Aminergic Modulation in Lobster Stomatogastric Ganglion. II. Target Neurons of Dopamine, Octopamine, and Serotonin Within the Pyloric Circuit

ROBERT E. FLAMM AND RONALD M. HARRIS-WARRICK
Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853

SUMMARY AND CONCLUSIONS

1. In the preceding paper, we describe how dopamine, octopamine, and serotonin modulate the neural circuit generating a well-described motor pattern, the pyloric rhythm of the stomatogastric ganglion in the spiny lobster, Panulirus interruptus (14). In this paper, we identify the neurons within the pyloric circuit that are directly affected by each amine.

2. We accomplished this by isolating each pyloric neuron from all known synaptic input, using a combination of Lucifer yellow photoinactivation of presynaptic neurons and pharmacological blockade by pyloric neurotransmitters. Dopamine, octopamine, and serotonin were bath applied to the preparation, and the responses of synaptically isolated neurons were recorded.

3. Each amine had a unique constellation of effects on the neurons of the pyloric circuit. Almost every neuron in the circuit was directly affected by each amine. Dopamine and octopamine modulated every neuron, whereas serotonin affected four of the six cell types.

4. Each amine had multiple effects among pyloric neurons including the induction of endogenous rhythmic bursting activity, initiation or enhancement of tonic firing activity, and inhibition accompanied by hyperpolarization.

5. All three amines induced rhythmic bursting in one neuron (the AB neuron), but the form of the underlying slow-wave membrane-potential oscillations was different with octopamine than with dopamine and serotonin.

6. Our knowledge of the effects of each amine on each pyloric neuron, combined with the extensive knowledge of the synaptic organization of the pyloric circuit, has allowed us to explain qualitatively the major aspects of the unique variants of the pyloric motor rhythm that each amine produces in the synaptically intact circuit (14).

INTRODUCTION

A number of neuromodulators have now been shown to interact with neuronal circuits generating simple motor patterns. Biogenic amines and peptides can activate these circuits to generate motor patterns in vivo and in vitro (28, 35, 60, 64–66). They can also produce qualitative changes in ongoing motor patterns by enhancing or inhibiting neuronal activity, changing cycle frequency, and inducing phase changes (2, 4, 11, 14, 21, 24, 47). These effects of neuromodulators on central pattern generator (CPG) circuits could underlie the plasticity of these motor patterns in the intact animal, allowing its behavior to adapt to the changing demands of the environment.

Neuromodulators can induce these changes in motor output, at least in part, by influencing one or more of the neurons that comprise the neural circuit generating the motor pattern. Many studies have investigated the mechanisms by which modulators alter the activity of single neurons (for reviews, see Refs. 8, 23, 31, 51). Several different aspects of neuronal function can be influenced by neuromodulators, including changes in the efficacy of syn-
Target Neurons of Amines in Pyloric Circuit

aptic transmission (5, 12, 16, 20, 29, 58, 59), changes in neuronal excitability mediated by long-term conductance changes (23, 26, 27, 43), and enabling or disabling of plateau potential or burst-generating capabilities (7, 9, 25, 33, 39, 52, 61, 67). Although these effects of modulators on single neurons have been thoroughly investigated, little work has been done to place them within the context of an integrated CPG circuit. Thus, the degree of complexity of neuromodulator effects on the different neurons of a functional circuit is largely unknown (39, 50, 61).

We are analyzing the modulation by biogenic amines of the pyloric circuit of the stomatogastric ganglion (STG) from the spiny lobster, Panulirus interruptus. This is probably the best understood CPG circuit at present. It genie amines of the pyloric circuit of the stomata
gastric ganglion (STG) from the spiny lobster, Panulirus interruptus. This is probably the best understood CPG circuit at present, it consists of 14 identifiable neurons grouped into six major classes. The patterns of synaptic connectivity within the circuit have been elucidated (10, 41, 42, 46), and much is known about the membrane properties of the component neurons (17, 45, 54, 55). In addition, only two neurotransmitters (acetylcholine and glutamate) are used by the 14 neurons in the circuit (34, 36-38). However, several compounds have been detected and proposed as modulators of the pyloric circuit, including dopamine (1, 3, 15, 32), octopamine (3), serotonin (4, 62, 63), histamine (6), and the peptides proctolin and FMRFamide (24, 40). In the previous paper (14), we have given a detailed description of the modulation of the pyloric motor pattern by dopamine, octopamine, and serotonin. Each amine produced a unique variant of the pyloric motor pattern, redefining the pyloric CPG by activating or inhibiting pyloric neurons and changing phase relationships between neurons.

The next step in an analysis of the cellular mechanisms of CPG modulation is to identify the neuron(s) that are directly affected by each amine. When a neuromodulator is added to a circuit, a neuron's activity could be affected either directly, as a consequence of the modulator's action on the neuron itself, or indirectly, as a consequence of changes in synaptic input to the neuron from other cells that are directly modulated. To determine the direct target neurons of the amines in the pyloric circuit, we have isolated each pyloric neuron from synaptic input of all the other pyloric neurons. We accomplished this by using a combination of two techniques, pharmacological blockade of the pyloric circuit's neurotransmitters (34, 36-38), and Lucifer yellow photoinactivation of individual pyloric neurons (44, 45). Modulatory input from other ganglia was greatly reduced or eliminated with a sucrose block on the stomatogastric nerve (STN), the only input nerve to the STG (56). This functional isolation procedure retains the neuron in its normal location with intact dendrites and dendritic receptors, but without detectable synaptic input. Dopamine, octopamine, and serotonin were bath applied and the responses of each isolated pyloric neuron cell type monitored. These data were then used to provide a qualitative explanation for the variants of the pyloric rhythm produced by each amine in the synthetically intact pyloric circuit. Our results have been presented in abstract form (13).

Materials and Methods

Pacific spiny lobsters (Panulirus interruptus) were purchased from Pacific Biomarine and kept in marine aquaria (Aquarium Systems) at 15°C until use. The stomatogastric nervous system was removed from the animal, as described by Mulloney and Selverston (48), and perfused at 5 ml/min with cold (14°C) oxygenated saline (saline composition in mM: NaCl 479, KCl 12.8, CaCl2 13.7, Na2SO4 3.9, MgSO4 10.0, glucose 2.0, tris base 11.1, maleic acid 5.1, pH 7.35).

Synaptic isolation procedure

Three steps were used for the synaptic isolation of a pyloric neuron. These are shown schematically for the ventral dilator (VD) neuron in Fig. 1A. For simplicity, this figure shows only the synapses to the VD from other pyloric neurons. First, a long-term sucrose block (14) was applied to the stomatogastric nerve for at least 1 h to eliminate descending modulatory inputs to the STG from other ganglia. Second, Lucifer yellow photoinactivation (44) was used to kill neurons with electrical connections to the VD or to kill presynaptic cholinergic neurons [the 2 pyloric dilators (PDs) and, in other experiments, the VD (36, 37, 38)]. Lucifer yellow CH (3–5% in double-distilled H2O; microelectrodes back-filled with 0.2 M LiCl) was ejected from bevelled microelectrodes (70–100 MΩ) with 5–10 nA hyperpolarizing current pulses of 500–700 ms duration at 1 Hz for 20 to 30 min. Following Lucifer injection, the cells were exposed for 30 min to bright blue light (wavelength = 390–490 nm; 50 W mercury lamp) via an epifluorescence attachment to the dissecting microscope. A cell was judged killed by three criteria (44). 1) Its action potentials in nerve
roots were abolished; 2) its membrane potential was zero; and 3) synaptic potentials in postsynaptic cells were abolished. Although several cells in the pyloric CPG are electrically coupled, they are not dye coupled (45). After photoinactivation of one of the four electrically coupled neurons, the surviving neurons have a transient decrease in input resistance that reverses in ~1 h (45). Photoinactivation has no detectable effect on postsynaptic cells, other than the elimination of IPSPs (10). Third, pharmacological blockade was used to block the remaining pyloric synaptic connections to the neuron under study. The anterior burster (AB), lateral pyloric (LP), eight pyloric (PY), and inferior cardiac (IC) neurons are glutamatergic (34, 38), and their synaptic effects are antagonized by $10^{-6}$ M picrotoxin (10, 38). Picrotoxin was dissolved in saline just before use and bath applied for at least 60 min before amine application and continued throughout the experiment. No observable side effects were observed with picrotoxin. The two PD and the VD neurons are cholinergic (36-38), and in a few experiments, $10^{-3}$ M atropine was used to block cholinergic synapses (10, 38). However, atropine had a number of side effects when bath applied for prolonged periods at this concentration, and its use was abandoned in favor of photoinactivation of cholinergic neurons. The results obtained with atropine were essentially the same as those from experiments using photoinactivation of cholinergic neurons. The time interval between the completion of the isolation procedure and amine application was at least 1 h.

With these procedures, no synaptic input to any isolated pyloric neuron was detectable for the remainder of the experiment. However, three potential sources of synaptic input still remain. First, residual activity in descending axons from other ganglia may remain after sucrose block of the STN; however, addition of $10^{-7}$ M tetrodotoxin to the sucrose pool or to the commissural and esophageal ganglia and stomatogastric nerve produced the same level of activity in pyloric neurons as the sucrose block (discussed in Ref. 14). In addition, although spike activity in the STN is greatly reduced or absent, terminals of these axons could conceivably release transmitter in a tonic, nonspiking fashion. Amines could modulate this hypothetical nonspiking release. Second, the STG contains neurons from another CPG, the gastric circuit. Weak chemical synaptic inhibition from two gastric neurons, the lateral gastric (LG) and medial gastric (MG), onto the PD and LP neurons has been reported (56). The LG and MG neurons use glutamate as a transmitter, and their synaptic effects appear to be blocked by picrotoxin (34). Thus, these synapses are blocked by our standard isolation procedure. In addition, a weak electrotonic connection between the VD and the two lateral posterior gastric neurons (LPGs) has been reported (22), but no evidence of this coupling was seen in our experiments. No other synapses from neurons within the STG onto pyloric neurons have been found. Third, very weak electrotonic coupling between most pyloric neurons has been reported (22). For most of our experiments, there were no potential changes in an isolated neuron that were time locked to spikes or slow-wave potentials in other neurons. In the rare cases in which we did see evidence for this weak coupling, the results were not included in our analysis. Despite these potential problems, we believe that our synaptic isolation procedure is superior to two alternatives, physical isolation and culturing of somata, or synaptic blockade with low-calcium/high-magnesium saline. Our preparation retains the original neuropil with all its receptors and channels, and avoids potential tissue-culture artifacts. In addition, calcium-dependent modulatory mechanisms can be detected and analyzed with our normal saline.

Dopamine HCl (10^-4 M), octopamine HCl (10^-4 M), and serotonin creatinine phosphate (10^-4 M) were dissolved in cold lobster saline containing 10^-6 M picrotoxin just before use and bath applied separately for 8-10 min at a perfusion rate of 5 ml/min. Intracellular and extracellular recording techniques and data collection and analysis were described in Ref. 14. All experiments were repeated at least three times. Lucifer yellow CH was a kind gift of Dr. W. Stewart. All other chemicals were obtained from Sigma Chemical Co.

RESULTS

Activity of synaptically isolated pyloric neurons

An example of synaptic isolation of the VD neuron is shown in Fig. 1B. Figure 1B1 shows the rhythm of synaptic activity of the synaptically intact pyloric circuit. The VD was firing rhythmic bursts of action potentials. It was isolated by 1) a sucrose block on the STN, 2) photoinactivation of the AB and two PDs, and 3) picrotoxin blockade of glutamatergic input from the lateral pyloric (LP) and inferior cardiac (IC) neurons. After isolation, the VD fired tonically at a low frequency, with no detectible synaptic input (Fig. 1B2).

The columns entitled “Control” in Figs. 2, 5, and 7 illustrate the activity of each class of pyloric neuron under conditions of synaptic isolation. All pyloric neurons normally burst vigorously when these synaptic connections were intact (Fig. 1B1). In synaptic isolation (Fig. 2, Control) most neurons were silent or fired tonically at a low rate. The AB neuron displayed variable activity; it was usually in-
FIG. 1. Diagram of the synaptic isolation procedure using the VD neuron as an example. A: 3 methods are used to isolate the VD. 1) sucrose block on the stomatogastric nerve; 2) Lucifer yellow photoinactivation of neurons with electrical connections to the VD; and 3) pharmacological blockade of glutamatergic synapses onto the VD with 10^{-6} M picrotoxin. B: effect on pyloric neuron activity. B1 and B2 are from different preparations. B1: pyloric activity in a synaptically intact preparation. The pattern consists of a repetitive sequence of bursts from all pyloric neurons. The VD (intracellular recording) fires in bursts with 2 phases of inhibition: the VD is inhibited by the LP early in its burst, and a second phase of inhibition from the AB (simultaneous with the PD burst), which terminates the VD burst. B2: when a VD cell is isolated from all synaptic input, only tonic activity of the VD and LP (extracellular recording) remains. Note that no synaptic PSPs are detectable in the intracellular VD recording.

A neuron was judged to be a direct target for a particular amine if its activity in synaptic isolation was significantly altered by that amine. These changes consisted of the initiation, enhancement, or abolition of tonic spike activity and the initiation of endogenous bursting. The effects of dopamine and serotonin on isolated PD and AB neurons have been previously reported (39) and are in agreement with our results.

**Targets of the amines in the pyloric circuit**

A neuron was judged to be a direct target for a particular amine if its activity in synaptic isolation was significantly altered by that amine. These changes consisted of the initiation, enhancement, or abolition of tonic spike activity and the initiation of endogenous bursting. The effects of dopamine and serotonin on isolated PD and AB neurons have been previously reported (39) and are in agreement with our results.

**Neuronal targets of dopamine.** Dopamine (10^{-4} M) directly affected all six classes of pyloric neurons (Figs. 2 and 3). A wide range of effects was seen among pyloric neurons including the initiation of endogenous bursting pacemaker potentials, activation and enhancement of tonic spike activity, and the abolition of spiking. Endogenous bursting was induced in the AB neuron. The bursting pacemaker potentials were characterized by large-amplitude membrane-potential oscillations (22 mV in Fig. 2) and by attenuated (3-mV) action potentials. The peak and trough potentials of the slow-wave membrane-potential oscillations reached levels that were both depolarized and hyperpolarized from the membrane potential seen in the control (−50 mV in Fig. 2). In the one instance where the isolated AB was bursting before dopamine was
FIG. 2. The effect of 10^{-4} M dopamine on synaptically isolated pyloric neurons. Intracellular recordings are taken from different experiments; control and dopamine traces for each cell are from the same experiment. Effect with synapses intact: summary of the effects of 10^{-4} M dopamine of each neuron in the synaptically intact pyloric circuit (data summarized from Ref. 14). Control: activity of synaptically isolated neurons before dopamine addition. 10^{-4} M dopamine: activity 7-10 min after 10^{-4} M dopamine began to superfuse over the isolated neurons. All neurons are directly affected by dopamine. Dopamine induces bursting in the AB, inhibits the PD and VD, and enhances or activates tonic spike activity in the LP, PYs, and IC.

added to the bath, dopamine increased the oscillation amplitude by depolarizing the peak and hyperpolarizing the trough of the slow-wave membrane-potential oscillations (not shown). The PD and VD neurons were inhibited by dopamine. Tonic spike activity was abolished in both cells. The membrane potential of the PD was hyperpolarized ~10 mV (from -62 to -72 mV in Fig. 2), whereas the VD was usually hyperpolarized by no more than 5 mV. Dopamine initiated or enhanced tonic spike activity in the LP, PY, and IC neurons. The LP and PYs were both very strongly activated with increases in spike frequency and depolarizations from 2 to 10 mV. The IC was usually less strongly activated; spike activity was often initiated, and it was depolarized by no more than 5 mV. Thus every neuron in the pyloric circuit is modulated by dopamine.

FIG. 3. Effects of 10^{-4} M dopamine on tonic spike activity of pyloric motor neurons. Numbers in parentheses indicate the sample size. Bars, SEM; -, control; +, bath application of 10^{-4} M dopamine.
Dopamine can elicit two different stable motor patterns at different concentrations (14). In particular, the PD and VD neurons showed enhanced activity (along with the AB, LP, and PY neurons) at $10^{-5}$ M dopamine but were hyperpolarized and usually silent in $10^{-4}$ M dopamine (Fig. 7 in Ref. 14). These dual effects of dopamine on the PD and VD neurons could be caused by two different mechanisms. First, dopamine could exert a concentration-dependent dual action directly on these neurons, with excitation at $10^{-6}$ to $10^{-5}$ M and inhibition at $10^{-4}$ M. Second, dual effects could arise from a combination of a direct amnergic action (for example, inhibition) and indirect synaptic effects (for example, electrical coupling of an unaffected PD to a highly excited AB cell). Bath application of dopamine on synaptically isolated PD and VD neurons was used to distinguish between these mechanisms (Fig. 4A). Dopamine had no effect on the PD neuron at $10^{-6}$ M, caused a small hyperpolarization and decreased the spike frequency at $10^{-5}$ M, and significantly hyperpolarized and abolished spike activity at $10^{-4}$ M. Experiments performed on isolated VDs produced similar results (Fig. 4B). Thus, dopamine had a purely inhibitory effect on the PD and VD neurons. The apparent excitatory effect of $10^{-5}$ M dopamine on the PD and VD neurons in the synthaptically intact pyloric circuit appears to be a consequence of synaptic input from other pyloric neurons (see DISCUSSION).

**Neuronal Targets of Octopamine.** Octopamine ($10^{-4}$ M) also directly affected all six classes of pyloric neurons. In contrast to dopamine, all pyloric neurons were at least weakly excited by octopamine (Fig. 5 and 6). As in dopamine, the AB was the only cell that was induced to fire rhythmic bursts of action potentials. These were characterized by small-amplitude, slow-wave membrane-potential oscillations (~10 mV) and by spikes that were 4 mV in amplitude. The peak-to-trough membrane-potential oscillations induced by octopamine (~40 to ~50 mV in Fig. 5) were both depolarized from the preoctopamine membrane potential. In the one case where the isolated AB was bursting before octopamine was added to the bath, octopamine de-

---

**FIG. 4.** Effects of increasing concentrations of dopamine on synaptically isolated PD and VD neurons. A: for PD, an inhibitory effect is detected in $10^{-5}$ M dopamine; increasing the concentration of dopamine increases its inhibitory effect on the PD. B: for VD, a slight hyperpolarization is produced in $10^{-6}$ M dopamine. Like the PD, the VD is increasingly inhibited by increasing the concentration of dopamine.
Effect with Synapses Intact

**Control**

<table>
<thead>
<tr>
<th>Neuron</th>
<th>Activity</th>
<th><strong>10^{-4} M Octopamine</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>VD</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>PY</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>O</td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 5.** The effect of $10^{-4} M$ octopamine on synaptically isolated pyloric neurons. Intracellular recordings are taken from different experiments; control and octopamine traces for each cell are from the same experiment. Effect with synapses intact: summary of the effects of $10^{-4} M$ octopamine on each neuron in the synaptically intact circuit (data summarized from Ref. 14). Control: activity of synaptically isolated neurons. $10^{-4} M$ octopamine: activity 7–10 min after $10^{-4} M$ octopamine began to be superfused over the isolated cells. All neurons are at least weakly excited in octopamine. Bursting is induced in the AB. All other neurons spike tonically.

Increased the slow-wave amplitude from 10 to 6 mV and depolarized the membrane potential of the trough phase. Both the PDs and VD were weakly excited by octopamine (Fig. 5), each showing slight increases in tonic spike activity (Fig. 6). The PD was depolarized by no more than 2 mV, whereas the VD neuron showed no significant change in membrane potential. LP spike activity was greatly enhanced (Fig. 5); its spike frequency increased from 2.1 to 5.6 spikes/s (Fig. 6). This increase in activity was accompanied by depolarizations of up to 8 mV. The PY neurons displayed mixed responses to octopamine. In 8 of 10 experiments, PY spike activity, recorded ex-

**FIG. 6.** Effects of $10^{-4} M$ octopamine on tonic spike activity of pyloric motor neurons. Numbers in parentheses indicate sample size. Bars, SEM; -, control; +, bath application of $10^{-4} M$ octopamine.
Effect with Synapses Intact | Control | $10^{-5}$M Serotonin

AB + | | 

PD + | |  

VD - | |  

LP - | |  

PY O | |  

IC + | | 

FIG. 7. The effect of $10^{-5}$ M serotonin on synaptically isolated pyloric neurons. Intracellular recordings are from different experiments; control and serotonin data for each cell are from the same experiment. Effect with synapses intact: summary of the effects of $10^{-5}$ M serotonin on each neuron in the synaptically intact circuit (data summarized from Ref. 14). Control: activity of synaptically isolated neurons. $10^{-5}$ serotonin: activity 7–10 min after $10^{-5}$ M serotonin began to superfuse over the isolated neurons. Serotonin excites the AB and IC, inhibits the VD and LP, and has no significant effect on the PDs and PYs.

tracellularly from the PY nerve (PYN), was initiated by octopamine. However, the spike frequency, $6.0 \pm 0.3$ spikes/s (SEM, $n = 10$), was much lower than the activity produced by dopamine, $16.2 \pm 0.4$ spikes/s (SEM, $n = 9$). In two of four intracellular recordings, the PY membrane potential did not change, and no spike activity was observed. In two experiments, the PY neuron depolarized 2–10 mV, and spike activity was initiated or enhanced (Fig. 5). These results suggest that the eight PYs do not respond uniformly to octopamine because some cells are activated, whereas others appear to be unaffected (see DISCUSSION).

FIG. 8. Effects of $10^{-5}$ M serotonin on tonic spike activity of pyloric motor neurons. Numbers in parentheses indicate the sample size. Bars, SEM; -, control; +, bath application of $10^{-7}$ M serotonin.
The IC neuron was weakly activated by octopamine, spike activity being initiated in three of four preparations. The membrane potential of the IC showed no significant change.

**NEURONAL TARGETS OF SEROTONIN.** Serotonin, like dopamine, produced a range of excitatory and inhibitory effects on isolated pyloric neurons. It was the only one of the three amines that did not appear to modulate all of the pyloric neurons. Like dopamine and octopamine, serotonin induced endogenous burst activity in the AB (Fig. 7). Bursting was characterized by large-amplitude membrane-potential oscillations (17 mV in Fig. 7) and by attenuated (3-mV) action potentials. The peak and trough values of the slow-wave oscillations were depolarized and hyperpolarized, respectively, from the preserotonin membrane potential. The only other cell that was excited by serotonin was the IC neuron (Figs. 7 and 8). Spiking was often initiated in this cell, but changes in resting potential were insignificant. The PD neuron appeared to be completely unaffected by serotonin, with no significant change in spike activity or membrane potential (Figs. 7 and 8). The PY neurons also appeared to show no obvious effect. These cells were usually silent both before and during serotonin superfusion (Figs. 7 and 8). A slight hyperpolarization was observed in intracellular recordings from two PYs; however, this was not reproducible. Both the VD and LP were inhibited by serotonin. The VD was hyperpolarized by 8–10 mV, and spike activity was always abolished, whereas the LP was hyperpolarized by 2–4 mV, and spike activity was greatly reduced or abolished.

**DISCUSSION**

We are interested in characterizing the cellular mechanisms for modulation of motor patterns generated in the central nervous system. As the first step towards this goal, we analyzed the changes in pyloric motor pattern induced by dopamine, octopamine, and serotonin (14). In this paper, we have completed the next step in this analysis by identifying the target neurons for these amines in the pyloric circuit. We can now place these direct amine effects on neurons in the context of the modulated pyloric motor pattern and describe some general points concerning neuromodulation of motor circuits.

Figure 9 shows the variants on the pyloric circuit generated by dopamine, octopamine, and serotonin. The anatomically defined circuit and the neurons in this circuit that are active during a long-term sucrose block of the STN are shown in Fig. 9. Control. For each amine (Fig. 9), the target neurons in the circuit are shown on the left; each cell is either stippled for excitation, striped for inhibition, or clear for no observed effect. The column on the right shows the functional circuit for each amine, that is, the subset of pyloric neurons that is active and participating in an amine-induced motor pattern (see discussion in Ref. 14). The synaptic connections between pyloric neurons are included in both columns to aid in the following discussion. We do not know whether they are modulated by amines, but we suspect that they are affected (50).

A number of general conclusions may be drawn at this point: 1) Each amine can generate a unique variant of the pyloric rhythm that is different from those generated by the other amines. Similarly, histamine (6) and the peptides proctolin and FMRFamide (24) can produce variants of the pyloric rhythm. In addition, dopamine and octopamine can each generate at least two different, stable patterns, depending on the concentration of the amine (14). 2) These variants arise from each amine’s unique constellation of effects on the pyloric circuit. In essence, the anatomically defined pyloric circuit is selectively sculpted to produce a different functional CPG circuit by each amine. 3) Almost every neuron in the pyloric circuit is directly affected by each amine. The only apparent exceptions (see below) are that serotonin does not change the activity of isolated PD (39) and PY neurons, and octopamine excites some but not all PY neurons. Thus, there is no single target for an amine in this circuit. 4) Each amine can have multiple physiological effects even within this simple, 14-neuron circuit. For example, dopamine has at least three actions on different neurons. It induces endogenous rhythmic bursting in the AB, initiates or enhances tonic spike activity in the LP, PY, and (to a lesser extent) IC, and hyperpolarizes and inhibits the PD and VD neurons. Serotonin has a similar multiplicity of effects. Octopamine excites all the pyloric neurons, but there are both qualitative and quantitative differences in its effects on different cells. Bursting is initiated in the AB, and
strong tonic firing is seen in the LP. Much weaker excitation of tonic spike activity is seen in the PD, VD, IC, and some PY neurons, with no effect on some PYs. Multiple effects of a neuromodulator also occur in the lobster cardiac ganglion, where the peptide proctolin elicits both short- and long-term excitatory responses in different neurons (61). 5) Although we have no data concerning aminergic modulation of synaptic function in the pyloric circuit, such a role must be considered likely, given the extensive roles of these amines in synaptic modulation in other invertebrate systems (5, 16, 20, 30, 59). The cholinergic...
APM neuron in *Jasus lalandii* modulates synaptic efficacy in the pyloric circuit (7, 50). This could provide further mechanisms for modulation and restructuring of the pyloric CPG. Amine effects on isolated neurons must be interpreted within the context of the anatomically defined CPG circuit in order to understand the functional consequences of an amine on the motor pattern. A detailed knowledge of the synaptic interactions within the circuit (10, 42, 46) as well as of the unique membrane characteristics of pyloric neurons (17, 45, 54, 55) must be integrated with the amine effects on each neuron in order to understand how the functional CPG is generated. This point is discussed in detail in the next section.

Reconstruction of the amine-modulated pyloric circuit

**DOPAMINE.** With 10^{-4} M dopamine, the neurons that are active in the synaptically intact circuit (AB, LP, PYs, and IC) are all directly excited by dopamine. The neurons that are inactive in the synaptically intact circuit are all directly inhibited by dopamine (PDS and VD). Thus, the direct actions of dopamine on the pyloric neurons are sufficient to generate the basic pattern (i.e., which neurons are active or inactive) of the synaptically intact system.

In the synaptically intact pyloric circuit, the motor pattern obtained with 10^{-5} M dopamine differs from that seen with 10^{-4} M dopamine in that activity of the PDs and VD is enhanced rather than inhibited (Fig. 6 in Ref. 14). This excitation does not arise from a dual effect of dopamine directly on the PD and VD neurons. Dopamine has a purely inhibitory effect on these neurons that is maximal at 10^{-4} M (Fig. 4). The increase in PD burst activity in 10^{-5} M dopamine probably results from the strong electrotonic coupling between the PD and AB neurons. AB burst activity is enhanced by 10^{-5} M dopamine in the synaptically intact circuit. Thus, the AB can enhance PD activity due to their electrotonic coupling and outweigh the weak direct inhibition of PD by dopamine at this concentration. Enhanced AB burst activity can also account for the residual PD activity that is sometimes observed in 10^{-4} M dopamine in the synaptically intact system (for example, Fig. 4A in Ref. 14). The weak enhancement of VD activity in 10^{-5} M dopamine is more difficult to explain, but it may result from enhanced postinhibitory rebound. All pyloric neurons show postinhibitory rebound, and the extent of the rebound effect depends on the amplitude of the hyperpolarization (56). The VD receives inhibitory synaptic input from several neurons (primarily AB and LP) that are excited by 10^{-5} M dopamine. This increased inhibitory input may enhance rebound excitation, accounting for increased VD activity in 10^{-5} M dopamine. Both these cases reveal the importance of the interaction of direct amine actions, circuit connectivity, and intrinsic membrane properties in the production of amine-modulated motor patterns.

**OCTOPAMINE.** Both direct excitation of pyloric neurons and indirect inhibition caused by changes in activity of presynaptic neurons are important in the generation of the motor pattern by 10^{-4} M octopamine. Octopamine strongly activates the isolated AB and LP neurons, with weaker excitation of the remaining pyloric neurons (PD, VD, IC, and PY). In the synaptically intact preparation, this quantitative difference in excitation allows AB and LP to dominate the motor pattern (Fig. 7 in Ref. 14). The two PD neurons, although only weakly excited to fire tonically by octopamine, are electrically coupled to the bursting AB cell; as a consequence, they burst actively in the intact circuit. The similar shape of the underlying slow-wave membrane potentials in the AB and PD cells in the synaptically intact circuit (Fig. 7 in Ref. 14) thus arises from the endogenous bursting pacemaker potentials in the activated AB neuron. The isolated LP fires tonically at high frequency with octopamine. This neuron also fires tonically throughout most of the cycle in the synaptically intact circuit, pausing only when inhibited by the bursting AB and PD cells (Fig. 7 in Ref. 14). This octopaminergic activation of the AB (and indirectly the PD) and LP neurons results in a strong synaptic inhibition of all the other pyloric neurons. Thus, although the VD and some of the PY neurons are weakly excited by octopamine, they are inhibited in the intact circuit by a constant barrage of IPSPs from the AB, PD, and LP neurons. The isolated IC neuron is weakly excited by octopamine but is silent in the intact circuit. We cannot fully explain this, as AB/PD inhibition lasts only
about half of the cycle, and the LP does not inhibit the IC. Further work will be needed to explain this discrepancy.

Lower concentrations of octopamine (10^{-6} to 10^{-5} M) elicited a different motor pattern in the intact pyloric circuit, with enhanced activity of PY neurons and bursting of the VD neuron (Fig. 9 in Ref. 14). Only with 10^{-4} M octopamine were these cells inhibited. Octopamine does not have an inhibitory effect on isolated VD and PY neurons. However, the LP neuron, which inhibits these cells, was less strongly activated with 10^{-3} M octopamine than with 10^{-4} M octopamine. Therefore, the enhanced activity of these neurons at lower octopamine concentrations reflects a direct excitatory effect of octopamine, which is masked by synaptic inhibition from the highly activated AB, PD, and LP cells at higher concentrations.

Octopamine's effect on isolated PY neurons was quite variable; spike activity was initiated in some PYs, but no apparent effect was seen in others. This variability may be due to a physiological heterogeneity of PY neurons. Two subclasses of PY neurons have been described, called the early pylorics (PEs) and the late pylorics (PLs) on the basis of their time of firing during the PY burst (22). Although we did not categorize the PY neurons into subclasses, the variable effects of octopamine on the PYs could result from intrinsic differences in the responsiveness to octopamine between these two subclasses.

SEROTONIN. The effects of serotonin on the pyloric rhythm can also be explained by interpreting the direct effects of the amine within the context of the synaptic connectivity of the pyloric circuit. In the synaptically intact circuit, the AB and PD neurons burst strongly, and the IC often fired weakly about half way through the cycle, while the VD, LP, and PY neurons were inhibited or inactive (14). Our results with synaptically isolated neurons showed that serotonin elicits endogenous bursting in the AB and tonic activity in the IC, inhibits both the VD and LP, and has no significant effect on the PYs. These effects are consistent in isolated and synaptically intact conditions. The synaptically isolated PD is unaffected by serotonin (39; Fig. 6), but its activity is enhanced in the synaptically intact circuit. This enhanced activity can be attributed to the strong electrotonic coupling between the PD neurons and the activated AB neuron. It should be stressed that although we did not see any significant direct effect of serotonin on the PD and PY neurons, we cannot rigorously conclude that these neurons are not affected by this amine. For example, voltage-dependent conductances could be modulated by serotonin but are not detectable in the basal state we obtained with our experimental isolation procedure. In addition, because the PYs were usually inactive in control conditions an inhibitory effect of serotonin would be difficult to measure.

Generation of bursting induced by the amines

All three amines induced endogenous rhythmic bursting in the AB neuron. Interestingly, the shape of the underlying bursting pacemaker potential in octopamine was different from those induced by dopamine and serotonin (Fig. 10). The most obvious difference is in the membrane-potential oscillation amplitude in which dopamine and serotonin induced 20-mV oscillations and octopamine induced only 10-mV oscillations. Also, in octopamine both the peak and trough phase of the slow-wave membrane-potential oscillations are depolarized from the preamine membrane.
potential, whereas in dopamine and serotonin the trough phase reaches a level that is hyperpolarized from the preamine membrane potential. Since the AB was synaptically isolated, these differences in bursting must result from different actions of the amines on the AB. Thus, at least partially different ionic conductances appear to underlie the bursting mechanisms induced by different amines in the AB. These results also indicate that a neuron is capable of bursting by more than one ionic mechanism. The generation of different amplitudes of bursting pacemaker potentials may be important in generating plasticity in pyloric motor output, especially in light of the importance of graded nonspiking synaptic transmission in this system. Many pyloric neurons release transmitter at rest and as a continuous function of membrane potential (18, 19). A relatively normal pyloric rhythm can be observed with tetrodotoxin in the bathing medium to abolish action potentials in the ganglion (1, 53). A twofold change in the amplitude of the AB bursting pacemaker potential would thus have a strong effect on nonspiking inhibition of its postsynaptic cells. This in turn could affect the overall motor pattern.

In our experiments with synaptically isolated neurons, only the AB produced bursting pacemaker potentials in response to the amines. The other isolated neurons, when active, spiked tonically. In contrast, in the synaptically intact pyloric circuit (with a long-term sucrose block on the STN), all pyloric neurons displayed burst activity in response to amines (14). These results suggest that, with the exception of the AB cell, synaptic interactions are critical for amine-induced bursting in pyloric neurons in the intact pyloric circuit. This apparent bursting can result from several mechanisms: 1) the PD fires in bursts due to its strong electrotonic coupling with the endogenous burster, AB; 2) other cells are tonically activated by amines but are periodically inhibited by the AB/PD group or other cells; 3) cells can show postinhibitory rebound to initiate apparent bursting (56). The significance of synaptic interactions for the production of oscillatory activity in the pyloric circuit has been previously described (1, 53, 57).

Although each amine alone could not induce endogenous burst activity in synaptically isolated pyloric neurons (except for the AB), it remains possible that they may do so under different conditions. A number of these neurons have been termed "conditional bursters," because they are capable of endogenous bursting when properly stimulated or modulated (45). For example, oscillatory activity was initiated in partially isolated PD, LP, and VD neurons by tonic stomatogastric nerve stimulation (45), and synaptically isolated PDs were induced to burst by bath application of the muscarinic agonist pilocarpine (39). In our experiments, every attempt was made to eliminate other modulatory inputs to the synaptically isolated neurons. This inevitably resulted in the loss of ionic mechanisms that could be involved in production of endogenous bursting. For example, the ability to elicit plateau potentials with short depolarizing current injection is lost after a tonic sucrose block on the STN (54, 55). Whether the amines could activate such conductances was not investigated, although dopamine is thought not to induce plateau potential capabilities in the sucrose-blocked STG (57). Thus, the effects of amines could vary with the state of the neuron (i.e., which ionic conductances are expressed and can thus be modulated). In addition, combinations of modulators, including amines (1, 3, 6, 15, 32) and peptides (24, 40), may have effects that cannot be predicted from the sum of effects of the modulators in isolation (14).

Cellular mechanisms for aminergic action

A major result of this work is that a single amine can produce a number of different responses in neurons within a single 14-neuron circuit. We have not yet characterized the detailed actions of these amines beyond making the distinction between excitatory and inhibitory responses. However, it might be useful to speculate on how the amines might induce their diverse effects on different cells.

The most obvious hypothesis for this diversity is that each amine influences different ionic conductances in each target neuron by interacting with different receptors and/or biochemical mechanisms. An alternative mechanism would involve identical receptors and biochemical mechanisms in all cells but different participants in the reaction in different cells. For example, excitation and inhibition could both be mediated by cAMP-dependent phosphorylation, but different proteins could be phosphorylated. A third hypothesis
is that an amine would generate different cellular responses due to heterogeneity in non-target ionic conductances and biochemical mechanisms. For example, different pyloric neurons have different abilities to support endogenous bursting pacemaker potentials (45). In this paper, we show that the pyloric neurons responded differently to synaptic isolation, with some cells inactive and others spiking tonically or bursting weakly before the amines were applied. Thus, certain pyloric neurons have passive or voltage-dependent conductances that are not found in other pyloric neurons. Because of these intrinsic differences, an amine might induce the same ionic or biochemical effect in two neurons, but the resulting activity of the neurons might be different. In addition, a second neuromodulator could induce a unique activity state in a neuron, such that subsequent application of an amine would produce a physiological response that is different than when the amine is acting alone. Our reported effects of amines on identified neurons must thus be considered a minimal set; other effects may only be detectable under different experimental conditions. Experiments on the ionic and biochemical mechanisms of amine modulation of these cells should further our understanding of the mechanisms by which a modulator can induce such diverse effects among interacting neurons.

Conclusion

In conclusion, we have analyzed the effects of dopamine, octopamine, and serotonin on synaptically isolated neurons from the pyloric circuit in order to understand how these amines produce unique variants of the pyloric motor pattern. Our results have demonstrated the extraordinary degree of complexity involved in neuromodulation of a simple circuit. Nearly every neuron in the circuit is affected by each amine, and each amine can have different physiological effects among different neurons. It is clear that the behavioral consequences of amine modulation require that the amine effects be interpreted within the context of the circuit and the state of the system. These conclusions may be applicable to more complex motor systems, where complete identification of the neuronal components of the motor circuit is not yet possible. If so, we predict that there will be neither a single target nor a single mechanism of action of a neuromodulator in a motor circuit. As more components of a complex circuit are identified, increasing complexity of neuromodulation of the circuit may be expected.

ACKNOWLEDGMENTS

We thank E. Marder and J. Eisen for invaluable advice, E. Marder for suggestions on the manuscript, M. Nelson for assistance in figure preparation, and B. Seeley for preparation of the manuscript.

This work was supported by National Institute of Health Grant NS-17323 and Hatch Grant NYC-191410 to R.M. Harris-Warwick.

Received 13 June 1985; accepted in final form 3 December 1985.

REFERENCES

10. EISEN, J. S. AND MARDER, E. Mechanisms underlying


44. Miller, J. P. and Selverston, A. I. Mechanisms underlying pattern generation in lobster stomatogastric


