Elevated temperature alters the ionic dependence of amine-induced pacemaker activity in a conditional burster neuron

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Summary. The anterior burster neuron of the lobster (Panulirus interruptus) stomatogastric ganglion is a conditional burster that functions as the primary pacemaker for the pyloric motor network. When modulatory inputs to this cell are blocked, it loses its bursting properties and becomes quiescent. Applications of the monoamines, dopamine, octopamine or serotonin restore rhythmic bursting in this cell (Flamm and Harris-Warrick 1986). At 15 °C, serotonin- and octopamine-induced oscillations depend critically upon sodium entry (blocked by low sodium saline or tetrodotoxin); dopamine-induced oscillations depend upon calcium entry (blocked by reduced extracellular calcium; Harris-Warrick and Flamm 1987). We show here that the ionic dependence of amine-induced oscillations in the anterior burster cell differs at 15 and 21 °C. At 21 °C, all amines have the potential to induce rhythmic oscillations in saline containing tetrodotoxin. At the elevated temperature and in tetrodotoxin, both calcium and sodium currents are essential for the maintenance of dopamine-induced oscillations; serotonin-induced oscillations do not depend upon either calcium or sodium alone; octopamine-induced oscillations do not depend upon calcium and show a variable dependence upon sodium. Thus, multiple ionic mechanisms, which vary with both the modulator and the ambient temperature, can be recruited to support rhythmic activity in a conditional burster neuron.

Key words: Conditional burster - Temperature - Stomatogastric ganglion - Lobster - Pyloric motor circuit - Amines

Introduction

The anterior burster (AB) interneuron is a conditional burster neuron in the lobster stomatogastric ganglion (STG) and functions as the primary pacemaker for the pyloric motor rhythm (Miller 1987; Bal et al. 1988). The rhythmic oscillatory activity of this cell is dependent upon modulatory input from higher ganglia. When these inputs are blocked, rhythmic output from the pyloric network stops; the AB cell loses its bursting capability and becomes silent (Robertson and Moulins 1981; Moulins and Cournil 1982; Nagy and Miller 1987). Under such conditions, bath application of modulatory compounds, including dopamine (DA), serotonin (5HT), octopamine (Oct), proctolin, and musecinic agonists, can restore rhythmic pacemaker potentials in the quiescent AB cell (reviews: Harris-Warrick 1988; Marder and Meyrand 1989). When the AB cell is isolated from all known synaptic inputs, DA, 5HT, and Oct (Flamm and Harris-Warrick 1986) and proctolin (Hooper and Marder 1987) can initiate the cell’s rhythmic activity, demonstrating that this activity is a conditional endogenous property of the AB cell.

Harris-Warrick and Flamm (1987) investigated the ionic mechanisms of the bursting activity induced by DA, 5HT and Oct at 15 °C in AB cells from the California spiny lobster, Panulirus interruptus. They showed that bursting induced by DA was critically dependent upon Ca2+ currents. Rhythmic oscillations continued in the presence of tetrodotoxin (TTX) but were blocked by treatments which even moderately reduced calcium currents. In contrast, they found that Oct and 5HT-induced bursting in the AB cell was Na+-dependent. Bursting induced by these two amines was blocked by TTX and by reduced extracellular Na+, but not abolished by modest reductions in extracellular Ca2+. Other differences between the three amines were also seen: thus, DA, 5HT and Oct could induce bursting activity in the AB cell by different ionic mechanisms.

At 21 °C, we have observed that all 3 amines can induce rhythmic activity from the pyloric circuit of spiny

Abbreviations: AB anterior burster; PD pyloric dilator; PY pyloric constrictor; DA dopamine; 5HT serotonin; Oct octopamine; STG stomatogastric ganglion; TTX tetrodotoxin; GSP graded synaptic potential

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lobsters in saline containing TTX. Since 5HT and Oct did not induce rhythmic oscillations in TTX-treated AB cells at 15 °C, we re-investigated the ionic mechanisms underlying the oscillations induced by all 3 amines at the elevated temperature. Our experimental temperature of 21 °C is at the top of the range (12–21 °C) that *Panulirus interruptus* lives in (personal observations). We performed similar experiments to those of Harris-Warrick and Flamm (1987) to allow comparisons of results obtained at the 2 different temperatures. We show that the ionic mechanisms of AB cell oscillatory activity induced by DA, 5HT and Oct at 15 °C differ qualitatively and quantitatively from those at 21 °C. We suggest that not only can different modulators maintain conditional pacemaker activity in a single burster neuron by different mechanisms (Harris-Warrick and Flamm 1987), but that the same modulator can itself use different ionic mechanisms at different temperatures to maintain and shape pacemaker activity.

**Materials and methods**

California spiny lobsters (*Panulirus interruptus*) were purchased from Marins Inc. Long Beach, CA and kept in marine aquaria at 15 °C. The stomatogastric nerve system was dissected from the lobster (as described by Selverstone et al. 1976) and placed in a preparation dish filled with *Panulirus* saline of the following composition (mM): NaCl 479, KCl 12.8, CaCl2 13.7, Na2SO4 3.9, MgSO4·10H2O 10.0, HCl 2.1, KH2PO4 1.5, pH 7.35 (Mallony and Selverston 1974). The STG was desheathed, enclosed in a 1 ml pool of saline walled by vaseline, and constantly perfused at 5 ml/min with oxygenated saline.

Standard intracellular techniques were used for voltage recordings (3 M KCl-filled microelectrodes, 10–20 MΩ). The cell bodies of the pyloric motor neurons were identified during rhythmic pyloric activity by: (1) matching the timing of action potentials recorded intracellularly from the cell body with action potentials recorded extracellularly from an appropriate motor nerve root; (2) the timing of spike activity within the pyloric rhythm; and (3) the synaptic connectivity. The cell body of the AB interneuron was identified by the cell's synchronous firing with the pyloric dilator (PD) motor neurons (which are tightly coupled electrically to the AB cell) and the characteristic shape of the AB membrane potential oscillation and action potentials. After AB identification, detectable sources of synaptic input were eliminated by perfusion of the STG with 10^{-4} M TTX-saline solution, and 6-carboxyfluorescein-esterified photo-inactivation (Miller and Selverston 1979; Flamm and Harris-Warrick 1986) of the 3 cells (2 PD cells and the ventral dilator motor neuron) that exhibit significant electrical coupling to the AB cell (Maloney 1987). All experiments were performed on synthetically isolated AB cells. We maintained the experimental temperature at 21 °C with a pellicon device.

Anions were dissolved, just before their application, in the appropriate solution containing 10^{-7} M TTX for a final concentration of: DA 10^{-4}, 5HT 10^{-4}, and Oct 10^{-4} M. These are the same amine concentrations used by Harris-Warrick and Flamm (1987) and are saturating concentrations for burst induction in the AB cell. DA was initially applied in all experiments to ensure that in the quiescent TTX-treated state, the electrode was positioned in a healthy AB cell that was capable of oscillating. The amplitude of an amine-induced oscillation was measured from the peak depolarization to the trough hyperpolarization; the duration of a single oscillation was measured at half-amplitude; and frequency was determined from the time taken for 10 oscillations to occur. We did not apply all the amines to all the cells, and in some experiments, 5HT and Oct failed to induce AB cell oscillations. When oscillations were not induced by 5HT and Oct, DA was applied to ensure that the AB cell was still capable of producing oscillations. If the cell did not produce oscillations after DA application, the previous data were discarded. A wash period of at least 30 min with TTX-saline separated applications of different amines. An experiment series using any one amine was only run once with each preparation.

We changed the concentration of extracellular Na^+ and Ca^2+ by substituting Tris^- or n-methyl-D-glucamine for Na^+ and Mg^2+ for Ca^2+ in the TTX-saline. Data from the ion substitution experiments were analyzed after 10 min perfusion with the altered saline. This was the time when we considered that a complete exchange of solutions had occurred within the STG neuropil. It was determined from the effects of reduced Ca^2+ saline (25% of normal Ca^2+, high Mg^2+) on the amplitude of the graded synaptic potential (GSP) between pyloric circuit neurons. The time course for the effects of reduced Ca^2+ on GSP amplitude and on AB oscillations should be similar because synaptic contacts between pyloric cells and the currents underlying AB bursting both occur in neuropil processes of King (1976); extracellular Ca^2+ modulation of the time course of reduced transmission in low Ca^2+ saline is shown in Fig. 1. Here, the absolute peak amplitude of a GSP (Johnson and Harris-Warrick 1989) at a PD to pyloric constrictor (PY) cell synapse before perfusion with reduced Ca^2+ saline was 1.8 mV. Immediately following entry of reduced Ca^2+ saline into the small pool containing the STG, the GSP amplitude began to decline until a steady-state value of 0.25 mV was reached in 7.5 min. Maximum recovery of the GSP amplitude occurred within 7 min of re-perfusing the STG with the normal saline. At 3 other PD synapses (2 with different post-synaptic PY cells and 1 with a post-synaptic inferior cardiac cell), GSPs were maximally reduced within 5 to 7 min of reduced Ca^2+ saline perfusion. Recovery of these synapses occurred within 5 to 8 min after return to normal saline. Longer perfusion times than 10 min sometimes caused additional changes in oscillatory properties, but since these changes may arise from secondary responses of the cell to altered Ca^2+ and Na^+ concentrations (modulated, for example, by the membrane Ca^2+ pump; Carafoli 1991, or the Na^-K^- pump: Hirsberger et al. 1991) they were not studied further. Amine-induced AB oscillations stopped after return to the control TTX-saline, and the effects of reduced Na^+ and Ca^2+ treatments were reversible. Statistical comparisons were made with Student’s t-tests and analysis of variance (with subsequent protected
Results

DA-, SHT- and Oct-induced AB oscillations at 21 °C

In many AB cells, DA, SHT and Oct could all induce rhythmic oscillations at 21 °C in saline containing 10⁻⁷ M TTX (Fig. 2A). This is in striking contrast to experiments carried out at 15 °C, where only DA could induce or maintain membrane potential oscillations in the presence of TTX (Harris-Warrick and Flamm 1987). In all of our experiments at 21 °C, DA induced rhythmic oscillations in isolated AB cells; SHT and Oct induced oscillations in approximately half the experiments in which they were applied (SHT, 7 of 13 applications; Oct, 6 of 10 applications). In experiments where SHT and Oct were both applied, all the cells induced to oscillate by SHT also oscillated with Oct, but Oct-induced oscillations were not always accompanied by SHT-induced oscillations (Fig. 2B).

There was a significant effect of temperature on all slow wave oscillation parameters (Table 1). At 21 °C, oscillation frequencies were significantly faster (F₁,₄₄ = 28.2), amplitudes were significantly larger (F₁,₄₄ = 6.8), and trough values were significantly higher (F₁,₄₄ = 5.3) than corresponding values at 15 °C (Harris-Warrick and Flamm 1987). The enhancement of oscillation frequency with elevated temperature is a typical result for endogenous oscillators (Adams and Benson 1985) unless extreme temperatures are approached (Fletcher and Ram 1991).

Since DA was applied to every preparation in our experiments, we statistically compared the characteristics of the oscillations produced by SHT or Oct with those produced by DA at 21 °C (Table 1). DA produced significantly higher frequency oscillations than did SHT, while the Oct-induced cycle frequency was essentially the same as with DA. The amplitudes of the slow wave oscillations were highest with DA, intermediate with SHT, and lowest for Oct; the SHT- and Oct-induced amplitudes were significantly smaller than the DA amplitudes. This rank order of oscillation amplitudes was also seen at 15 °C (Harris-Warrick and Flamm 1987; Table 1).

In addition to the cycle frequency, we measured the duration of the oscillations at half-amplitude. This measurement allowed us to distinguish infrequent and brief oscillations with long hyperpolarized inter-oscillation intervals from more regular oscillations with more even depolarized and hyperpolarized components. Indeed, SHT had the same half-amplitude duration as DA, despite a significantly lower cycle frequency (Table 1), indicating that the cell spent a relatively greater fraction of its cycle at hyperpolarized levels in SHT (Fig. 2A). In contrast, the Oct-induced oscillations had a significantly longer half-amplitude duration than DA, despite the similarity in cycle frequency. This quantifies the differences in shape of the oscillations induced by the two amines (Fig. 2): DA-induced oscillations had a clearly biphasic rising phase, with a narrow, almost spike-like peak, while the Oct-induced oscillations had much less (Fig. 2A) or no (Fig. 2B) second rapid component to the rising phase, causing the cell to have a much broader depolarizing oscillation.

Oscillation characteristics in general were more variable for SHT and Oct than they were for DA. This is indicated by some of the SEM values for the mean os-

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Amine</th>
<th>DA (10⁻⁴M)</th>
<th>SHT (10⁻³M)</th>
<th>Oct (10⁻⁴M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
<td>2.0 ± 0.2³</td>
<td>1.4 ± 0.3²</td>
<td>1.9 ± 0.2⁵</td>
<td>[1.14 ± 0.1]</td>
</tr>
<tr>
<td></td>
<td>[1.14 ± 0.1]</td>
<td>[0.79 ± 0.1]</td>
<td>[1.9 ± 0.2]</td>
<td>[1.14 ± 0.1]</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>17.3 ± 1.3</td>
<td>15.4 ± 2.4</td>
<td>11.0 ± 1.5</td>
<td>[14.2 ± 1.4]</td>
</tr>
<tr>
<td></td>
<td>[14.2 ± 1.4]</td>
<td>[12.2 ± 1.1]</td>
<td>[11.0 ± 1.5]</td>
<td>[14.2 ± 1.4]</td>
</tr>
<tr>
<td>Half-amplitude duration (ms)</td>
<td>210 ± 10.4</td>
<td>220 ± 13.6</td>
<td>273 ± 17.₈</td>
<td>[210 ± 10.4]</td>
</tr>
<tr>
<td></td>
<td>[210 ± 10.4]</td>
<td>[220 ± 13.6]</td>
<td>[273 ± 17.₈]</td>
<td>[210 ± 10.4]</td>
</tr>
<tr>
<td>Trough value (mV)</td>
<td>-0.8 ± 0.8</td>
<td>-0.7 ± 1.5</td>
<td>5.0 ± 1.7</td>
<td>[-1.6 ± 0.7]</td>
</tr>
<tr>
<td></td>
<td>[-1.6 ± 0.7]</td>
<td>[-1.7 ± 0.6]</td>
<td>[5.0 ± 1.7]</td>
<td>[-1.6 ± 0.7]</td>
</tr>
</tbody>
</table>

* Oscillation trough value measured relative to the pre-amine resting potential. Positive values indicate a depolarization from the pre-amine resting potential and negative values indicate a hyperpolarization from the pre-amine resting potential.
* Significantly different from the mean for the same parameter of DA-induced oscillations at 21° C (<0.05, Student’s paired t test).
* Significantly different from the mean for the same parameter measured at 15° C (P <0.05, protected t tests). An analysis of variance demonstrated that temperature had a significant main effect on the frequency, amplitude and trough value of AB oscillations.
cillation characteristics (Table 1), and shown for Oct-induced oscillations in Fig. 2.

In those AB cells where Oct and SHT did not induce rhythmic oscillations, they induced mean AB depolarizations of 3.7 ± 0.9 and 7.3 ± 3.2 mV, respectively, and induced active membrane responses that were not present before these amines were applied. Figure 3A shows the response of an AB cell to a 1 nA step of depolarizing current before and during Oct application. Current injection before Oct application produced little active membrane response. After Oct application, the same amplitude current injection produced an initial, pronounced oscillation followed by a smaller, increasingly damped oscillations. In 2 experiments, SHT-induced oscillations but these were not maintained and disappeared in the continued presence of SHT. However, active oscillatory properties were still present that were not evident before SHT application. Figure 3B shows the response to a 2 nA depolarizing current step before and during SHT application in one of these AB cells. Even though the cell has a stable resting potential, it responds to depolarization with a set of damped oscillations. Thus, even when SHT and Oct did not induce or maintain rhythmic oscillations, they still induced an active state that was different from the control state. There were no significant differences in the pre-amine resting potentials of those AB cells that did or did not oscillate after SHT or Oct application. Mean pre-amine resting potentials of cells induced to oscillate were (mV): DA, 55 ± 1.6; SHT, 57 ± 2.4; Oct, 55 ± 2.3.

**Ionic dependence of amine-induced oscillations at 21°C**

We conducted ion substitution experiments varying extracellular Ca\(^{2+}\) and Na\(^+\). These experiments indicated that the ionic dependence of amine-induced oscillations in AB cells at 21°C differ from those at 15°C (Harris-Warrick and Flamm 1987). At 21°C, DA-induced oscillations were abolished by reduction in either Na\(^+\) or Ca\(^{2+}\). In contrast, most SHT- and some Oct-induced oscillations at 21°C were not abolished by reductions in either Na\(^+\) or Ca\(^{2+}\).

**DA-induced AB oscillations**

In 4 of 5 experiments, isolated AB cells perfused with TTX-saline containing 25% of normal Ca\(^{2+}\) (replaced with elevated Mg\(^{2+}\)) showed a complete block of DA-induced oscillations within the 10 min perfusion period. These oscillations ceased with a final resting potential near the peak value of the oscillation in control TTX-saline (Fig. 4A). The onset of the block was characterized by a progressive depolarization of the interburst trough, with little change in the oscillation peak. The oscillation frequency was constant until immediately before the cessation of oscillation; the half-amplitude duration increased as the oscillations became broader until the oscillations ceased (Figs. 4B and 4A). The final mean resting potential from these 4 experiments thus resulted almost entirely from a depolarization of the oscillation trough that was 91 ± 9% of the distance from the trough to the peak amplitude in normal saline. In the fifth experiment, reduced Ca\(^{2+}\) treatment did not completely block DA-induced oscillations within the 10 min perfusion time but the effects of low Ca\(^{2+}\) followed a similar pattern.

The response of DA-induced oscillations to superfusion with 50% Na\(^+\)-TTX saline was variable. Oscillations were completely blocked in 3 of 5 experiments (Figs. 4B and 5B). This sensitivity of the DA-induced oscillations to low Na\(^+\) saline at 21°C is in marked contrast to the results at 15°C, where the same treatment did not block DA-induced oscillations in any experiments (Harris-Warrick and Flamm 1987). At 21°C, the cell stopped oscillating at a resting potential that was significantly more hyperpolarized than that seen after reduced Ca\(^{2+}\) treatment and in fact, only a few mV depolarized from the pre-amine resting potential (about 15 ± 8.7% of the distance from trough to peak amplitude in normal saline;
Fig. 5. Time course of reduced extracellular Ca\(^{2+}\) (A) and Na\(^{+}\) (B) block of DA-induced rhythmic activity in isolated AB cells from different preparations at 21 °C. In top graphs, open symbols (+) indicate oscillation peak values and closed symbols (*) indicate oscillation trough values. In bottom graphs, open symbols (+) indicate half-amplitude oscillation duration. Pre data points indicate values before amine application, and time data points indicate measurements of DA-induced oscillations after 5 min in control TTX-saline. Reduced ion solutions arrive at the preparation at time 0.

Fig. 4B). As the DA-induced oscillations were progressively blocked by reduced Na\(^{+}\), the oscillation peak value became more and more hyperpolarized, and the oscillations broadened, as measured by an increase in half-width duration. In contrast, there was little change in oscillation frequency until the oscillations stopped (Fig. 5B).

In two other experiments, the reduced Na\(^{+}\) treatment did not block DA-induced oscillations after 10 min perfusion but did dramatically alter the properties of the oscillations. In both cases, the oscillation frequency slowed and the oscillation duration broadened, while the oscillation amplitude either did not change or was reduced towards intermediate values. These results suggest that both Ca\(^{2+}\)- and Na\(^{+}\)-dependent mechanisms can play important roles in the maintenance of DA-induced oscillations at 21 °C.

SHT-induced oscillations

Neither reduced Ca\(^{2+}\) nor Na\(^{+}\) treatments were effective in eliminating SHT-induced oscillations. In 4 of 4 experiments, SHT-induced oscillations continued after 10 min perfusion with 25% Ca\(^{2+}\) (high Mg\(^{2+}\)) saline. This treatment did, however, cause changes in the characteristics of the oscillation. The traces in Fig. 6A show, for example, that after 10 min of reduced Ca\(^{2+}\) treatment, the AB cell had a reduction in oscillation amplitude due to a significant depolarization of the oscillation trough value, a greatly decreased oscillation frequency and increased oscillation duration. However, there was great quantitative variability in the effects of reduced Ca\(^{2+}\) treatment on SHT-induced oscillations, as seen by the relatively large SEMs in Table 2. As a consequence, the only statistically significant changes in SHT-induced oscillations upon changing to low Ca\(^{2+}\) saline were a depolarization of the oscillation trough value and an increased oscillation duration (Table 2). These effects were

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean% Change From Control</th>
<th>Amplitude</th>
<th>Mean% of Control</th>
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<tbody>
<tr>
<td></td>
<td>Peak</td>
<td>Trough</td>
<td>Frequency</td>
</tr>
<tr>
<td>SHT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25% Ca(^{2+})</td>
<td>+13 ± 18.0</td>
<td>+43 ± 7.3*</td>
<td>72 ± 11.8</td>
</tr>
<tr>
<td>50% Na(^{+})</td>
<td>-17 ± 16.8</td>
<td>-24 ± 5.0*</td>
<td>58 ± 11.9*</td>
</tr>
<tr>
<td>Oct</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25% Ca(^{2+})</td>
<td>+41 ± 15.8*</td>
<td>+31 ± 11.4*</td>
<td>112 ± 21.4</td>
</tr>
<tr>
<td>50% Na(^{+})</td>
<td>-38 ± 2.0</td>
<td>-7 ± 0.6</td>
<td>60 ± 7.0</td>
</tr>
<tr>
<td>(^{(*)})</td>
<td>Expressed as the percentage of the control oscillation amplitude. Positive peak or trough values indicate a depolarization from the pre-treatment level and negative values indicate a hyperpolarization from the pre-treatment values.</td>
<td></td>
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<tr>
<td>(^{*})</td>
<td>Control values are significantly different from reduced ion treatment values</td>
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</table>

Table 2. Characteristics of AB cell oscillations induced by SHT and Oct after treatment with reduced calcium or reduced sodium salines. Means (± SEM) are from only those experiments in which the treatment did not block the oscillations within 10 min perfusion time.
at least partially reversible upon return to normal saline (Fig. 6).

SHT-induced oscillations also continued in reduced Na⁺ saline (3/3 experiments). The example in Fig. 6B shows that 50% Na⁺ saline reduced the oscillation amplitude, by a lessening of both peak and trough values, could decrease the oscillation frequency and could increase the oscillation half-amplitude duration. Due to variability in the different experiments (as seen in the large SEM values in Table 2), only the decrease in oscillation amplitude, and depolarization of the oscillation trough were statistically significant.

Oct-induced oscillations

Reduced Ca²⁺ saline significantly altered, but did not eliminate, oscillations induced by Oct. Oscillations were maintained but significantly slowed in reduced Ca²⁺ saline in 4 of 4 experiments. In the example of Fig. 7A, this treatment caused a slight increase in oscillation amplitude, a large decrease in oscillation frequency and an increased in oscillation duration, due primarily to the greatly reduced rate of depolarization after the peak of the oscillation. The whole oscillation was depolarized by 3 mV. Table 2 shows that reduced Ca²⁺ saline caused a statistically significant decrease in mean oscillation frequency and an increase in mean oscillation duration. In addition, there were significant depolarizations of both the peak and trough oscillation values, although the mean oscillation amplitude was only modestly affected, showing that the entire oscillation was depolarized.

Reduced Na⁺ treatment had more variable effects. In 3 of 5 experiments, this treatment decreased Oct-induced oscillations while leaving the final resting potential at varying levels (14 ± 18% of the distance from the trough to peak amplitude in normal saline). The example of Fig. 7B shows a final resting potential (within 5 min after switching to 50% Na⁺) approximately halfway between the original oscillation peak and trough levels. In 2 other experiments, reduced Na⁺ treatment did not block Oct-induced oscillations within 10 min. However, the oscillation duration was prolonged and the peak amplitude reduced somewhat relative to the control values in normal saline (Table 2).

Discussion

Our results indicate that the underlying ionic mechanisms by which amines generate rhythmic activity in the pyloric AB neuron are different at 15°C and 21°C. First, at 15°C, pacemaker potentials induced by SHT and Oct, but not DA, are critically dependent upon voltage-gated Na⁺ channels; bursting induced by SHT and Oct is eliminated by TTX (Harris-Warrick and Flamm 1987). In contrast, at 21°C, all 3 amines are able to induce pacemaker potentials in TTX (Fig. 2). This indicates that at the elevated temperature, rhythmic activity induced by SHT and Oct, as well as DA, does not depend critically on TTX-sensitive Na⁺ channels.

Second, the characteristics of the pacemaker potentials induced by each amine differ at the two temperatures. At 21°C, pacemaker potentials are more frequent, of larger amplitude, and have trough values less hyperpolarized than at 15°C (Table 1). The rank order of oscillation amplitudes remains the same at the two temperatures: DA induces the largest amplitude oscillations, followed by SHT and then Oct. The rank order of oscillation frequency, however, differs between the two temperatures. At 15°C, the oscillation frequency is highest with DA, followed by SHT and then Oct. At 21°C, both DA- and Oct-induced oscillation frequencies are faster than that induced by SHT (Table 1).

The responses of amine-induced oscillations to reduced Ca²⁺ and Na⁺ treatments also differ at the two temperatures. At 15°C, DA-induced bursting is critically dependent upon Ca²⁺ entry; reducing the extracellular Ca²⁺ concentration to 25% consistently eliminates rhythmic activity (Harris-Warrick and Flamm 1987). DA-induced potentials are not blocked by TTX, and a 50% reduction of extracellular Na⁺ only moderately affects burst amplitude with little effect on burst frequency. A further reduction in extracellular Na⁺ concentration to 25% of normal causes a greater reduction in burst amplitude and frequency but still does not block DA-induced bursting. These results led Harris-Warrick and Flamm (1987) to conclude that Na⁺ entry is not essential for DA-induced bursting, but does play a role in shaping the oscillation via TTX-insensitive channels. At 21°C, we found that rhythmic oscillations induced by DA are critically dependent upon Ca²⁺, as seen at 15°C. In addition, a 50% reduction in extracellular Na⁺ had much more marked effects on the oscillation at 21°C, actually blocking rhythmic activity in the majority of preparations (Figs. 4 and 5). Our results suggest that both Ca²⁺ and Na⁺ currents are essential in the maintenance of rhythmic activity induced by DA at the elevated temperature.

At both 15°C and 21°C, reduced extracellular Ca²⁺ has qualitatively similar effects on SHT- and Oct-induced AB bursting. Both amines retain rhythmic oscillations in 25% Ca²⁺ saline (Figs. 6 and 7). At 15°C, low Ca²⁺
reduces the burst amplitude, due to a depolarization of the burst trough, and also reduces the burst frequency and the rate of burst repolarization. Bursting induced by these amines is only eliminated when extracellular Ca
sup+ is reduced to 10% or lower (Harris-Warrick and Flamm 1987). At the higher temperature, significant changes in oscillation characteristics also occur in 25% Ca
sup+ for both amines (Table 2). Thus, at both temperatures, Ca
sup+ plays an important role in shaping the oscillation characteristics of 5HT- and Oct-induced bursting.

Reduced Na
sup+ treatment, however, reveals a striking difference between 5HT- and Oct-induced oscillations at the two temperatures. When extracellular Na
sup+ is reduced to 50% at 15 °C, bursting induced by these two amines is rapidly terminated (Harris-Warrick and Flamm 1987). Burst amplitudes are progressively reduced by hyperpolarization of the burst peak and depolarization of the burst trough, and the burst frequency declines continuously until bursting stops at a membrane potential somewhat depolarized from the burst trough level. Thus, the 5HT- and Oct-induced bursts are critically dependent upon Na
sup+ entry at the lower temperature. At 21 °C, however, the 5HT-induced oscillations were not blocked by reduced Na
sup+ treatment in any of our experiments (Fig. 6, Table 2). Thus at 21 °C, Na
sup+ no longer plays an essential role in maintaining 5HT-induced oscillations that it plays at 15 °C, although it still shapes the oscillation characteristics. Reduced Na
sup+ can, however, alter the characteristics of these oscillations at the higher temperature: it significantly reduces burst amplitude and depolarizes the trough level of 5HT-induced oscillations (Table 2). At 21 °C, Oct-induced oscillations showed a variable response to low Na
sup+ saline, with 3 cells terminating their oscillations and two cells showing marked changes in oscillation characteristics. This variability contrasts with the uniform elimination of bursting with low Na
sup+ at 15 °C, suggesting a subtle change of emphasis in the role of Na
sup+ in bursting. It also demonstrates that Oct- and 5HT-induced oscillations do not use identical ionic mechanisms at the higher temperatures, since 5HT-induced oscillations are not blocked by low Na
sup+ while Oct-induced oscillations can be blocked under the same conditions.

There is variability between AB cells in the ability of the amines to induce TTX-resistant oscillations at the elevated temperature. We do not understand the sources of this variability. It may be due to inherent differences in the intrinsic properties of AB cells from different preparations, or to some unknown experimental variable that changes their neuromodulatory responses. Despite this variability, it is still apparent that each amine uses a different balance of currents to support bursting at 21 °C.

Our comparison of rhythmic activity at 15 and 21 °C suggests that shifts in the balance of ionic currents underlying conditional bursting occur in the AB neuron: all of the amine-induced oscillations have Ca
sup++ and Na
sup+- dependent components whose relative importance varies with temperature. The induction of oscillations by all 3 amines in the presence of TTX, the enhanced amplitudes, frequencies and depolarized trough potentials of oscillations at 21 °C compared to 15 °C, all suggest that a greater depolarizing drive is present to support rhythmic activity induced by the amines at the higher temperature. Our results suggest that a TTX-insensitive, Na
sup+-dependent current supplies this additional depolarizing drive for all 3 amines. With all 3 amines, reduced Na
sup+ treatment causes a hyperpolarization of the oscillation peak level. In DA or Oct, this can sometimes cause rhythmic activity to stop at a resting potential close to the original resting potential.

Na
sup+ currents are thought to be at least partially responsible for the depolarizing phase of pacemaker potentials during normal bursting from the AB/PD pyloric pacemaker group (Herrmann 1979; Gola and Selverston 1981; Harris-Warrick and Flamm 1987). They are also a major component of the depolarizing drive for endogenous bursting in other cells (Smith et al. 1975; Barker and Gainer 1975; Colmers et al. 1982; Benson and Adams 1989), including those showing conditional rhythmic activity in TTX and involved in motor pattern generation (Wallén and Grillner 1987). Important currents for bursting carried completely or partially by Na
sup+ can be temperature sensitive. For example, the slow inward current responsible for the depolarizing drive of bursting Aplysia cells, which is at least partially carried by Na
sup+, is very sensitive to temperature; it is abolished upon cooling from 20 to 10 °C (Wilson and Wachtel 1974). Warming from 22 to 25 °C can induce this current and generate bursting in cells that do not display rhythmic activity at the lower temperature (Wilson and Wachtel 1974). Also, the transient depolarizing current which drives the burst after the first action potential and underlies the depolarizing after-potential in Aplysia bursting cells has a Na
sup+ component and is temperature sensitive (Lewis 1984). Another temperature sensitive current with a Na
sup+ component that contributes to the frequency of rhythmic activity is the hyperpolarization-activated current described in vertebrate heart muscle (I., DiFrancesco and Ojeda 1980) and leech heart interneurons (Angstadt and Calabrese 1989). Finally, a slow Na
sup+ current which is activated by DA and which increases with elevated temperature from 11 to 30 °C is found in Aplysia neurons (Matsumoto et al. 1988a, b).

The ion substitution experiments indicate that Ca
sup++-dependent currents may play a more important role in the repolarization of the conditional AB burst than in sustaining the depolarizing drive. Ca
sup++ currents are thought to be (or to activate) the main depolarizing drives for bursting in some neurons (reviews: Cooke 1988; Partridge and Swandulla 1988; Benson and Adams 1989; Andrew 1989; see also Arbas and Calabrese 1987; Angstadt and Stretton 1989). However, this does not appear to be the case for pyloric pacemaker bursting (Herrmann 1979; Gola and Selverston 1981; Harris-Warrick and Flamm 1987). When reduced Ca
sup++ treatment blocks oscillations, the AB cell remains at a potential near the peak depolarization of the original oscillation (Gola and Selverston 1981; Harris-Warrick and Flamm 1987). This has also been shown for conditional bursting induced by n-methyl-D-aspartate in lympephy motor neurons (Wallén and Grillner 1987). We showed that at 21 °C in TTX, 5HT- and Oct-induced oscillations undergo a depolarization of both oscillation peak and
trough levels in 25% Ca\textsuperscript{2+} saline (Table 2). These data, along with the greatly prolonged duration and apparent reduced slope of repolarization (Figs. 6 and 7) of the pacemaker potentials with reduced Ca\textsuperscript{2+} treatments suggest that Ca\textsuperscript{2+}-dependent currents play an important role in evoking the repolarization phase of the AB oscillation. This phase is probably dominated by Ca\textsuperscript{2+}-activated potassium currents (I_{Kca}) as pacemaker waves suggested for the AB/PD pacemaker group (Hermann 1979; Gola and Silverston 1981; Harris-Warrick and Flamm 1987), and other bursting cells (Gorman et al. 1982; Tazaki and Cooke 1983; Smith and Thompson 1987). Ca\textsuperscript{2+}-activated potassium currents are temperature sensitive in some bursting neurons (Johnston 1980; Thompson et al. 1986).

Conditional burster neurons are thought to be involved in a variety of rhythmic motor outputs, ranging from locomotion to respiration in invertebrates and vertebrates (Harris-Warrick 1988; Jacklet 1989). In pokilotherms, such rhythmic motor outputs are typically very temperature-dependent. For example, the frequencies of heartbeat in leeches (Arbas and Calabrese 1984), wing beat in flying locusts (Foster and Robertson 1990), parapodial swimming in Aplysia (von der Porten et al. 1982) and the pyloric motor rhythm in spiny lobsters (Johnson, Peck and Harris-Warrick, unpublished observations) all increase with elevated temperature. This may reflect increased activity and metabolic processes as these animals adapt their behaviors to the higher temperatures. Since the pyloric motor frequency is set mainly (though not solely) by the burst frequency of the AB neuron (Bus et al. 1988), changes in its oscillatory frequency may help adjust the frequency of the pyloric motor pattern to ambient temperature conditions. In addition, changes in other burst characteristics, such as burst amplitude, can cause changes in the phase relationships for activity of follower cells of the pyloric network (Eisen and Marder 1984), perhaps further adapting the pattern to ambient conditions. These changes in pacemaker frequency and amplitude at elevated temperatures may come about through altered neuromodulatory input to a system. Certainly there are many neuromodulatory substances that could accomplish this in the lobster STG (Harris-Warrick and Marder 1991). Our results suggest that modifications in motor circuit output with elevated temperature can be mediated by a single neuromodulator acting by different ionic mechanisms at different temperatures (see also Parsons and Pinkser 1989). In our experiments, the amines may have recruited additional depolarizing currents to increase oscillation frequency and amplitude at the elevated temperature.

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