6 ± 6.5 (\( n = 8 \); \( t = 2.8, P = 0.026 \)). As in the control + Cs condition above, there were no changes in any parameters in any of the other cell types studied.
AMINE MODULATION OF $I_h$

DISCUSSION

These experiments show that, although $I_h$ is present in all the pyloric neurons, it is a small current with a hyperpolarized $V_{Act}$ and a slow $\tau_{Act}$. Blocking $I_h$ in the ongoing pyloric rhythm produces only small and variable increases in cycle period. Under more defined modulatory conditions, such as during DA application, blocking $I_h$ produces more reliable changes in period. These results suggest that the role of $I_h$ in setting the cycle frequency may depend on the modulatory conditions at the time, ranging from no effect to a modest acceleration.

$I_h$ parameters in unmodulated pyloric neurons

Analysis of our voltage-clamp data from synaptically isolated pyloric neurons revealed that the biophysical parameters of $I_h$ vary among the different cell types of the pyloric network. The pyloric neurons differed in the amplitude, $V_{Act}$ and slope parameters of $I_h$. Although we do not know the molecular basis of these cell-specific differences in $I_h$ parameters, one possibility is differences in expression of alternate splice variants of the $I_h$ channel gene. The honey bee $I_h$ gene shows a significant amount of alternate splicing of its RNA (Gisselmann et al. 2004) and we recently detected alternate splicing in the $I_h$ gene (Ouyang et al.), which gives rise to currents with varying $V_{Act}$, slope, and $\tau_{Act}$ values when expressed in Xenopus oocytes (Q. Ouyang, personal communication). Alternatively, there might be differential expression of auxiliary subunits for $I_h$ among pyloric cell types, such as homologs of KCNE2 that have been shown to modify HCN channel properties in vertebrates (Decher et al. 2003). Finally, it is possible that the physical location of the channels varies between neuron types and, as a result, our measurements from the soma might be different.

Amine modulation of $I_h$

Swensen and Marder (2001, 2000) showed in the crab that a set of peptides modulate the same current but in different subsets of the pyloric network resulting in unique variants of the pyloric rhythm. In the same fashion, we found that each of the monoamines affected $I_h$ in a different subset of pyloric neurons. DA had more marked effects on more neurons than did OCT and 5HT. The major modulatory effect was to depolarize $V_{Act}$ and accelerate $\tau_{Act}$ with lesser effects on $g_{max}$ and no effects on the slope of the voltage activation curve. All of these effects would increase $I_h$ at less-hyperpolarized voltages.

DA modulated $I_h$ in the AB, PY, and VD neurons. Most significantly, it enhanced $I_h$ in the AB cell by depolarizing $V_{Act}$ by 20 mV, increasing the maximal conductance by 50% and accelerating the rate of activation. Weaker effects were seen in the PY and VD neurons. It is interesting to note that DA depolarized $V_{Act}$ in the PY as much as in the AB, but because PY’s control $V_{Act}$ was 14 mV more hyperpolarized than that of the AB, the shift in $V_{Act}$ in the PY neurons caused little change in $I_h$ in the normal physiological range of the neurons. All of these changes could enhance the depolarizing effect of $I_h$ in these cells, but especially in the AB, which is the major pacemaker of the pyloric rhythm (Ayali and Harris-Warrick 1999). In the AB neuron, the DA-evoked shift in $V_{Act}$ was sufficient to allow significantly more activation of $I_h$ in the physiologically relevant voltage range, than under control conditions. Although the rate of activation was accelerated somewhat when measured at −100 mV, activation is still so slow in the physiologically relevant voltage range (> −55 mV) that the current is likely to act as a tonic inward leak current, even in the presence of DA.
OCT modulation of $I_h$ was much more subtle: it depolarized the $V_{Ac}$ and accelerated the rate of activation of $I_h$ in the VD and IC neurons, but not into a physiologically relevant voltage range. These smaller changes mirror the more subtle changes one sees with OCT on the pyloric rhythm in general (Flamm and Harris-Warrick 1986a). 5HT affected only one neuron, the IC, where it depolarized the $V_{Ac}$. This change is also not likely to be sufficient for 5HT to significantly enhance $I_h$ under normal physiological conditions.

Other researchers have also found that the major effect of neuromodulators on $I_h$ is to shift the $V_{Ac}$ in the depolarizing direction (Santoro and Tibbs 1999), in most cases as a result of direct binding of cAMP, which depolarizes the channel’s $V_{Ac}$ (for review see Accili et al. 2002; DiFrancesco and Tortora 1991; Ingram and Williams 1996). We do not know the second-messenger mechanisms underlying amine actions in the pyloric network. Some of their cellular effects can be mimicked by elevations in cAMP (Flamm et al. 1987). However, optically measured cell-specific cAMP elevations in response to monoamines (Hempel et al. 1996) do not correlate simply with the cell-specific $I_h$ responses to monoamines described here.

Role of $I_h$ in the cycling pyloric rhythm

When the STG is dissected along with the commissural and esophageal ganglia, modulatory inputs from these ganglia support an actively cycling pyloric rhythm; however, pyloric cycle frequency varies from preparation to preparation, presumably arising from differences in the exact modulatory inputs that are active after each dissection (Miller 1987). If $I_h$ is active during pyloric cycling, we would expect that blocking $I_h$ with Cs$^+$ would slow the pyloric rhythm by reducing the rate of rebound to the next burst. In theory, $I_h$ block could also cause the neurons to hyperpolarize and change their phasing, burst duration, and number of action potentials (Harris-Warrick et al. 1995b). In our experiments, we found that the only important physiological parameter that did change during $I_h$ block with Cs$^+$ was the period, which typically increased by roughly 8% (Fig. 5C).

Figure 5C also shows that there was a significant amount of variability (as seen by the very large SD) in the percentage change in period on blocking $I_h$. We propose that this is attributed to the variable (and undefined) modulatory state of our different preparations. Significantly, in some preparations (three of 13) there was no detectable effect of Cs$^+$ on cycle frequency, suggesting that in these preparations $I_h$ is functionally silent. In others Cs$^+$ caused a significant prolongation of cycle period, similar to the response we saw with DA. For several reasons, we think that this is a consequence of blocking $I_h$ in the AB, the primary pacemaker neuron. First, there were no significant changes in any of the other parameters of the pyloric rhythm (trough voltages, phasing, burst duration, or number of action potentials). Second, our voltage-clamp experiments showed that in the isolated AB neuron, activation of $I_h$ could be shifted to the physiologically relevant voltage range by DA. These two findings suggested to us that in these control rhythmic preparations, Cs$^+$ was blocking $I_h$ in the AB that had been shifted into a physiologically relevant range by some undefined neuromodulatory input and that this blockade would slow the cycle frequency.

To test this, we generated a more uniform baseline condition by isolating the STG and initiating the rhythm with DA, which had the largest effects of the three amines on $I_h$, particularly on the AB pacemaker neuron. When we then added Cs$^+$ to block $I_h$, it showed a larger and more reliable change in activity: the period lengthened by 13.5% and the SD of the percentage change with Cs$^+$ was smaller (4.1 vs. 10.5%). As in the control + Cs$^+$ condition, there were no other important changes in other rhythm parameters. These findings lead us to believe that the Cs$^+$-induced slowing in period is again primarily ascribed to blocking $I_h$ in the AB and that the main effect of DA modulation of $I_h$ is to enhance the oscillation frequency of the pacemaker AB neuron by shifting the $V_{Ac}$ of $I_h$ into the physiological range where it could affect the cycle frequency.

DA modulation of $I_h$ in the LP neuron was previously studied by Harris-Warrick et al. (1995b). Like the AB, PY, and VD neurons studied in voltage clamp in this study, DA depolarized $V_{Ac}$ in LP (from −113 to −93 mV) and decreased $\tau_{Ac}$ (from 2.4 to 0.96 s). In the synaptically isolated LP neuron, postinhibitory rebound after large hyperpolarizing steps was enhanced by DA as a combination of enhanced $I_h$ and decreased $I_A$ (Harris-Warrick et al. 1995b). In the present study, Cs$^+$ block caused a 13% increase in the pyloric cycle period when activated solely by DA. It may be that in addition to DA’s effects on $I_h$ in the AB, Cs$^+$ block of $I_h$ in the LP could also contribute to changing the period of the STG oscillations by its inhibitory synapse on the PD–AB pacemaker kernel.

In vertebrate systems, $I_h$ has a strong pacemaker function in a number of systems—resulting from a more depolarized $V_{Ac}$ and relatively faster $\tau_{Ac}$—so it activates and deactivates during ongoing oscillations (Ludwig et al. 1998; Santoro et al. 1998). In the thalamus, $I_h$ is modulated to contribute to the generation of spindle waves: these are bursts of synchronized action potentials separated by long depolarized periods when the neurons are silent. The silence is generated by a persistent afterdepolarization that blocks spike generation, arising from activation of persistent $I_h$ by cAMP accumulation, generated in a Ca$^{2+}$-dependent manner during the spindle burst (Luthi and McCormick 1998b, 1999). cAMP shifts the $V_{Ac}$ to depolarized voltages, so that $I_h$ is active at more depolarized voltages, just as monoamines do in selected pyloric neurons. However, in the lobster pyloric network, $I_h$ is small, activates slowly, and at much more hyperpolarized voltages. Because of this, it has been modeled as a depolarizing leak current (Zhang et al. 2003).

Our experiments suggest that the role $I_h$ plays in the pyloric motor pattern is not fixed, but varies with the modulatory conditions. Our voltage-clamp and Cs$^+$ blockade data indicate that when the appropriate modulatory inputs are not active, $I_h$ plays little or no role in shaping the motor pattern; because of its hyperpolarized voltage activation range, it is essentially silent under these conditions. However, when inputs such as those releasing DA become active, the voltage dependency of $I_h$ activation is shifted, at least in the AB neuron, such that the current is active in the physiologically relevant voltage range, and now helps to affect the cycle frequency. Thus $I_h$ is conditionally active in pyloric neurons, playing a significant role only under modulatory conditions that allow it to be activated in the physiologically relevant voltage range.
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Present addresses: E. Stevens, New York Medical College (School of Medicine), Valhalla, NY 10595; S. Repicky, University of Miami School of Medicine, Dept. of Molecular and Cellular Pharmacology, Miami, FL 33101.

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