The Journal of Neuroscience, July 1990, 10(7): 2066–2076

Aminergic Modulation of Graded Synaptic Transmission in the Lobster Stomatogastric Ganglion

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Graded chemical synaptic transmission is important for establishing the motor patterns produced by the pyloric central pattern generator (CPG) circuit of the lobster stomatogastric ganglion (Raper, 1979; Anderson and Barker, 1981; Graubard et al., 1983). We examined the modulatory effects of the amines dopamine (DA), serotonin (5-HT), and octopamine (Oct) on graded synaptic transmission at all the central chemical synapses made by the pyloric dilator (PD) neuron onto its follower cells, using synaptic input–output curves measured from cell somata. DA strongly reduced the graded synaptic strength at all the PD synapses. DA reduction of chemical synaptic strength from PD onto the inferior cardiac (IC) neuron could change the sign of synaptic interaction between these 2 cells from inhibitory to excitatory by uncovering a weak electrical connection. 5-HT had weaker and more variable effects, reducing graded synaptic strength from the PD onto the lateral pyloric and pyloric neurons and enhancing the weak synapse from the PD to the IC cell. Oct strongly enhanced the graded synaptic strength at all the PD central synapses. Oct enhancement of graded synaptic strength between the PD and IC cells could also change the sign of the interaction: weak, excitatory electrical coupling, which was sometimes dominant before Oct, was masked by the enhanced chemical inhibitory interaction during Oct application. Measurements of electrical coupling between 2 PD cells and between 2 postsynaptic cells suggest that Oct does not change the input resistance of these cells and may act directly at the PD synapses. The effects of DA and 5-HT are most easily explained by their general reductions in pre- and postsynaptic input resistance. DA, 5-HT, and Oct each produce a distinct pyloric motor pattern (Flamm and Harris-Warrick, 1986a). These amine-induced motor patterns may be explained by the unique actions of each amine on the intrinsic membrane properties of different pyloric CPG neurons (Flamm and Harris-Warrick, 1986b) and by modulation of graded synaptic transmission between the pyloric neurons.

Received Aug. 3, 1989; revised Dec. 18, 1989; accepted Dec. 28, 1989.

We thank Dr. Paul Katz for many helpful discussions and for drawing an earlier version of Figure 1*A*, Barbara Seely for preparing the final manuscript, and Stephen Singer for preparing the final figures. This work was supported by National Research Service Award NS07859 to B.R.J. and NIH Grant NS17323 and Hatch Act Grant NYC-191410 to R.M.H.-W.

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Graded chemical synaptic transmission, where transmitter is released as a continuous function of presynaptic voltage, is an important means of synaptic communication in invertebrate and vertebrate nervous systems. Many neurons in invertebrates use graded chemical transmission for the production of rhythmic motor patterns (reviewed in Simmers, 1981; Wilson and Phillips, 1983; Siegler, 1985; see also Paul and Mulloney, 1985; Spencer, 1988; DiCaprio, 1989), for neuromuscular transmission (Davis and Stretton, 1989), and for transmission of sensory information (reviewed in Bush, 1981; Shaw, 1981; see also Wilkens, 1988). In vertebrates, graded chemical synaptic interactions have been described in the retina (Fain, 1981), in the acoustico lateralis system (Russell, 1981), and between cells in the olfactory bulb (Shepherd, 1981). Graded synaptic transmission is especially useful for subtle neuronal interactions, allowing precise, continuous control of postsynaptic membrane potential (Pearson, 1986). Despite the apparent importance of graded chemical synaptic transmission, little is known about the effects of common neuromodulatory substances on the strength of this form of intercellular communication. In contrast, the effects of amines and other modulators on action-potential-evoked synaptic transmission are well documented (reviewed in Kandel and Schwartz, 1982; Vizi, 1984; Gribkoff and Dudek, 1987; Koketsu, 1987; Kow and Pfaff, 1988).

The purpose of our study was to examine the modulatory actions of amines on graded synaptic transmission in a neural network where graded chemical synaptic interactions are important for network function, and where even small changes in synaptic strength could have functional consequences for the network output. The pyloric circuit in the stomatogastric ganglion (STG) generates rhythmic movements in the foregut of decapod crustaceans (Claiborne and Ayers, 1987). This motor circuit is a small, well-defined central pattern generator (CPG) network composed of 14 neurons in 6 classes whose synaptic interconnectivity is known in detail (Mulloney, 1987; Fig. 1*A*). All 14 neurons generate action potentials to send signals to distant targets (muscles or neurons in other ganglia). However, within the STG these spiking neurons use graded transmission to communicate with each other (Maynard and Walton, 1975; Graubard, 1978; Graubard et al., 1980, 1983). As a consequence, the motor patterns produced by the pyloric CPG are thought to be orchestrated primarily by graded synaptic interactions (Raper, 1979; Anderson and Barker, 1981; Russell and Graubard, 1987; Hartline et al., 1988). These interactions occur on small processes in the neuropil of the STG; the cell bodies do not receive any synaptic inputs (King, 1976). Thus, passive spread of current is an important determinant of the amplitude of the response (both for information transfer between input

and output sites in the neuropil and for our recordings made from distant cell bodies). We have examined the effects of the amines dopamine (DA), serotonin (5-HT), and octopamine (Oct) on graded chemical synaptic strength at all the central synapses made by the pyloric dilator (PD) motoneuron. This neuron, as part of the primary pacemaker group, helps to provide the major timing cues for the pyloric motor pattern (Miller, 1987; Hartline et al., 1988). It makes cholinergic inhibitory synapses on 3 classes of follower cells; the lateral pyloric (LP), the pyloric (PY) and the inferior cardiac (IC) motoneurons (Fig. 1). DA, 5-HT, and Oct are endogenous modulators in the lobster nervous system (Kravitz, 1988); each can induce a distinct pyloric motor pattern when bath-applied to the STG (Flamm and Harris-Warrick, 1986a). These amines act, at least in part, by each having a unique constellation of effects on the intrinsic membrane properties of the different pyloric CPG neurons (Flamm and Harris-Warrick, 1986b). Here we demonstrate that, in addition, DA, 5-HT, and Oct also modulate the strength of graded chemical synaptic interactions within the pyloric motor circuit.

Materials and Methods

Pacific spiny lobsters (*Panulirus interruptus*) were purchased from Marinus Inc. (Long Beach, CA) and maintained in marine aquaria at 15°C. The stomatogastric nervous system was dissected and placed in a preparation dish filled with oxygenated *Panulirus* saline of the following composition (mM): NaCl, 479; KCl, 12.8; $CaCl_2$, 13.7; Na_2SO_4, 3.9; $MgSO_4$, 10.0; glucose, 2.0; Tris base, 11.1; maleic acid, 5.1, pH 7.35 (Mulloney and Selverston, 1974). The STG was desheathed and enclosed in a small (1 ml) pool of saline walled by Vaseline. This allowed rapid exchange of solution over the ganglion at a perfusion rate of approximately 5 ml/min. Standard intracellular techniques were used for current injection into and voltage recordings from motoneuron cell bodies. Both KAc- (4 M, 30–40 MΩ) and KCl- (3 M, 10–20 MΩ) filled electrodes were used. The cell bodies of the PD neurons and their synaptic partners were identified during rhythmic pyloric activity (generated with descending inputs from other ganglia intact) by (1) matching action potentials recorded extracellularly from an appropriate motor nerve root and intracellularly from the soma; (2) the timing of spike activity within the pyloric rhythm; (3) the characteristic shape of membrane potential oscillations and action potential amplitudes; and (4) the synaptic connectivity. The PY cell population in these experiments was a mixture of early (PE) and late (PL) firing PYs (Hartline et al., 1987). We saw no differences in an amine's effects on PD-PY synaptic strength between different PY cell types.

Following cell identification, we replaced the saline in a Vaseline-walled pool surrounding the input nerve to the STG with 1 M sucrose or 10^{-7} M TTX. This procedure stopped rhythmic pyloric activity by eliminating all descending modulatory inputs to the STG (Russell, 1979; Nagy and Miller, 1987); ascending modulatory inputs from muscle stretch receptors (Katz et al., 1989) were eliminated in the initial dissection by removing the appropriate nerves. The STG was superfused with 10^{-7} M TTX-saline to block action-potential-evoked synaptic transmission. We isolated the PD cell's inhibitory synapses as follows (Fig. 1*B*). In most experiments, the anterior burster (AB) interneuron and the ventral dilator (VD) motoneuron of the pyloric circuit were killed by intracellular iontophoresis of 5,6-carboxyfluorescein and illumination with bright blue light (Miller and Selverston, 1979; Flamm and Harris-Warrick, 1986b). In addition, picrotoxin (PTX, 5×10^{-6} M) was added to the TTX-saline perfusing the STG to block synapses from glutamatergic pyloric cells (AB, LP, PY, IC) within the pyloric circuit (Bidaut, 1980; Eisen and Marder, 1982; Marder and Eisen, 1984a). These treatments (Fig. 1*B*) (1) isolate the cholinergic PD neurons and their synaptic partners from all detectable sources of synaptic input (Flamm and Harris-Warrick, 1986b); (2) ensure that measured synaptic effects are direct; and (3) allow measurements to be made without interference from rhythmic activity induced by DA and 5-HT in TTX-treated AB cells at elevated temperatures (Harris-Warrick and Flamm, 1987; Johnson and Harris-Warrick, unpublished observations). In a few experiments, the VD cell was not killed; this had no detectable effect on the magnitude of amine effects on PD synaptic strength.

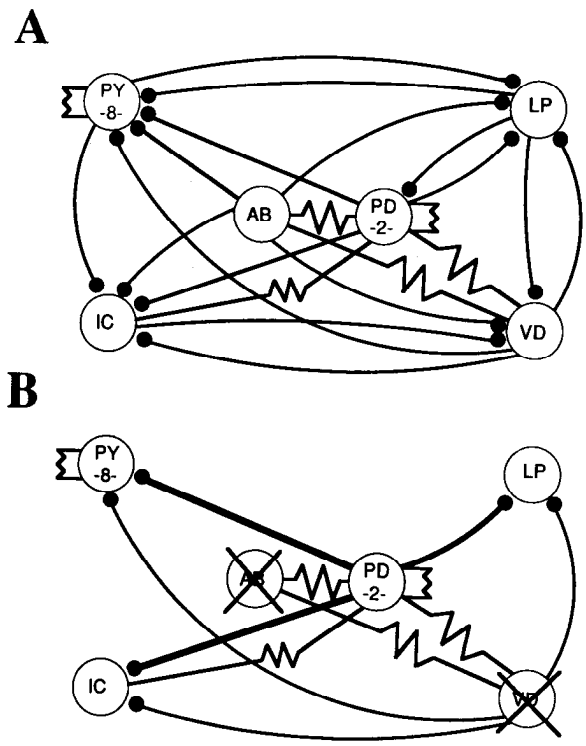

Figure 1. *A*, Summary of the synaptic connections in the pyloric central pattern generator of *Panulirus interruptus* (modified from Mulloney, 1987). There are 8 PY and 2 PD neurons and 1 of each other cell type. *Resistor symbols* indicate electrotonic connections between and among cell types; *filled circles* indicate inhibitory chemical synapses. The PD cell makes chemical inhibitory synapses with LP, PY, and IC cells, and makes a weak electrical connection with the IC cell. *B*, Diagrammatic representation of the procedures used to isolate the PD cell's chemical inhibitory synapses. *Crosses* through the AB and VD cells indicate their photoinactivation; all glutamatergic synaptic connections are absent to indicate their blockade by picrotoxin.

Graded synaptic inhibition from PD onto LP, PY, and IC was measured at 19–23°C. Graded transmission is very temperature-sensitive and falls rapidly in amplitude at temperatures below 19°C (Johnson and Harris-Warrick, 1989). Graded synaptic strength was determined from input-output (I/O) curves (Fig. 3), gathered with 2 presynaptic electrodes (for current injection and voltage recording) and 1 postsynaptic electrode to record the graded synaptic potential (GSP). I/O curves were constructed from 1 sec presynaptic polarizations (square pulses, 0.2 Hz stimulation rate) of varying amplitude and sign, plotted against the peak amplitude of the postsynaptic polarization. The presynaptic stimulation series always began with the weakest polarizations; these were steadily increased until the strongest polarization levels were reached. Preliminary experiments indicated that there was no obvious decrement in postsynaptic responses to 1 sec depolarizing square pulses repeated at 0.2 Hz. Comparisons of GSP amplitudes between the control, amine, and wash conditions were made at the level of PD depolarization which elicited the maximum postsynaptic polarization in the control condition (see Results). The slope of an I/O curve was obtained from a simple regression line through the data points with measurable GSPs. The threshold for a detectable postsynaptic response was calculated as the x-intercept point from this regression line unless there was transmitter release at the PD cell's resting potential (Graubard et al., 1983). This was indicated by a depolarization of a postsynaptic cell upon presynaptic hyperpolarization (example, Fig. 3*B*, PD-PY). In this case, the threshold for transmitter release was estimated from the point at which the I/O curve became flat. Our estimations of the response threshold are not exact, since PD depolarizations evoked in the soma decrement somewhat en route to the release sites in the neuropil. However, they provide points of comparison between the control and amine conditions. The dependence of the GSP amplitude on the postsynaptic membrane po-

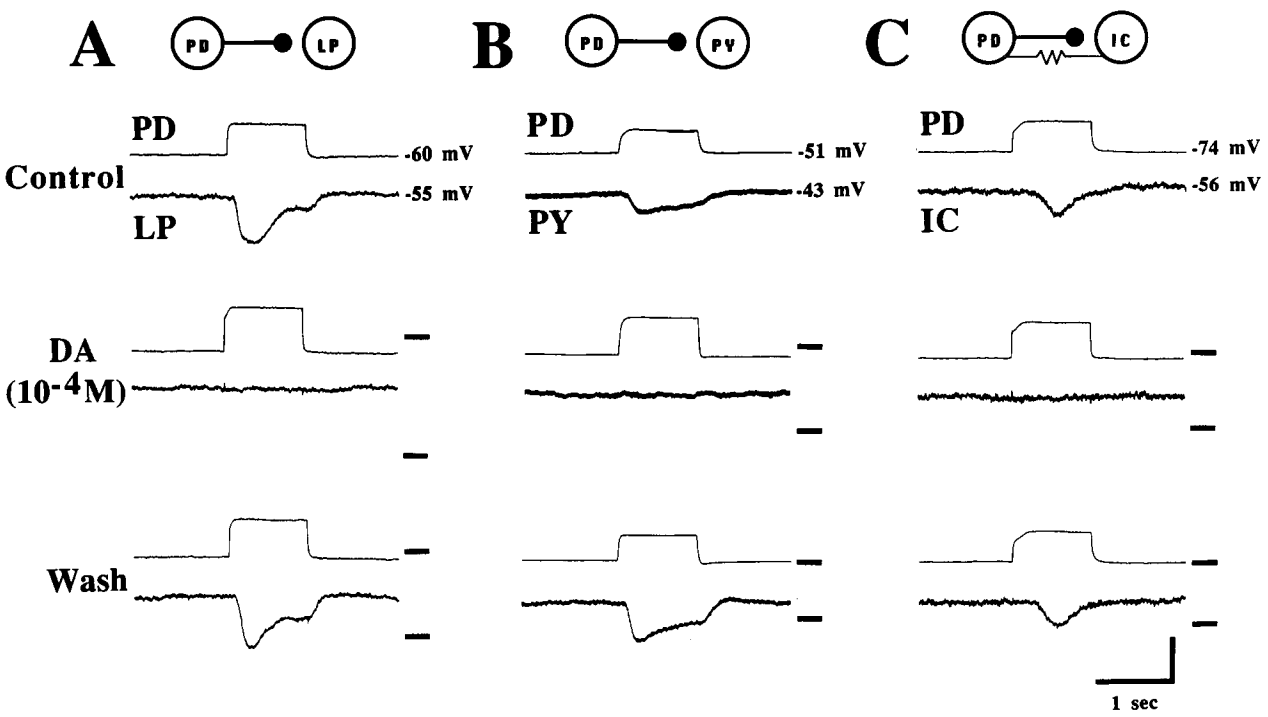

Figure 2. Dopamine reduction of graded chemical transmission from PD onto LP (*A*), PY (*B*), and IC (*C*). Pairs of traces show a 1-sec presynaptic PD depolarization and the postsynaptic responses. *Top pairs of traces,* Control before DA application; *middle pairs,* 5-min perfusion with DA (10^{-4} M); *bottom pairs,* wash after 7 (*A*), 10 (*B*), and 30 (*C*) min. In each series of traces, the PD cell was depolarized to the same absolute peak value; the depolarizing step size varies due to direct effects of DA on the resting potential of the PD cell. Resting potentials for both cells in each pair are indicated for the control traces; lines to the *right* of the amine and wash traces are the control resting potentials. Variations in PD cell resting potential may be at least partially due to damage caused by double-electrode penetrations. Voltage calibration bar: *A,* 30 mV (PD) and 6 mV (LP); *B,* 33 mV (PD) and 6 mV (PY); and *C,* 43 mV (PD) and 4 mV (IC). In *C,* the relatively long GSP latency in IC after PD depolarization is probably due to weak electrical coupling between PD and IC cells (see text and Fig. 3, PD-IC). Degrees of synaptic isolation: *A* and *C,* AB and VD cells killed and PTX present; *B,* AB cell killed and PTX present.

tential was determined in LP neurons with 2 postsynaptic electrodes (for current injection and voltage recording), and 1 PD presynaptic electrode to deliver 1 sec depolarizing pulses of constant current. All data are from the first application of an amine to a preparation; compared to the first application, subsequent Oct applications were often not as effective in changing synaptic strength. I/O curves were compared in the TTX-control saline, after 5 min in an amine-TTX solution, and after a wash period varying from 6 to 30 min. Data reported in the Results are only from synapses where amine effects reversed during the wash period. Test concentrations of the amines were as follows: DA, 10^{-4} M; 5-HT and Oct, 10^{-5} M. All the amines were obtained from Sigma Chemical Co. and were dissolved in TTX or TTX/PTX saline immediately before test application. TTX and TTX/PTX control salines were usually prepared the day of an experiment, but occasionally unused control saline was stored and refrigerated for up to a week for use in subsequent experiments. Statistical comparisons of resting potential differences between the control and amine conditions were made with the Student's *t*-test; significant differences were accepted at $p < 0.05$. All values are given as mean ± SD.

Results

General characteristics of GSPs

As described by Graubard et al. (1983), a 1-sec depolarizing step delivered to a PD neuron elicited an initial peak synaptic hyperpolarization in its follower cells, which decayed to a plateau that was maintained through the stimulation period (Figs. 2, 4). The latency of the peak polarization and the amplitudes of both the peak and the plateau hyperpolarizations depended on the amplitude of the presynaptic depolarization. With small PD depolarizations, when chemical synaptic transmission was weak, or after GSP reduction by amines, a distinct plateau phase of the GSP was often absent (Fig. 2*C*). With large PD depolarizations GSPs reached a maximum amplitude; further PD depolarization elicited reduced GSPs (Fig. 3*A,* PD-PY and PD-IC; *B* and *C,* PD-IC). This may be due to weak electrical coupling betwen the pyloric CPG cells (Mulloney, 1987), which would tend to offset the PD cell's inhibition of its follower cells (Fig. 3, *A, C,* PD-IC, for example). The PD-IC interaction was weak and variable. In some preparations, chemical inhibition was stronger than electrical coupling, such that the IC hyperpolarized upon PD depolarization (example, Fig. 2). In other preparations, the electrical coupling was stronger and IC depolarized upon PD depolarization (Fig. 3*C,* PD-IC). Electrical coupling was clearly observed at 7 of 12 PD-IC synapses (including preliminary experiments).

Effects of DA on GSPs

DA (10^{-4} M) greatly reduced or abolished GSPs in all 3 cells postsynaptic to the PD neurons (Figs. 2, 3*A*). This effect was quickly reversible (within 7 min) upon wash with the TTX-saline solution. Accompanying this GSP reduction, DA significantly hyperpolarized PD cells an average of 4 ± 3.8 mV (n = 11) and significantly depolarized LP, PY, and IC cells an average of 11 ± 2 mV (n = 4), 4 ± 1 mV (n = 4), and 6 ± 1.5 mV (n = 3), respectively. Similar effects of DA on membrane potential were previously reported by Flamm and Harris-Warrick (1986b). In 3 of 4 preparations, the GSP at the PD-LP synapse was completely abolished by DA (Fig. 2*A*). Figure 3*A* (PD-LP) shows the I/O curve for a PD-LP synapse in control and DA conditions;

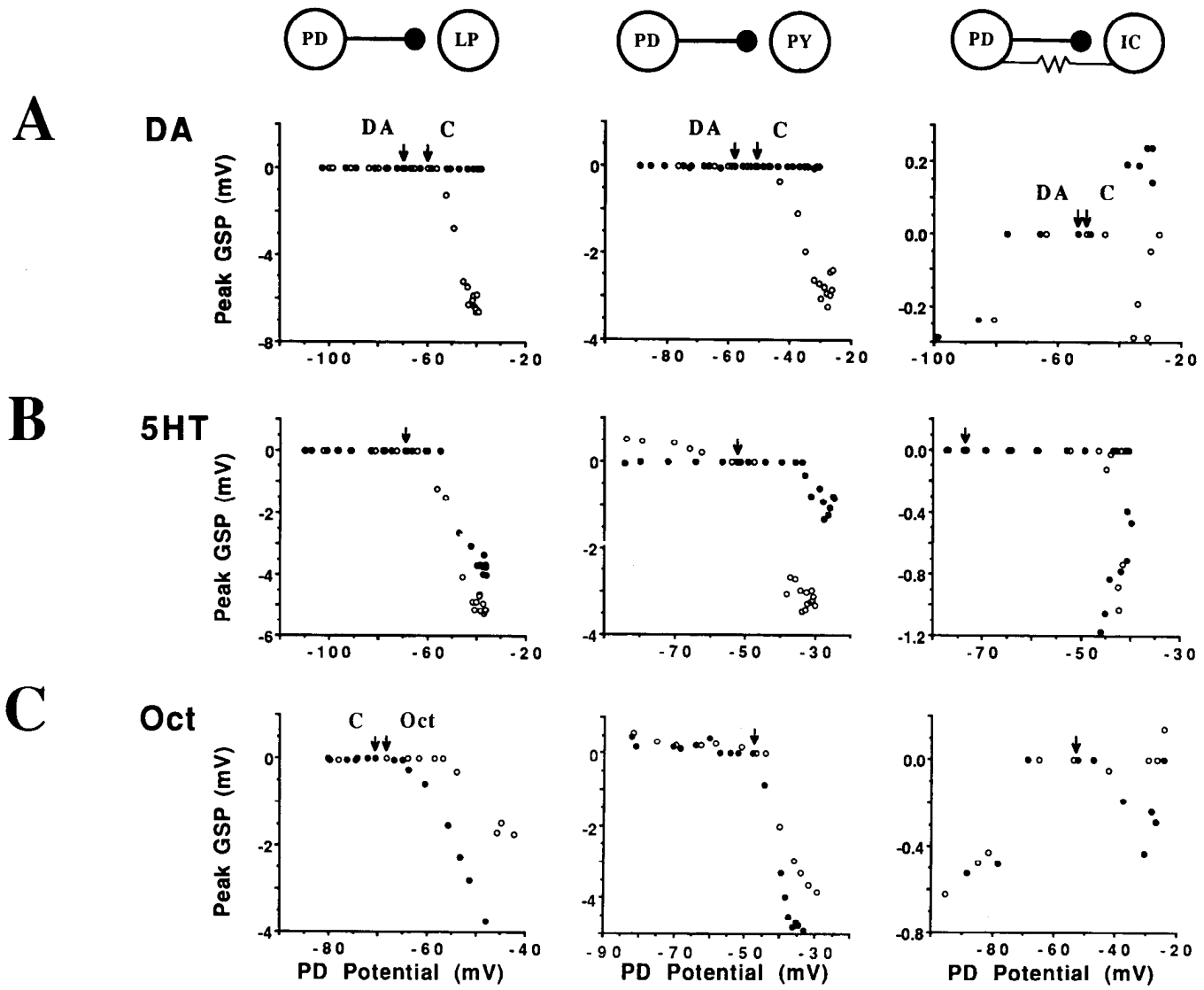

Figure 3. Amine effects on input–output curves (peak GSP amplitude in postsynaptic cell plotted against PD peak potential) for graded chemical transmission from PD onto LP, PY, and IC. *A*, DA (10^{-4} M). *B*, 5-HT (10^{-5} M). *C*, Oct (10^{-5} M). *Open circles* indicate measurements before amine application, and *filled circles* are measurements after 5 min of amine superfusion. *Arrows* indicate PD resting potential in control and amine conditions; *single arrow* indicates the same PD resting potentials in control and amine conditions. Control and amine resting potentials (mV) for LP in *A*: −62 and −54; *B*: −51 and −53; *C*: −67 and −62; for PY in *A*: −58 and −54; *B*: −63 and −62; *C*: −61 and −58; for IC in *A*: −61 and −53; *B*: −50 and −47; *C*: −68 and −64. The PD-IC graphs show weak electrical coupling between these 2 cells, as described in the text. Degrees of synaptic isolation: PD-LP: AB and VD cells killed and PTX present in *A* and *B*, VD cell killed and PTX present in *C*. PD-PY: AB cell killed and PTX present in *A* and *B*, AB and VD cells killed and PTX present in *C*. PD-IC: AB and VD cells killed in *A*, *B*, and *C*.

this demonstrates the complete abolition of GSPs in the LP cell over a wide range of presynaptic PD depolarizations. In a fourth preparation, DA reduced the peak GSP amplitude in the LP to 34% of the control value. DA shifted the threshold for a detectable GSP response at this synapse by 3 mV in the depolarizing direction and reduced the slope of the I/O curve to 43% of the control. A change in the threshold for detectable GSPs suggests a direct action of DA on the presynaptic PD cell, but other interpretations are possible (see Discussion).

DA also abolished GSPs at 3 of 4 PD-PY synapses. An example is shown in Figure 2*B*. The I/O curve in Figure 3*A* (PD-PY) shows that the PD-PY GSPs are completely abolished by DA over a range of presynaptic depolarizations. At a fourth PD-PY synapse, DA reduced the peak GSP amplitude to 14% of the control value. At this synapse, DA shifted the threshold for detectable postsynaptic response 13 mV in the depolarizing direction and reduced the slope of the I/O curve to 55% of the control value. Such a strong shift in the threshold again suggests a presynaptic site of DA action at the PD-PY synapse, although it does not exclude an additional postsynaptic action (see Discussion). At 3 PD-IC synapses, GSPs were completely abolished by DA. In Figure 2*C*, a small GSP lacking a pronounced plateau phase is reversibly abolished in DA. In addition to this chemical synaptic inhibition, PD and IC cells were often found to be weakly coupled electrically. The I/O curves in Figure 3*A* (PD-IC) show that when the small GSP in the IC cell is abolished by DA, weak electrical coupling is revealed. DA thus changes the sign of the synaptic coupling between these PD and IC cells.

Effects of 5-HT on GSPs

5-HT (10^{-5} M) reduced graded synaptic strength from PD to LP and PY, but this effect was weaker and more variable than the reductions caused by DA. 5-HT-induced GSP reduction reversed within 10 min upon wash with the TTX-saline solution. 5-HT did not significantly affect the resting potential of 10 PD cells [average hyperpolarization of 1 ± 2.4 mV ($p = 0.30$), and weakly (but not significantly) hyperpolarized the LP and PY cells an average of 2 ± 1.4 mV ($p = 0.18$, $n = 4$) and 2 ± 3.6 mV ($p = 0.50$, $n = 3$), respectively (see also Flamm and Harris-Warrick, 1986b)]. 5-HT reductions in GSP amplitude in 3 LP cells ranged from complete abolition of the GSPs (2 cells) to a reduction to 73% of the control value in a third cell. The I/O curves in Figure 3*B* (PD-LP) are from this third cell. At this synapse, 5-HT shifted the response threshold 4 mV in the depolarizing direction with only a 14% reduction in the slope of the I/O curve. This implicates an action of 5-HT on the presynaptic PD cell (see Discussion). 5-HT reduced the GSP amplitudes at 3 PD-PY synapses to an average of 54% of controls (range, 24–73%). At these synapses, the 5-HT effect on the response threshold varied from no change to a large shift (18 mV) in the depolarizing direction. The slope of the I/O curves for these synapses decreased to 56% of the mean control slope (range, 50–65% of control). Figure 3*B* (PD-PY) shows the I/O curve for the PD-PY synapse with the largest reduction in peak GSP amplitude (to 24% of control). At this synapse, 5-HT shifted the threshold for postsynaptic response 18 mV in the depolarizing direction. The control I/O curve shows transmitter release at rest (the PY depolarizes with PD hyperpolarization) which is lost during 5-HT application (no PY depolarization with PD hyperpolarization). The slope of the I/O curve was decreased to 54% of the control value at this synapse. Although variable, these results suggest that 5-HT, like DA, may act to reduce transmitter release from the PD neuron onto the LP and PY cells.

In contrast, 5-HT weakly enhanced chemical synaptic strength at PD-IC synapses. The I/O curves in Figure 3*B* (PD-IC) show a weak electrical connection upon PD hyperpolarization in both control and 5-HT curves. In the control, a small GSP is obtained with PD depolarization; this GSP is weakly enhanced by 5-HT. In 2 preparations, 5-HT depolarized IC cells an average of 5 mV compared to the control value, caused the response threshold to shift in the hyperpolarizing direction (10 and 5 mV), and increased the slopes of the I/O curves by an average of 19%.

Effects of Oct on GSPs

Oct (10^{-5} M) consistently enhanced the strength of graded synaptic transmission between the PD neurons and their follower cells. This effect completely reversed within the standard 30-min wash period. Oct caused a mean depolarization of all cells (Flamm and Harris-Warrick, 1986b): PD cells 3 ± 4.8 mV ($p = 0.09$, $n = 10$), LP cells 5 ± 1.2 mV ($p = 0.03$, $n = 3$), PY cells 2 ± 1.6 mV ($p = 0.16$, $n = 3$), and IC cells 3 ± 1.5 mV ($p = 0.09$; $n = 3$).

At 3 PD-LP synapses, Oct increased the peak GSP amplitude by a mean of 57% (range, 27–116%). At these synapses, the threshold for detectable response was shifted by varying amounts in the hyperpolarizing direction (1, 6, and 11 mV); Oct only slightly increased the slopes of the I/O curves by a mean value of 15% (range, 57–158% of control slope; Fig. 3*C*, PD-LP). Hyperpolarizing shifts in the response threshold suggest that Oct may act at least in part on the presynaptic PD cells (see Discussion). However, some of the increase in peak GSP amplitude may be accounted for by the simultaneous Oct-induced depolarization of the postsynaptic LP cell (Fig. 4*A*). Such a postsynaptic depolarization would increase the driving force for the K^+ conductance increase underlying the PD-evoked inhibitory potential (Eisen and Marder, 1982). The mean Oct-induced depolarization in LP cells would increase the driving force of the PD-evoked GSPs by a mean value of 25% (range, 9–45%), based on an approximate reversal potential of −78 mV for graded transmission (Graubard et al., 1983). This small increase in driving force does not fully account for the 57% increase in the mean peak GSP amplitude during Oct superfusion. We demonstrated this directly by comparing the GSP amplitudes at the same LP resting potential before and during Oct application. In Figure 4*B*, an LP cell was held at −55 mV by current injection: Oct still increased the PD-evoked GSP amplitude to 20% above the control value. Reversal potential measurements for the PD-evoked GSP in another LP cell showed a similar reversal potential (−75 to −80 mV) for the GSP in the presence and absence of Oct (Fig. 4*C*). The slopes of the regression lines through the control and Oct measurements in Figure 4*C* are −0.25 and −0.52, respectively, proving that Oct-induced depolarization of the LP cell cannot alone account for the increased amplitude of the GSP.

Oct increased the peak amplitude of the GSP at 4 PD-PY synapses by a mean value of 50% (range, 28–87%). Oct did not consistently change the response threshold for these cells (range, 3 mV depolarization to 4 mV hyperpolarization) but increased the slope of the I/O curve by an average of 56% [range, 7% decrease to 190% increase in slope; Fig. 3*C* (PD-PY)]. As with the LP cell, Oct directly depolarizes synaptically isolated PY cells (Flamm and Harris-Warrick, 1986b), and this could account for part of the increase in GSP amplitude. Under our experimental conditions, an Oct-induced depolarization of the PY cells would increase the GSP driving force by 10% (range, 7% decrease to 18% increase). At 1 PD-PY synapse, the Oct-induced depolarization of the PD cell caused transmitter release at rest.

As seen at the PD-LP and PD-PY synapses, Oct enhanced graded synaptic transmission from the PD onto the IC cell in 3 experiments. The control I/O curve in Figure 3*C* (PD-IC) shows weak electrical coupling between the PD and IC cells for both hyperpolarizing and depolarizing PD current injection. That is, in this preparation, the electrical coupling between the PD and IC was stronger than the chemical inhibition, so no net inhibitory potential was observed upon PD depolarization. During Oct application, however, graded synaptic inhibition was sufficiently enhanced to evoke a hyperpolarizing response in the IC cell with PD depolarizations beyond −47 mV. Thus, in this cell, Oct reverses the net sign of the synaptic communication between the PD and IC. A similar result was seen in a second IC cell, where, with Oct, a hyperpolarizing response was again seen in the IC cell at presynaptic depolarizations that evoked only small depolarizations in the control condition. At a third PD-IC synapse, Oct enhanced the maximal GSP amplitude by 81%, shifted the response threshold 2 mV in the hyperpolarizing direction and increased the slope of the I/O curve by 42%. Thus, although the PD-IC synapse is weak, Oct has a quantitatively strong effect to enhance it.

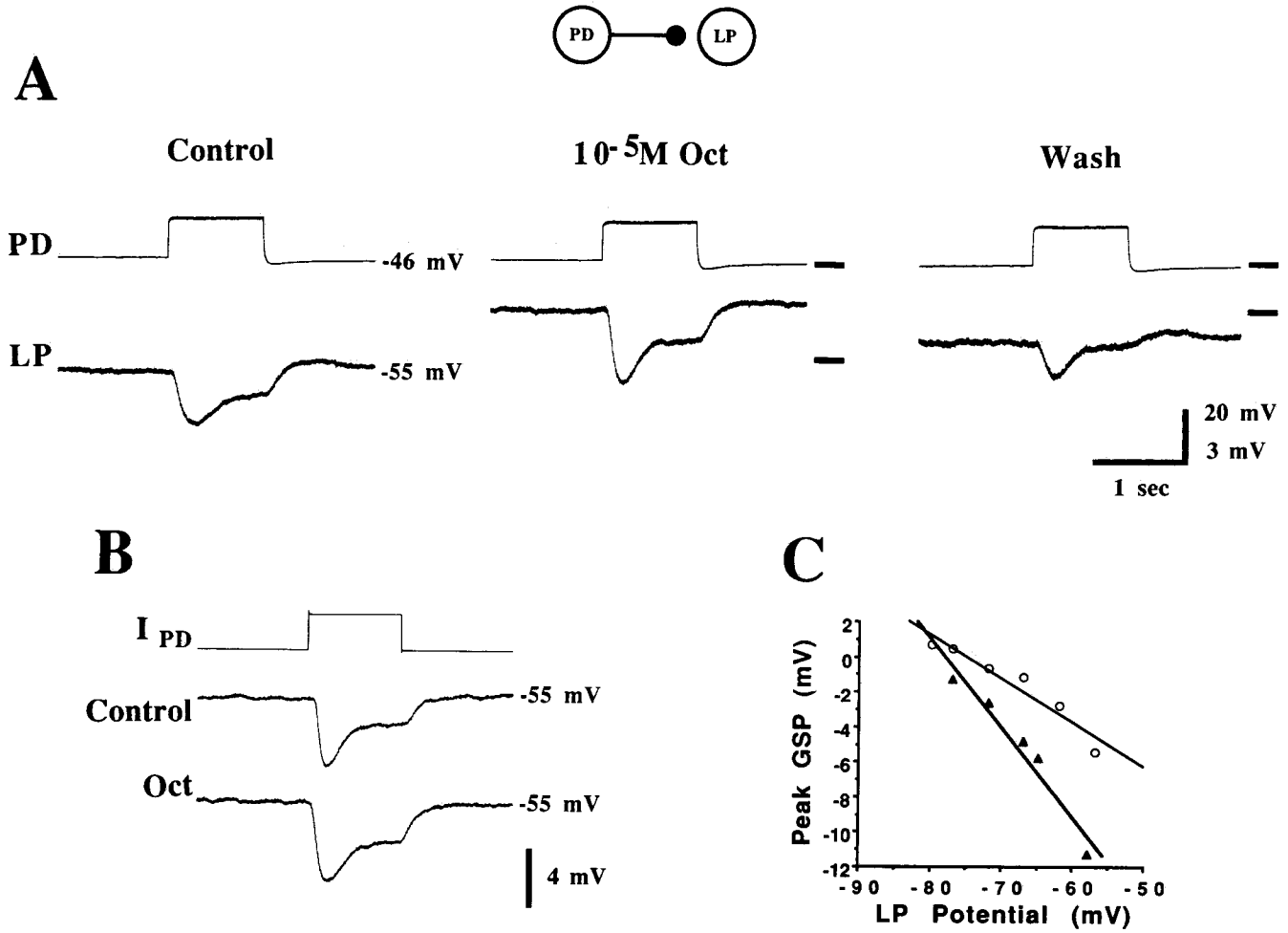

Figure 4. Oct (10^{-5} M) enhancement of graded chemical synaptic strength at the PD-LP synapse. *A*, LP GSPs in response to 1-sec PD depolarizations before Oct application (*Control*), after 5 min of Oct superfusion, and after 30 min wash. The PD and LP cell resting potentials indicated for the control traces are shown by *black bars* in the other traces. AB and VD cells killed for synaptic isolation. *B*, LP cell maintained at −55 mV potential by current injection. GSP in response to 1 sec, 10 nA depolarizing current injection to the PD, before (*Control*) and after 5 min Oct superfusion. AB cell killed and PTX added for synaptic isolation. *C*, Plot of GSP amplitude versus LP potential in response to 15 nA, 1-sec PD depolarizing current pulses before (*open circles*) and after 5 min Oct superfusion (*closed diamonds*). *C* is from a different preparation than *B*, and shows the greatest enhancement by Oct of LP GSPs that we obtained. AB cell killed for synaptic isolation.

Effects of amines on electrical coupling between PD cells

In a preliminary study of the possible sites and mechanisms of amine action, we measured the input resistance from the PD and PY cell somata. PD cell somata showed no statistically significant input resistance changes with Oct, 5-HT, or DA application. Mean PD control and amine input resistances (MΩ) for Oct were 5.1 ± 4.3 and 4.8 ± 3.8 ($p = 0.25$, $n = 8$); for 5-HT, 7.2 ± 3.5 and 6.9 ± 3.3 ($p = 0.7$, $n = 8$), and for DA, 4.9 ± 2.7 and 4.6 ± 2.7 ($p = 0.4$, $n = 9$). No statistically significant input resistance changes were seen in PY somata with 5-HT and DA; mean PY control and amine input resistances (MΩ) for 5-HT were 5.7 ± 3.5 and 4.5 ± 2.5 ($p = 0.3$, $n = 3$), and for DA, 4.6 ± 4.0 and 4.5 ± 3.9 ($p = 0.9$, $n = 4$). In 3 experiments with PY somata, the input resistance (MΩ) increased continuously from control (4.5 ± 0.7), through Oct superfusion (5.2 ± 0.05) to the wash (8.5 ± 1.8). We have no explanation for this result, but it does not correlate with the enhancement of the PD-PY GSP upon Oct superfusion and full reversal of this effect during the wash (Fig. 4). Because no synapses are found on the soma of these cells, and little or no response is seen when amines are pressure-ejected locally over the soma (R. Harris-Warrick, unpublished observations), these soma measurements probably do not reflect input resistance changes that could occur in the electrically distant neuropil.

In an attempt to detect input resistance changes in the neuropil, we took advantage of the fact that the 2 PD cells and some of the PY cells are known to be electrically coupled to cells of their own type (Fig. 1; Mulloney, 1987). We measured the strength of PD-PD and PY-PY electrical coupling, since amine-induced changes in the general input resistance in the neuropil of a cell would affect both chemical synaptic transmission and electrical coupling to like cells in the same way, even if it cannot be detected from the soma input resistance measurements. Oct caused little or no change in electrical coupling between the 2 PD cells or between 2 PY cells (Fig. 5*A*). Weak reductions in electrical coupling were seen with 5-HT between 2 PD cells, but little change in electrical coupling was

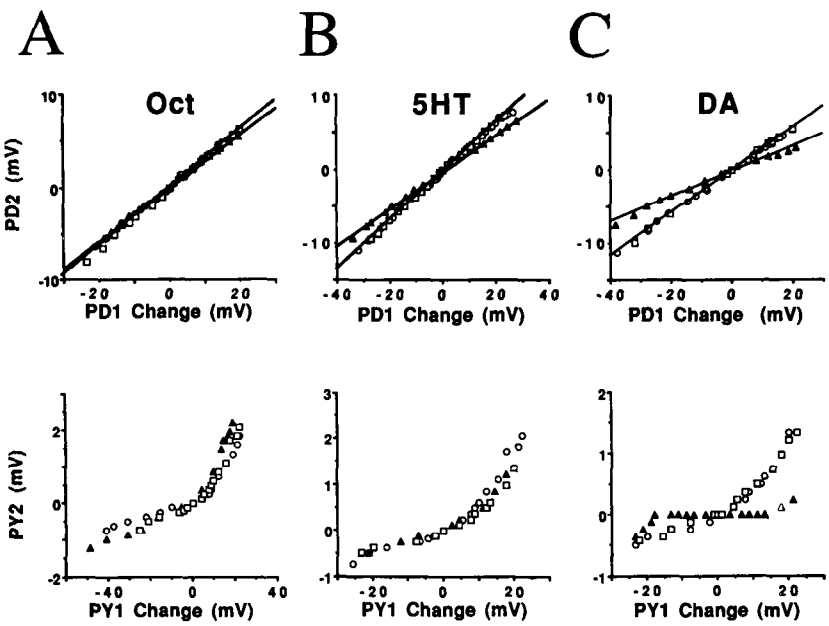

Figure 5. Amine effects on electrical coupling between 2 PD cells and 2 PY cells. Peak potential changes in PD2 or PY2 are plotted against the amplitude of 1-sec PD1 or PY1 peak polarizations of varying sign and amplitude. *Open circles* and *boxes* indicate control and wash measurements, respectively, while *filled triangles* indicate amine measurements. AB and VD cells killed for synaptic isolation of PD cells; AB cell killed and PTX added for isolation of PY cells. *A*, Oct (10^{-5} M). Slopes of the regression lines for PD coupling: control, 0.31; Oct, 0.29; wash, 0.33. *B*, 5-HT (10^{-5} M). Slopes of the regression lines for PD coupling: control, 0.33; 5-HT, 0.26; wash, 0.33. *C*, DA (10^{-4} M). Slopes of regression lines for PD coupling: control, 0.29; DA, 0.17; wash, 0.29. Slopes of regression lines have not been included for PY coupling because of the apparent rectification in coupling upon depolarization of these cells, which we have not yet explored in detail.

seen between 2 PY cells (Fig. 5*B*). In contrast, strong reductions were seen with DA for both PD-PD coupling and PY-PY coupling (Fig. 5*C*).

Our results suggest that, at least for the PD-PY synapse, generalized input resistance increases in pre- or postsynaptic cells cannot account for the enhancement in synaptic strength caused by Oct. The apparent input resistance decreases caused by DA and perhaps 5-HT in pre- and/or postsynaptic cells (as seen in the electrical coupling measurements) may, however, contribute to the observed reductions in synaptic strength.

Discussion

We have shown that amines can modulate the strength of graded chemical interactions between neurons of the pyloric motor circuit of the lobster STG. Specifically, we have described the effects of the endogenous modulators DA, 5-HT, and Oct (Beltz, 1988; Kravitz, 1988; Harris-Warrick et al., 1989) on graded chemical synaptic transmission at all the central output synapses of an important neuron in the pyloric circuit, the PD cell. With the use of cell-isolation procedures, we are confident that these amines unambiguously affect the strength of synaptic interactions between the identified cell pairs that we studied. DA strongly reduced the strength of graded chemical transmission from the PD neuron onto all of its follower cells. 5-HT had weaker and more variable effects, reducing graded synaptic strength from the PD onto the LP and PY and enhancing the synapse from the PD to the IC cell. Oct strongly enhanced the graded chemical synaptic strength at all of the PD central synapses.

Mechanisms of amine action

Graded chemical transmission could be modulated by 2 nonexclusive general classes of actions: (1) Direct actions at the synapse itself could modulate either presynaptic transmitter release or postsynaptic responsiveness, and (2) generalized changes in the cell membrane resistance would affect the passive spread of current from input sites to output sites in a cell. In the STG, there are no synapses directly on the somata, where we made our recordings. Synaptic interactions occur at a distance in the neuropil (King, 1976), so amine changes in the passive spread of current would not only affect neuronal I/O properties, but also our soma measurements of the amplitude of the pre- and postsynaptic response. As a consequence of this technical difficulty, our experiments have not directly addressed the mechanisms or sites of amine action in modulating the efficacy of synaptic transmission. However, we do have indirect evidence suggesting that Oct may act directly at a synaptic site to enhance the GSP amplitude from PD onto its follower cells. The Oct-enhanced GSPs are not explained by simple increases in driving force caused by the Oct-induced depolarization of postsynaptic cells. Calculated increases in driving force in Oct-depolarized postsynaptic cells do not fully account for the Oct-enhanced GSPs, although this does contribute to the effect. Nor is the Oct-induced enhancement of GSP amplitude explained by generalized increases in pre- or postsynaptic input resistance: Oct does not significantly change the input resistance measured in the soma of the PD or PY cells; it has little or no effect on the slopes of the I/O curves for electrical coupling between 2 PD cells or between between 2 PY cells (Fig. 5*A*). These electrical coupling measurements could indicate amine-induced input resistance changes at the biologically relevant sites in the synaptic neuropil more accurately than *I–V* plots from the electrically distant cell somata which lack direct synapses.

Although these results suggest that Oct acts directly at the synapse, it remains unclear whether its actions are pre- and/or postsynaptic. The threshold values and slopes of the I/O curves (Fig. 3) give some suggestions as to sites of action. Oct generally shifts the threshold for GSP generation in the hyperpolarizing direction (strongly for LP and IC, weakly for PY). This suggests that Oct is changing the threshold for transmitter release from PD terminals in a hyperpolarizing direction. Oct is known to enhance transmitter release at crustacean neuromuscular junctions (Breen and Atwood, 1983; Fischer and Florey, 1983; Harris-Warrick and Kravitz, 1984). Oct also increases the slope of the I/O curves (Fig. 3*C*), which could result either from en-

hanced transmitter release or from enhanced postsynaptic responsiveness. Thus, our results suggest that Oct enhances transmitter release from the PD terminals, but they do not exclude an additional enhancement of postsynaptic responsiveness.

In contrast, the actions of DA to reduce synaptic transmission at all the PD synapses and of 5-HT to reduce synaptic transmission onto the LP and PY cells are most easily explained by their reduction of the general input resistance of the pre- and postsynaptic cells. Although DA does not significantly change the input resistances measured in the somata of both PD and PY cells, it reduces the electrical coupling between 2 PD cells and between 2 PY cells (Fig. 5*C*). 5-HT has weaker effects, but can reduce electrical coupling between at least the presynaptic PD cells (Fig. 5*B*). The I/O curves for DA and 5-HT show a general shift of the threshold in a depolarizing direction and a reduction of the slope of the relation. These could both result from the decreased input resistance in the pre- and/or postsynaptic cells. A depolarizing shift in the threshold would result, because a larger depolarization of the PD soma would be necessary to adequately depolarize the electrically distant release sites. The reduced slope would result from loss of current during passive electrical flow in both pre- and postsynaptic cells. We cannot rule out, however, that in addition to reducing the general input resistance, these amines also reduce the strength of graded synaptic transmission by a direct synaptic action. It is also possible that the effects of DA and 5-HT on electrical coupling between PD cells and between PY cells result from changes in the junctional conductance rather than simply from input resistance decreases. DA and 5-HT directly affect junctional conductances in other systems (reviewed in Neyton and Trautmann, 1986; see also Colombaioni and Brunelli, 1988).

Functional consequences of amine changes in synaptic strength

Certainly more work needs to be done to understand the cellular mechanisms of amine modulation of synaptic strength in the STG. However, we emphasize that the exact cellular mechanisms of synaptic modulation by amines may make little difference to the functional consequences for altered motor patterns. Whether, for example, more or less transmitter is released by direct action at a presynaptic terminal or by greater or lesser current spread from electrogenic areas to release sites, the parameters of a motor rhythm that are dependent upon synaptic interactions between circuit components (rhythm frequency, firing phases) would still be altered. This is because graded chemical transmission is very dependent on the passive flow of current through pre- and postsynaptic cell processes (Rall, 1981). Of course, 1 advantage of a direct synaptic target over a generalized membrane resistance change lies in its ability to modulate a specific cell–cell interaction, and not bias all the inputs or outputs of a neuron.

The PD neurons are important for pyloric circuit function because they are usually part of the pacemaker driving the motor rhythm. The cycle frequency of the isolated pyloric CPG is determined mainly by the frequency of the endogenous membrane potential oscillations in the AB neuron (Hooper and Marder, 1987; Miller, 1987; Bal et al., 1988). Because of strong electrical junctions between the AB and the 2 PD cells (Fig. 1), these 3 cells oscillate together, imposing their burst frequency on the rest of the pyloric cells and establishing much of the firing phase relationships for follower cells through the combined strength of their inhibitory synapses (Miller, 1987). Changes in synaptic strength from the pacemaker group would thus be expected to alter fundamental properties of the pyloric motor pattern. Eisen and Marder (1984) have shown that indeed this is the case: changes in synaptic output from the AB-PD pacemaker group onto its follower cells can cause marked phase shifts in the activity of the follower cells. The AB evokes a rapid glutamatergic IPSP, while the PD evokes a cholinergic IPSP with much slower rising and falling phases. DA selectively inhibits the PD cells in the isolated pyloric circuit; as a consequence, the follower cells fire earlier in the pyloric cycle, because the remaining IPSP from the AB has a faster time course than the combined synaptic inhibition from both the AB and PD cells. When PD cell activity is enhanced by stimulating excitatory inputs, the onset of follower activity is delayed because of the prolonged time course of the PD-induced IPSP (Eisen and Marder, 1984).

It has been previously shown that DA, 5-HT, and Oct each generate a unique motor pattern from the pyloric circuit (Flamm and Harris-Warrick, 1986a) and that these motor patterns are due at least in part to amine-induced changes in intrinsic electrical excitability of the component neurons (Flamm and Harris-Warrick, 1986b; Harris-Warrick and Flamm, 1987). DA generates a motor pattern from the isolated pyloric circuit that can be partially explained by its direct action on isolated pyloric cells; cells excited by this amine (AB, LP, PY, and IC) are active, and cells inhibited by DA (PD and VD) are inactive (Flamm and Harris-Warrick, 1986b). The activity of follower cells in the DA-induced rhythm is phase-advanced compared with the rhythm when descending inputs from other ganglia are intact (Eisen and Marder, 1984; Flamm and Harris-Warrick, 1986a). Direct inhibition of the PD by DA (Eisen and Marder, 1984; Flamm and Harris-Warrick, 1986b), as well as reductions in its synaptic efficacy onto follower cells, would explain this phase advance of the follower cells. The DA-induced phase advance of the IC cell is further augmented by abolition of the chemical inhibitory synapse that unmasks a simultaneous electrotonic junction [Fig. 3*A* (PD-IC)]. Under normal conditions, the I/O curve for the PD-IC pair resembles over at least part of its range the full-wave rectification described for the PY-to-LP mixed electrical–chemical synapse in this circuit (Graubard and Hartline, 1987). This inverted U-shaped transfer function, where hyperpolarization of the postsynaptic cell is obtained regardless of the sign of the presynaptic polarization, is changed by DA into a weak, simple electrical coupling. In this state, as the pacemaker group depolarizes to fire, the PD-IC electrical connection would pull the IC cell towards its threshold for firing. We stress that rather than just changing the quantitative strength of the PD-IC synapse, DA can change the sign of the synaptic interaction during depolarizations of the PD cell.

The motor pattern generated from the pyloric CPG by 5-HT is dominated by rhythmic activity in the AB and PD cells, with weak activity in the IC cell (Flamm and Harris-Warrick, 1986a). The major characteristics of this pattern can also be explained by the direct effects of 5-HT on the excitability of the pyloric cells (Flamm and Harris-Warrick, 1986b). 5-HT causes rhythmic bursting in the isolated AB cell and tonic activity in the isolated IC cell. The firing pattern of the isolated PD cells is not directly affected by 5-HT, but in the intact circuit, they fire rhythmically because of their electrical coupling with the AB. The VD and LP cells are directly inhibited and silenced by 5-HT. 5-HT also does not change the firing pattern of the PY cells, which are usually silent in the intact circuit and remain so with

the amine. The weak enhancement of the graded synaptic interaction between PD and IC might contribute to the rhythmic activity of the IC during the 5-HT-induced rhythm; its tonic activation by 5-HT can be more effectively interrupted by increased cyclic inhibition from the PD. We note that the effects of 5-HT on graded synaptic transmission between the PD and PY cells and on electrical coupling between PD cells uncovers new targets of amine action that were not apparent from earlier studies; these showed no effect of 5-HT on synaptically isolated PD cells (Marder and Eisen, 1984b; Flamm and Harris-Warrick, 1986b). Oct, at the concentration we used in this study, induces a pyloric motor pattern in which all of the pyloric CPG neurons are active except for the IC (Flamm and Harris-Warrick, 1986a). Isolated pyloric cells are all directly excited by Oct, which explains the general activation of the cells in the Oct-induced rhythm (Flamm and Harris-Warrick, 1986b). The inactivity of the IC during Oct superfusion, despite its direct excitation by Oct, may be explained by the enhanced inhibition from the PD (and other) cells. In addition, Oct sometimes induces transmitter release at rest in the PD cell which could generate a tonic inhibition of the IC cell. At some PD-IC synapses, we again see an amine-induced change in the sign of the synaptic connection. In the example shown in Figure 3*C* (PD-IC), weak electrical coupling dominated over chemical synaptic inhibition before Oct application. During Oct superfusion, there was a pronounced synaptic inhibition of IC during PD depolarization, generating an inverted U-shaped transfer function that resembled the one described by Graubard and Hartline (1987).

Work is currently in progress to describe the effects of DA, 5-HT, and Oct on all of the graded synaptic interactions within the pyloric circuit. When this is complete, we hope to completely account for the motor patterns produced by these amines based on their effects on the intrinsic excitability and synaptic interactions of the pyloric circuit neurons. DA, 5-HT, and Oct all enhance neuromuscular transmission in peripheral muscles from a variety of different crustacean preparations (Dudel, 1965; Glusman and Kravitz, 1982; Breen and Atwood, 1983; Fischer and Florey, 1983; Dixon and Atwood, 1985; Miller et al., 1985). Similarly, in the stomach muscles innervated by the PD neuron (cpv1 and cpv2), all 3 amines, acting as circulating hormones, enhance the amplitude of excitatory postsynaptic potentials (Lingle, 1981; Govind and Lingle, 1987). Thus, DA and 5-HT have opposing actions at the PD's peripheral and central synapses. These differential effects on central and peripheral synapses of the same cell are consistent with idea that, in Crustacea, amines have generalized hormonal effects in the periphery to enhance muscle activity, while within the nervous system they appear to have more specific actions to modulate coordinated motor patterns (Harris-Warrick and Kravitz, 1984; Harris-Warrick, 1985).

Conclusion

Changes in synaptic efficacy are considered an important mechanism for generating a variety of motor patterns from even "simple," well-defined motor circuits (Harris-Warrick, 1988; Getting, 1989; Harris-Warrick and Johnson, 1989). Given the numerous modulatory substances that are present in input fibers to the STG and affect the pyloric circuit (reviewed in Marder, 1987; Marder and Meyrand, 1989; see also Turrigiano and Selverston, 1989) and other motor circuits of the STG nervous system (Heinzel, 1988; Dickinson and Marder, 1989; Turrigiano and Selverston, 1989), it is probable that other modulators will also affect graded synaptic strength to change the motor pattern produced by this CPG. Considering the importance of graded synaptic transmission in the production of rhythmic motor patterns in different animals and the widespread chemical modulation of motor circuits (Harris-Warrick, 1988), our results may prove to be of general significance in understanding how adaptive variants from a generic CPG program are produced (Harris-Warrick and Johnson, 1989). Modulation of graded chemical synaptic strength may also be important for local circuits in vertebrate nervous systems (Rakic, 1975) where neuronal interactions are mediated through passive spread of current in dendrites (Rall, 1981). Physiological and morphological studies indicate that dendrodendritic synapses which integrate local information are found in the olfactory bulb (Rall and Shepherd, 1968), the retina (Dowling and Werblin, 1969), thalamic nuclei (Ralston, 1971) and the motor cortex (Sloper, 1971). These probably function like the dendrodendritic synapses in the STG (King, 1976) that mediate reciprocal graded synaptic interactions such as occur between the PD and LP neurons. The well-defined, easily accessible motor circuits of the STG in the lobster thus may provide good model systems to examine both motor pattern production and the kinds of circuit interactions important for local neuronal integration in the vertebrate brain.

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