Amine Modulation of Glutamate Responses From Pyloric Motor Neurons in Lobster Stomatogastric Ganglion

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Johnson, Bruce R. and Ronald M. Harris-Warrick. Amine modulation of glutamate responses from pyloric motor neurons in lobster stomatogastric ganglion. J. Neurophysiol. 78: 3210–3221, 1997. The amines dopamine (DA), serotonin (5-HT), and octopamine (Oct) each elicit a distinctive motor pattern from a quiescent pyloric network in the lobster stomatogastric ganglion (STG). We previously have demonstrated that these amines alter the synaptic strength at multiple, distributed sites within the pyloric network that could contribute to the amine-induced motor patterns. Here, we examined the postsynaptic contribution to these changes in synaptic strength by determining how the amines modify responses of pyloric motor neurons to glutamate (Glu), one of the network transmitters, applied iontophoretically into the STG neuropil. Dopamine reduced the Glu responses of the pyloric dilator (PD), ventricular dilator (VD), and inferior cardiac (IC) neurons and enhanced the Glu responses of the lateral pyloric (LP) and pyloric constrictor (PY) neurons. The only effect of 5-HT was to reduce the Glu response of the PD neuron. Oct enhanced the Glu responses of the LP and PY neurons but did not affect the PD, VD, and IC responses. We also examined amine effects on the depolarizing responses to iontophoresed acetylcholine (ACh) in the PD and VD and found that they paralleled the amine effects on Glu responses in these neurons. This suggests that amine modulation of PD and VD responses to Glu and ACh may be explained by general changes in the ionic conductance of these neurons. We compare our results with our earlier work describing amine effects on synaptic strength and input resistance to show that amines act at both pre- and postsynaptic sites to modify graded synaptic transmission in the pyloric network.

INTRODUCTION

It is now well known that anatomically defined neural networks display great functional flexibility to alter their outputs. A single motor network can produce a variety of motor patterns; neurons from one motor network can be recruited to participate in another motor network, and neurons from several different networks can combine to produce novel conjoint networks and motor patterns (Dickinson 1995; Harris-Warrick 1994; Harris-Warrick et al. 1997; Meyrand et al. 1994; Weimann et al. 1991). These dynamic reconfigurations may arise from neuromodulator-induced changes in network building blocks (Getting 1989), which include modulation of ionic currents, alteration of the synaptic strength, and even whether or not anatomic synapses are functional within a network (Grillner et al. 1994; Harris-Warrick and Marder 1991; Marder and Calabrese 1996).

The synaptic connections within a network present multiple sites where a neuromodulator could change the network’s output (Dickinson et al. 1990; Grillner et al. 1994). We are examining neuromodulator-induced synaptic plasticity within a small, anatomically defined network, the pyloric central pattern generator (CPG) of the lobster stomatogastric ganglion (STG), to better understand how synaptic rewiring contributes to network flexibility. The pyloric CPG generates rhythmic foregut movements responsible for filtering food particles and for gastric fluid circulation (Johnson and Hooper 1992). In the spiny lobster, Panulirus interruptus, the synaptic connectivity between the six major cell types of this 14-neuron network is known completely (Miller 1987; Mulloney 1987) and includes graded and spike-evoked inhibitory chemical synapses as well as both rectifying and nonrectifying electrical synapses (Hartline and Graubard 1992; Hartline et al. 1988; Johnson et al. 1993a).

We have examined the ability of the amines dopamine (DA), serotonin (5-HT), and octopamine (Oct) to induce plasticity at synapses within the pyloric network. These amines are endogenous modulators in crustacea (Fingerman and Nagabhushanam 1992; Harris-Warrick et al. 1992; Kravitz 1988) and other animals. They initiate motor patterns from a quiescent pyloric network and modify ongoing pyloric motor patterns in a manner distinctive for each amine (Flamm and Harris-Warrick 1986a; Harris-Warrick et al. 1995a,b). This is accomplished partially by the unique constellation of effects each amine has on the intrinsic firing properties of the pyloric neurons (Anderson and Barker 1981; Flamm and Harris-Warrick 1986b; Harris-Warrick and Flamm 1987; Harris-Warrick et al. 1995a,b, 1997; Marder and Eisen 1984b). In addition, the synaptic interactions between the pyloric neurons are targets of amine modulation (Eisen and Marder 1984; Johnson et al. 1995). We previously demonstrated that DA, 5-HT, and Oct can each change graded synaptic strength at multiple, distributed synaptic sites within the pyloric network (Johnson et al. 1995). The amines fine-tune the gain of different chemical and electrical synapses, and they also abolish, functionally create, and even reverse the functional sign of selected pyloric graded synapses (Johnson and Harris-Warrick 1990; Johnson et al. 1993–1995). This synaptic rewiring acts in concert with amine actions on cellular membrane properties to shape the pyloric motor output (Johnson et al. 1995).

Our previous work did not directly address the cellular sites of amine action on the pyloric network synapses. Our current objective is to better define the pre- and postsynaptic contributions to amine-induced changes in graded synaptic strength. Graded synaptic transmission depends on the passive flow of current between input and output sites within a cell. Thus an amine could act presynaptically to modify transmission by two major mechanisms. First, it could act at the synapse itself to alter release by modifying terminal ionic conductances or the transmitter release machinery (Delaney et al. 1991). Second, it could act nonspecifically on
iontophoretically applied glutamate (Glu). Glu is the inhibitory transmitter of four of the six cell types [anterior burster (AB), lateral pyloric (LP), pyloric constrictor (PY), and inferior cardiac (IC)] in the pyloric network (Marder 1987). In addition, we examined amine effects on the pyloric dilator (PD) and ventricular dilator (VD) neurons’ depolarizing responses to acetylcholine (ACH) (Marder and Eisen 1984a; Marder and Paupardin-Tritsch 1978) to see whether they paralleled amine effects on other regions of the cell, including the recording site in the soma (Johnson and Harris-Warrick 1990).

To help distinguish between these possible sites of amine action, we examined the postsynaptic effects of amines on pyloric motor neuron responses to iontophoretically applied glutamate (Glu). Glu is the inhibitory transmitter of four of the six cell types [anterior burster (AB), lateral pyloric (LP), pyloric constrictor (PY), and inferior cardiac (IC)] in the pyloric network (Marder 1987). In addition, we examined amine effects on the pyloric dilator (PD) and ventricular dilator (VD) neurons’ depolarizing responses to acetylcholine (ACH) (Marder and Eisen 1984a; Marder and Paupardin-Tritsch 1978) to see whether they paralleled amine effects on Glu responses. We discuss these results along with our earlier work on amine modulation of input resistance (Harris-Warrick et al. 1995a,b; Johnson et al. 1993a, 1995) and electrical coupling (Johnson et al. 1993a) to define the possible sites of action and mechanisms underlying the distributed amine effects on the glutamatergic synapses within the pyloric network.

**METHODS**

Spiny lobsters (*P. interruptus*) were purchased from Don Tomlinson (San Diego, CA) and maintained in marine aquaria at 15°C. The stomatogastric nervous system was dissected as previously described by Selverston et al. (1976). The STG was desheathed, enclosed in a 1-ml petroleum jelly (Vaseline)-enclosed pool, and constantly perfused with oxygenated saline [which contained (in mM) 479 NaCl, 12.8 KCl, 13.7 CaCl₂, 3.9 NaSO₄, 10.0 MgSO₄, 2.0 glucose, and 11.2 tris(hydroxymethyl)aminomethane (Tris) base, pH 7.35 (Mulloney and Selverston 1974)] at 5 ml/min. The experimental temperature (19–20°C) was the same as in our earlier studies (Johnson and Harris-Warrick 1990; Johnson et al. 1994, 1995).

Standard intracellular techniques were used for cell identification, cell kills, and recording voltage responses (3 M KCl-filled microelectrodes, 10–20 MΩ) to iontophoretically applied transmitters. We identified the six major classes of pyloric neurons during rhythmic pyloric activity in normal *Panulirus* saline by the coincidence of extracellularly recorded action potentials from an appropriate motor root with intracellularly recorded action potentials in the soma, the timing of spiking activity during the pyloric rhythm, the characteristic shape and amplitude of membrane potential oscillations and action potentials, and the pattern of synaptic connectivity. The PY neurons in this study belonged to a subclass defined by their electrical coupling with the LP neuron and their depolarizing response to DA (late pyloric, PL; Hartline et al. 1987, or PY1 neurons, Levini et al. 1994).

After cell identification, we synthetically isolated the cell under study from the rest of the pyloric network (Johnson et al. 1995) by photoactivating neurons (Miller and Selverston 1979) that synapse, either chemically or electrically, onto that cell. We then superfused the preparation with 10⁻⁷ M tetrodotoxin (TTX)-saline to block spiking synaptic transmission and to abolish rhythmic pyloric activity by eliminating all descending (Nagy and Miller 1987; Russell 1979) and ascending inputs to the STG (Katz et al. 1989). However, not all neurons could be completely isolated in this manner. For example, it was not possible to identify and photoactivate all eight PY neurons in a single preparation. Thus we could not always eliminate possible contributions of PY activity to amine effects (Johnson et al. 1995) (see RESULTS). In addition, we could not eliminate possible local circuit interactions between pyloric neurons and terminals of descending inputs (Coleman et al. 1995; Nusbaum et al. 1992). However, we saw no evidence of descending inputs affecting our results. We used photoactivation to isolate cells from synaptic input instead of altering the Ca²⁺/Mg²⁺ content of the saline to block synaptic transmission because Ca²⁺ might be involved in the mechanism of modulator action (Zhang and Harris-Warrick 1995).

We used standard iontophoretic procedures (Curtis 1964; Marder and Eisen 1984a) to apply Glu or ACh in the STG neuropil. We examined hyperpolarizing voltage responses to iontophoretic application of 0.5 M Glu (pH 8), using 500- to 1,000-nA pulses of 300- to 700-nA positive current. In addition, we examined depolarizing responses to 0.1 M ACh (pH 4.5; 500- to 1,000-nA pulses of 300- to 700-nA negative current) in the PD and VD neurons; for these experiments, we added 10⁻⁷ M picrotoxin to the TTX saline to block Glu transmission (Bidaut 1980). Iontophoretic current injection levels and durations were adjusted to achieve the largest and fastest voltage response from a neuron. The amplitudes of our iontophoretic potentials reflect, in part, the distance of the iontophoretic electrode from the postsynaptic receptors. However, the iontophoretic amplitudes we obtained are close to those found for graded synaptic potentials from isolated pairs of pyloric neurons (Johnson et al. 1995). Transmitter leakage from the iontophoretic pipette was offset with braking currents of 5–10 nA. Amine solutions were prepared in TTX saline just before application to a final concentration of 10⁻⁴ M DA (DA-HCl), 10⁻⁵ M 5-HT (creatinine sulfate complex), and 10⁻⁵ M Oct (d,L-Oct), as in our earlier studies.

The voltage response to iontophoretically applied transmitter was recorded with two electrodes in a cell. One electrode monitored the cell’s voltage while the other injected tonic current to maintain the resting potential at –50 mV throughout the experiment. This was necessary to maintain a constant electrochemical driving force for the iontophoretic responses because amines change the resting potential of pyloric neurons (Flamm and Harris-Warrick 1986b; Johnson and Harris-Warrick 1990). In a few DA experiments on the PD and IC neurons, we used intracellular electrodes filled with 0.6 M K₂SO₄ and 0.02 M KCl. There was no difference between the results of these and the other experiments using 3 M KCl filled electrodes to control membrane potential. We assumed that the amines had no effect on the reversal potentials of the Glu and ACh responses because Oct has no effect on the reversal potential of ACh-mediated inhibition at the PD → LP synapse (Johnson and Harris-Warrick 1990) and DA and 5-HT have no effect on the reversal potential of the outward current induced by Glu in cultured STG neurons (Cleland and Selverston 1997).

Glutamate or ACh was iontophoresed every 60 s, and the cell’s response was recorded for a continuous data series that included measurements for a period in normal TTX saline before amine application (control), a 6-min period of amine bath application (treatment), and an extended wash period of ≈30 min with TTX saline (wash; see Fig. 2A for the 1st example). We averaged the six peak amplitude values of the iontophoretic responses for each 6-min period of a data series (see Fig. 2 for examples). Iontophoretic responses were filtered at 10 Hz, digitized, and analyzed with pClamp software (Axon Instruments). We compared the mean peak values from the treatment and the control periods using the analysis of variance (ANOVA) to examine the overall statistical significance of amine effects within our study. This was followed by "protected" t-tests to determine specific statistical differences between individual data groups. We used P < 0.05 (2-tailed probability) to accept means as statistically different; mean data are given as ±SE.
FIG. 1. Summary of amine effects on the responses of the pyloric motor neurons to iontophoretically applied glutamate (Glu). Mean percent change in the control peak response to Glu is shown for each neuron after treatment with 10⁻⁴ M dopamine (DA, A), 10⁻⁵ M serotonin (5-HT, B), and 10⁻⁴ M octopamine (Oct, C). Mean percent change of the lateral pyloric (LP) Glu response in A is shown for the 1st wash period instead of the treatment period (see text). * Statistically significant change from the mean control value; n, number of independent measurements (preparations).

RESULTS

General amine effects on iontophoretic responses to glutamate

DA, 5-HT, and Oct each had significant effects on the responses of the pyloric motor neurons to iontophoretically applied Glu (Fig. 1). DA altered the Glu responses of all pyloric motor neuron types: it decreased the Glu response in some neurons while increasing it in others (Fig. 1A). 5-HT weakened the Glu response in the VD neuron (Fig. 1B), whereas Oct enhanced the Glu responses of the LP and PY neurons (Fig. 1C). An overall ANOVA showed a statistically significant amine by neuron interaction for the change in Glu responses \( F(18,72) = 2.68, P < 0.01 \). In addition, each amine had statistically different effects from the other amines on the Glu responses of the different motor neurons. The detailed effects of each amine on the Glu response of each pyloric motor neuron are described below.

Dopamine

DA altered the Glu responses of all pyloric motor neuron types (Fig. 1A). The Glu responses of the PD, VD, and IC neurons were all statistically reduced by DA. The mean Glu response of the PD neuron was reduced to 55 ± 7% (range: 36–58%; \( n = 3 \)) of the control mean. Figure 2A shows an example of DA’s effect on a continuous data series from a PD experiment. Figure 2, insets (and of subsequent figures) shows examples of a neuron’s responses to Glu during the control \((a)\), treatment \((b)\), and wash \((c)\) conditions at the times indicated. Figure 2, A and B, shows that the greatest effect of DA on the Glu response occurs during the treatment period and that this effect gradually reverses during the wash.

The Glu responses of the PD neuron during DA treatment were significantly reduced to 57 ± 6% (range: 38–72%; \( n = 5 \)) of control values and did not appear to completely reverse until ~12 min into the wash (W2 period; Fig. 2C). The IC neuron Glu responses were reduced during DA application to 61 ± 6% (range: 47–75%; \( n = 4 \)) of control values. This effect appeared to reverse more quickly than the DA reduction of the PD and VD Glu responses (Fig. 2D).

In contrast, DA significantly increased the Glu responses of the PY neurons (Fig. 1A). This was the most dramatic effect of DA on any pyloric neuron type, with a mean enhancement to 236 ± 31% (range: 163–300%; \( n = 5 \)) of control values. Figure 3A shows an example of this enhancement for a PY cell with the sample traces (inset). The Glu response returned to control levels during the next 12 min of washout (Fig. 3B).

We concluded that DA also increased the mean Glu response of the LP neuron (Fig. 1A), despite a possible masking of this effect during the treatment period by PY neurons that inhibit the LP neuron. We examined the effect of DA on the LP in five preparations, three of which had all PY neurons intact. In all of these preparations, the control LP response to Glu was the typical smooth, transient hyperpolarization (Fig. 4, A and B, control). In two of the three preparations with intact PY neurons, the Glu response became biphasic during DA treatment, with an early depolarizing phase followed by a delayed hyperpolarizing phase (Fig. 4A, 6' DA). After only a brief wash period (1’), the depolarizing phase disappeared, leaving an enhanced hyperpolarizing response to Glu (Fig. 4A, 1’ wash). We have shown previously that DA causes some PY neurons to depolarize enough to evoke tonic transmitter release and tonically inhibit the LP neuron (Johnson et al. 1995). These tonically depolarized PY cells can be inhibited by Glu ionotophoresis. Thus we suggest that during DA perfusion, the initial response of the LP to Glu application is a combination of Glu-evoked disinhibition from tonic PY transmitter release and a direct Glu inhibition of LP, leading to the biphasic response shown in Fig. 4A (6' DA). We tested this idea in two preparations where we identified and killed four PY neurons in addition to the pacemaker group and the PD neuron. In these experiments, we saw no sign of a biphasic LP Glu response during DA perfusion (Fig. 4B). To avoid this PY cell contamination, we compared the control LP Glu response to that seen during the first DA wash period (W1) in all five preparations when the biphasic response was gone. The W1 period response was 149 ± 13% of the control...
response (range: 117–189%; n = 5; P < 0.05; Figs. 1A and 4C). Because of the way we made these measurements, we certainly underestimated DA’s enhancement of the LP Glu response during the treatment period.

**Serotonin**

5-HT significantly decreased the mean VD Glu response to 39 ± 3% of control values (range: 31–47%; n = 4) but had little or no effect on the Glu responses of the other pyloric neurons (Fig. 1B). An example of this 5-HT effect on the VD’s Glu response is shown in Fig. 5A. Here, a 60% reduction in VD responses to Glu is seen during 5-HT perfusion. This 5-HT effect was reversible but the mean Glu response only slowly returned to control levels during several 6-min wash periods (Fig. 5B), suggesting that the effects of 5-HT on the VD neuron are longer lasting than those of DA.

The mean Glu response of the other pyloric motor neurons was not significantly changed by 5-HT (Fig. 6). PD Glu responses during the treatment period ranged from 95 to 119% of control values (n = 4; Fig. 6A). IC Glu responses during the treatment period ranged from 81 to 219% of control values (n = 3). A single experiment showed a large and reversible enhancement of the IC Glu response, and this alone appeared to raise the mean Glu response during the treatment and first two wash periods (Fig. 6B). The Glu responses of PY (range: 74–96% of control values; n = 3) and LP (range: 74–107% of control values; n = 3) neurons also were not statistically changed by 5-HT (Fig. 6, C and D).

**Octopamine**

Oct significantly enhanced the Glu responses of the LP and PY neurons and had no effect on the other pyloric motor neurons (Fig. 1C). Figure 7A shows an example of Oct enhancement of the Glu response for an LP neuron. As with the other amines, Oct rapidly began to change the Glu response early in the treatment period and the response began to recover shortly after Oct perfusion ended. LP Glu responses during Oct application were increased to 139 ± 3% of control values (range: 133–149%; n = 3; Fig. 7B). The PY neuron Glu responses were less strongly enhanced by Oct (mean enhancement to 123 ± 5% of control values; range: 113–131%; n = 3; Fig. 7C).
Oct had no significant effect on any of the PD (range: 95–127% of control values; \( n = 4 \)) or VD (range: 94–111% of control values; \( n = 4 \)) Glu responses (Fig. 8, A and B). There was a gradual reduction in the mean Glu response of the IC neurons after Oct application (Fig. 8C) but because this did not affect IC responses during the treatment period (range: 92–109% of control values; \( n = 3 \)), we did not examine it further.

**Amine effects on the iontophoretic responses of the PD and VD neurons to ACh**

In different crustacean species, pyloric neurons receive at least three cholinergic inputs. The PD and VD neurons use ACh to inhibit other pyloric neurons (Marder 1987). Descending modulatory inputs can use ACh as an excitatory neuromodulator (Nagy and Dickinson 1983), and ascending sensory/modulatory neurons can use ACh as a fast nicotinic transmitter (Katz and Harris-Warrick 1989). We have been unable to obtain reliably pure inhibitory responses to iontophoresed ACh. However, we were able to obtain clear depolarizing responses to ACh in the PD and VD neurons. We examined amine effects on these depolarizing responses to see if the amines affected ACh and Glu responses of these neurons in the same way, as would be predicted if amines were acting indirectly by altering the general cellular conductance (Johnson et al. 1993a, 1995). Just as it did with the Glu responses, DA significantly reduced the ACh responses of both the PD and VD neurons (Fig. 9A) with an example in Fig. 9B. DA reduced the mean VD ACh response to \( 62 \pm 6\% \) of control values (range: 50 ± 72%; \( n = 4 \); Fig. 10A). Similarly, the PD mean ACh response was reduced to \( 61 \pm 6\% \) of control values (range: 50–69%; \( n = 3 \); Fig. 10D).

Serotonin significantly reduced the ACh response of the VD neuron to \( 66 \pm 10\% \) of control values (Fig. 9A, 5-HT; range: 47–85%; \( n = 4 \); Fig. 10B). There was no effect of 5-HT on the PD responses to ACh (Fig. 10E; 81–125% of control; \( n = 4 \)). Oct (Fig. 9A, Oct) had no significant effect on the ACh responses of either the PD (101–215% of control; \( n = 4 \)) or VD (Fig. 10C; range 77–95% of control values; \( n = 3 \)) neurons. A single experiment with a large and reversible increase in the PD ACh response dominated the mean values during the treatment and W1 periods (Fig. 10F). In summary, the qualitative effects of the amines on the PD and VD responses to iontophoresed ACh were similar.

**FIG. 4.** Dopamine effects on Glu responses from LP neurons. A: possible PY contamination of the LP response to Glu during DA application. Example LP traces are shown during control, DA, and early wash period when the LP was not synaptically isolated from any PY neurons. B: DA effects on LP responses to Glu when 4 PY neurons were photoinactivated. C: normalized mean Glu responses averaged over the different time periods for all DA experiments on LP neurons (\( n = 5 \)). * Statistically significant change from the mean control value. See Fig. 2 legend for time period definitions in C.

**FIG. 5.** 5-HT reduction of Glu responses from the VD neuron. A: 5-HT reduction of the Glu response from a single VD neuron. B: normalized mean Glu responses for 4 5-HT experiments on the VD neurons. * Statistically significant change from the mean control value. See Fig. 2 legend for time period definitions.
DISCUSSION

Modulation of graded transmission within the pyloric network

Graded chemical transmission is an important mechanism for synaptic information transfer in many neural networks (see references in Johnson et al. 1995; Juusola et al. 1996; Marder and Calabrese 1996; Takahata and Hisada 1991). Little is known, however, about how modulatory inputs influence graded synaptic strength, let alone the sites and mechanisms of graded synaptic modulation. The lobster STG motor networks provide model systems to examine the importance of modulatory shaping of graded chemical synapses for network output (Elson and Selverston 1992; Johnson et al. 1995).

We have described previously the effects of DA, 5-HT, and Oct on all the graded synaptic interactions within the pyloric network (Johnson and Harris-Warrick 1990; Johnson et al. 1993–1995). We showed that 1) each amine acts at multiple synapses to create a unique pattern of distributed effects across the network; 2) amines have complex actions to strengthen, weaken, or have no affect on different synapses; and 3) in addition to adjusting the gain of an operating synapse, DA and 5-HT can rewire the pyloric network by activating silent synapses or by completely abolishing synapses seen under our control conditions. However, our earlier work did not address the cellular targets of amine action to modify the pyloric synapses.

Sites and mechanisms of amine action at glutamatergic pyloric synapses

Our results help us begin to identify the targets of amine modulation of graded synaptic transmission in the pyloric network. Table 1 summarizes data that we can use to narrow the possible mechanisms and sites of amine action on the graded glutamatergic synapses from the AB, LP, PY, and IC neurons (Fig. 11). These include amine affects on cell input resistance and responses to iontophoretically applied Glu and ACh. In addition, we will mention results from our studies of amine modulation of electrical coupling between pyloric neurons (Johnson et al. 1993a) because general input resistance changes in a postsynaptic neuron that affect Glu responses also would be expected to affect electrical coupling of that cell to other pyloric cells (Johnson et al. 1993a).

Figure 11 summarizes our previous results on amine effects on the graded glutamatergic synapses within the pyloric network and incorporates the data on cellular effects of the amines to generate suggestions for synaptic sites of amine action. We do not indicate the cholinergic synapses from PD and VD because we could not reliably obtain inhibitory iontophoretic responses to ACh.

Dopamine

The effects of DA on cellular input resistance, excitability, and transmitter responses suggest that this amine uses both pre- and postsynaptic mechanisms to change the synaptic strength of the glutamatergic synapses (Table 1; Fig. 11A).

![Fig. 6.](image-url)
increase transmitter release from the AB’s synaptic terminals also could contribute to strengthen these synapses.

DA also enhances AB’s other two chemical synapses, AB → LP and AB → PY (Johnson et al. 1995). A parsimonious hypothesis is that DA enhances presynaptic release from all AB terminals, but we have no direct evidence for this. In addition, a postsynaptic site of DA action is indicated for these synapses because DA increases the Glu responses of the postsynaptic LP and PY neurons. This could result in part from increases in LP and PY input resistances (Table 1).

**FIG. 7.** Oct enhancement of the Glu responses from the LP and PY neurons. A: Oct enhancement of the LP Glu response in a single experiment. B and C: normalized mean Glu responses averaged for all Oct experiments from the PY (n = 3) and LP (n = 3) neurons. * Statistically significant change from the mean control value. See Fig. 2 legend for time period definitions.

**AB SYNAPSES.** DA’s functional creation of the AB → IC synapse and enhancement of the AB → VD synapse (Johnson et al. 1994, 1995) occur despite a reduction in VD and IC responses to applied Glu (Table 1; Fig. 11A). This strongly suggests a presynaptic action to increase transmitter release from the AB, which outweighs the postsynaptic reduction of Glu responsiveness (Fig. 11A). Dopamine causes a 20% increase in the AB input resistance (Johnson et al. 1993a) that could indirectly increase its transmitter output on current injection into the soma. However, a direct DA action to

**FIG. 8.** Oct’s lack of effect on the Glu responses of the PD, VD, and IC neurons. A–C: normalized mean Glu responses for all Oct experiments on the PD (n = 4), VD (n = 4), and IC (n = 4) neurons. See Fig. 2 legend for time period definitions.
synaptic enhancements, we suggest that DA directly enhances release from LP terminals. DA also enhances the LP → PY synapse. Although an enhanced transmitter release from LP terminals may contribute to this effect, DA is certainly acting postsynaptically to increase the PY Glu response (Fig. 11A).

PY SYNAPSES. DA enhances both the PY → IC and PY → LP chemical synapses (Johnson et al. 1994, 1995). Under our control conditions, the PY → IC synapse is silent despite IC responses to applied Glu. This synapse is activated by DA, despite DA’s reduction in the IC Glu response. So enhancement of PY → IC is another example of a strong presynaptic enhancement of release outweighing a postsynaptic reduction in response. Dopamine causes a 38% increase in PY input resistance in DA-responsive neurons (Harris-Warrick et al. 1995a) that indirectly could increase transmitter output. This also could contribute to DA’s strengthening of PY → LP, but at this synapse DA must also act postsynaptically to enhance the LP response to Glu, increase LP’s input resistance, and enhance isolated PY → LP electrical coupling (Fig. 11A).

IC SYNAPSES. DA weakens the IC → VD synapse (Fig. 11A). The DA reduction of the VD’s Glu response, and the VD’s input resistance suggest that this is mediated at least in part postsynaptically.

In conclusion, if we make the parsimonious assumption that all AB, LP, and PY presynaptic terminals are similarly affected by DA, then a presynaptic enhancement of transmitter release would contribute to the strengthening of all their output synapses. This would outweigh a postsynaptic reduction of transmitter responsiveness when the PD, VD, and IC are postsynaptic partners. However, both pre- and postsynaptic actions of DA could contribute to synaptic strength enhancement when LP and PY are postsynaptic partners (Fig. 11A). DA’s postsynaptic actions could occur by two mechanisms: specific modulation of Glu receptor activity or general modulation of membrane currents affecting current flow to the recording site. Cleland and Selverston (1997) have presented preliminary evidence from unidentified, voltage-clamped cultured STG neurons that DA specifically reduces the Glu receptor current. This could help explain DA’s reduction of Glu responsiveness in some neurons but not its enhancement in other neurons.

**Serotonin**

Serotonin modulation of glutamatergic graded synapses within the pyloric network (Fig. 11B) also appears to be accomplished by both pre- and postsynaptic actions.

AB SYNAPSES. Enhancement of synaptic strength at some AB synapses, including activation of a silent AB → IC synapse (Fig. 11B) (Johnson et al. 1995), must be by a presynaptic mechanism. The AB’s chemical synaptic partners showed either a significant decrease (VD) or no change in iontophoretic Glu responsiveness (LP, PY, IC; Table 1). The AB neuron shows a very modest 8% increase in input resistance during 5-HT treatment (Johnson et al. 1993b), and it would be surprising if this small change increased the depolarization in its terminals enough to activate silent synapses and cause >100% increases in AB → LP and AB → PY synaptic strength. Thus we suggest that 5-HT...
directly increases transmitter release at the AB synaptic terminals. We previously observed that some AB → VD graded chemical synapses were enhanced by 5-HT, whereas others were weakened (Johnson et al. 1994). This variability may reflect differences between preparations in the relative magnitude of 5-HT's opposing effects to increase AB transmitter release or to decrease input resistance and Glu responsiveness of the VD (Table 1).

**LP SYNAPSES.** The weakening of the LP → PD synapse by 5-HT (Johnson et al. 1995) also appears to occur presynaptically because 5-HT does not change the PD's response to applied Glu or ACh, does not change the input resistance of the PD neuron (Table 1), and does not affect electrical coupling from AB → PD or between the PD neurons (Johnson et al. 1993a) (Fig. 11B). 5-HT causes a 6% decrease in the LP’s input resistance, but this alone appears too small to account for the 50% weakening of the LP → PD synapse. Thus 5-HT may act directly at the nerve terminal to reduce transmitter release from the LP neuron onto PD.

5-HT also weakens the LP → VD synapse (Fig. 11B) (Johnson et al. 1995). This must be due in part to a postsynaptic action of 5-HT because 5-HT decreases the VD response to applied Glu and ACh and decreases the VD input resistance (Table 1). 5-HT also reduces both AB → VD and PD → VD electrical coupling (Fig. 11B) (Johnson et al. 1993a), which is consistent with a postsynaptic input resistance decrease contributing to the LP → VD weakening. However, a presynaptic action of 5-HT also must contribute to LP → VD weakening, because the LP → VD synapse always is abolished by 5-HT no matter how much LP is
TABLE 1. Summary of amine effects on pyloric neuron input resistance and responses to iontophoretically applied transmitters

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Upward and downward pointing arrows indicate increase or decrease in the measured parameter, respectively. Zero indicates no effect. Glu, glutamate; ACh, acetylcholine; DA, dopamine; 5-HT, serotonin; Oct, octopamine; AB, anterior burster; PD, pyloric dilator; VD, ventricular dilator; IC, inferior cardiac; PY, pyloric constrictor; LP, lateral pyloric. *Summarized from Harris-Warrick et al. 1995a,b; Johnson et al. 1993a, 1995.

depolarized (Johnson et al. 1995), whereas the VD remains responsive (though less so) to LP’s transmitter, Glu.

Although we have suggested that all the terminals of a pyloric neuron might be affected similarly by a neuromodulator, this may not always be true. The LP → PY synapse was essentially unaffected by 5-HT (Fig. 11B) despite the clear presynaptic reduction of release at LP → PD and LP → VD. Serotonin has no significant effect on the PY that could compensate for a presynaptic reduction of release. Thus it appears that presynaptic release at LP → PY is not greatly affected by 5-HT. Certainly different synaptic terminals of the same neuron can show specific properties, as previously noted for transmitter release (Davis and Murphey 1994; Katz et al. 1993). Terminal specific sensitivity to amines could allow differential modulation of transmitter release.

PY SYNAPSES. The PY → LP synapse is weakened by 5-HT (Fig. 11B), an effect that must be predominantly presynaptic because 5-HT does not change the LP Glu response (Table 1). Although this amine does reduce PY input resistance by ~10% (Johnson et al. 1993a), this input resistance reduction is not large enough to affect PY → PY or LP → PY electrical coupling and thus should not affect current flow into the terminal. Thus we suggest that 5-HT directly reduces PY transmitter release. 5-HT’s effects on the PY → IC synapse could not be determined because this synapse is silent under control conditions and 5-HT does not activate it (Johnson et al. 1995).

IC SYNAPSES. The weakening of the IC → VD synapse (Fig. 11B) appears to be at least in part postsynaptic because VD shows a reduced response to Glu (Table 1). This probably is due to a decreased VD input resistance, as suggested above for the LP → VD synapse. In their preliminary study of unidentified cultured STG neurons, Cleland and Selverston (1997) found no effect of 5-HT on Glu receptor currents.

Octopamine

Octopamine enhanced the graded synaptic strength of most of the glutamatergic synapses within the pyloric network (Johnson et al. 1995) (Fig. 11C), like the other amines, its effects appear to be mediated both pre- and postsynaptically.

FIG. 11. Summary of amine effects on graded synaptic strength, pyloric neuron excitability, input resistance, transmitter responses, and suggested presynaptic actions for DA (A), 5-HT (B), and Oct (C). Small open circles: terminals of inhibitory Glu synapses; †, †, and o indicate increased, reduced, or no effect, respectively, on transmitter release. Resistor symbols, nonrectifying electrical synapses; diode symbols, rectifying electrical synapses with the preferred direction of positive current flow indicated by the direction of the diode arrow. Thin, thickened, and dashed synaptic connections indicate no change, enhancement, and weakening, respectively, of synaptic strength. Two representations of synaptic strength at the same electrical synapse mean the amine’s effect depended on the presynaptic neuron. Pipette symbols: iontophoretic application of Glu, and Glu or ACh for the PD and VD neurons; †, †, and o indicate enhanced, weakened, and no effect, respectively, on transmitter responses. Open circles within cell symbols indicate increased (+), decreased (−), or no effect (o) on input resistance. Borders around cell symbols indicate increased (thickened border), decreased (dotted line border), or no effect (thin border) on cell excitability. Data summarized from Flamm and Harris-Warrick 1986b; Johnson et al. 1995; this paper.
AB SYNAPSES. Silent AB → IC synapses are not activated and the AB → VD synapse is not affected by Oct (Fig. 11C) (Johnson et al. 1995). If we then assume that none of the AB terminals are affected by Oct, the enhancement of the AB → LP and AB → PY synapses appears to be accomplished postsynaptically because the Glu responses of both postsynaptic LP and PY neurons are enhanced by Oct (Table 1). Oct increases LP input resistance by 20% (Johnson et al. 1995). However, PY → LP electrical coupling is not significantly affected by this modest input resistance increase (Johnson et al. 1993a). Because PY input resistance is not changed at all by Oct, this amine may enhance both LP and PY postsynaptic responses by a direct action on the Glu receptor. Cleland and Selverston (1997) did not examine the effects of Oct on Glu receptor currents from STG neurons.

LP SYNAPSES. A presynaptic mechanism of action is suggested for Oct’s enhancement of the LP’s output synapses (Fig. 11C). Oct enhances the LP → PD and LP → VD synapses (Fig. 11C) without a change in the Glu response of the PD and VD neurons (Table 1). As described above, the Oct-induced 20% increase in LP input resistance does not change PY → LP electrical coupling (Fig. 11C) and is thus unlikely to cause enhanced LP output. Thus Oct may act at the LP presynaptic terminal to increase transmitter release (Fig. 11C). Oct also may act postsynaptically to enhance the LP → PY synapse because the PY shows enhanced responsiveness to applied Glu (Table 1).

PY AND IC SYNAPSES. Enhancement of PY → LP chemical transmission probably is accomplished postsynaptically by the same mechanisms as suggested above for AB → LP. Oct has no effect on either silent PY → IC synapses or IC → VD graded synapses (Johnson et al. 1995).

Distributed amine effects algebraically sum to create synaptic strength changes

Our analysis of amine sites of action at pyloric synapses reinforces our earlier conclusion that distributed changes at many network sites can strongly alter network output (Johnson et al. 1995; Lockery and Sejnowski 1993). In addition to amines acting at multiple pyloric synapses to change motor output, we have now shown that amines can act at multiple pre- and postsynaptic sites to modify a particular graded synapse. Sometimes amines appear to have both pre- and postsynaptic effects that are in the same direction as the synaptic strength change (Fig. 11). Often, however, pre- and postsynaptic amine effects oppose each other (Fig. 11), and the final synaptic strength change reflects the dominating amine action. For example, DA reduces PD and VD responses to iontophoresed Glu but enhances LP’s glutamatergic synapses onto these cells. In addition, DA creates functional graded synapses from AB and PY onto the IC while reducing the IC’s Glu response. Thus DA must be enhancing presynaptic release to outweigh opposing postsynaptic actions to decrease synaptic strength. Our finding that 5-HT can increase or decrease AB → VD chemical synaptic strength in different preparations (Johnson et al. 1994) suggests that one site of action may not always be dominant. It is possible that other neuromodulators could selectively alter the pre- or postsynaptic targets, such that an amine’s effects could be quantitatively changed or even qualitatively reversed at some synapses. Variability in the strength of opposing modulator effects thus could contribute to variability in the characteristics of motor patterns.

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