Dopaminergic Modulation of Inhibitory Glutamate Receptors in the Lobster Stomatogastric Ganglion

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Cleland, Thomas A. and Allen I. Selverston. Dopaminergic modulation of inhibitory glutamate receptors in the lobster stomatogastric ganglion. J. Neurophysiol. 78: 3450–3452, 1997. The intrinsic rhythmicity of the spiny lobster stomatogastric ganglion (STG) is strongly influenced by the strengths of the graded synapses between identified cells within the neural network. These synaptic strengths can be powerfully influenced by chemical neuromodulators such as dopamine and serotonin. Most of the intraganglionic chemical synapses in the STG are mediated by postsynaptic inhibitory glutamate receptors (IGluRs). To determine whether or not direct effects on these IGluRs contribute to the modulation of synaptic strength, unidentified STG neurons were extracted into primary culture and the effects of these aminergic neuromodulators on the glutamate-evoked membrane current were assessed. Dopamine (100 μM) reliably and significantly reduced the whole cell slope conductance of all IGluRs tested. Serotonin (20 μM) never affected the IGlu response, although it clearly altered other cellular membrane properties. Although all identified STG neurons may not conform to these observations, the data reveal a specific dopaminergic modulatory pathway within cultured neurons that reduces IGluR slope conductance. The relationship between IGluR modulation and net synaptic modulation in situ contributes to an emerging model in which synaptic strengths can be multiply modulated at different functional sites, yielding a complex, distributed, and state-dependent regulatory structure.

INTRODUCTION

The crustacean stomatogastric ganglion (STG) is a small oscillatory neural network, the intrinsic rhythmicity of which is heavily influenced by graded synaptic transmission among its identified neurons (Hartline and Graubard 1992; Hartline et al. 1988). Biogenic monoamines, among other modulators, are capable of dramatically modifying the rhythmic output of the network via specific effects on identified cells and synapses within the ganglion (Eisen and Marder 1982; Flamm and Harris-Warrick 1986a,b). Aminergic effects on the functional strengths of identified synapses in the intact STG have been described in detail (Johnson and Harris-Warrick 1990; Johnson et al. 1995). In these studies, dopamine and serotonin each increased the strengths of some identified synapses and reduced those of others in replicable patterns. Such changes in synaptic strengths could be mediated by several mechanisms: altered neuritic input resistances, altered voltage-dependence of transmitter release from the presynaptic neuron, and changes in quantal size, as well as postsynaptic receptor modulation.

Most of the chemical synapses in this network are mediated by inhibitory glutamate receptors (IGluRs) (Cleland 1996; Cleland and Selverston 1995; Marder and Paupardin-Tritsch 1978). Dopaminergic effects on cellular responses to iontophoretically applied glutamate in situ, presumably mediated solely by “postsynaptic” input resistance and IGluR modulation, are also diverse and specific to particular identified cells (Johnson and Harris-Warrick 1997). In a sample of isolated, voltage-clamped STG neurons in culture, however, in which membrane currents can be directly measured, dopamine consistently suppressed IGluR currents. To the extent that this IGluR response is representative of postsynaptic glutamate receptors in situ, direct modulation of IGluRs is clearly only one component of a complex suite of pre- and postsynaptic effector mechanisms influencing net synaptic strength. The effects of serotonin on synaptic strengths in the STG, in contrast, may not involve direct IGluR modulation. Some of these data were previously published in abstract form (Cleland and Selverston 1996).

METHODS

Stomatogastric ganglia were isolated from the Pacific spiny lobster, Panulirus interruptus. Media and cultured neurons (3–6 day primary culture) were prepared as described in Cleland and Selverston (1995). Cells were clamped to each test potential for several seconds; agonists were delivered by picospritzer after voltage-dependent currents equilibrated. Fast Green (0.1% by weight), which had no observable physiological effect, was used to visually confirm agonist application. Aminergic modulators were bath-applied at standard concentrations known to powerfully evoke modulatory responses in the intact ganglion (100 μM dopamine, 10 μM serotonin are typical). The preparation was perfused with fresh saline after every agonist application. Two-electrode voltage-clamp recordings were made; both electrodes had R<sub>e</sub> ≈ 8–14 MΩ and were filled with a solution of 0.6 M K<sub>2</sub>SO<sub>4</sub> + 20 mM KCl. Neurons with R<sub>e</sub> ≤ 3 MΩ were discarded; although they could still show robust IGlu responses, their responses to modulators were invariably very weak or absent. Current output was low-pass filtered at 1 kHz. Data were digitized at 2 kHz and analyzed by computer.

Pooled data from multiple experiments were normalized for statistical purposes. Our use of Student’s t-test presumes a normal distribution of current deviations from fitted linear regression functions. Data were also corrected for electrode drift; total drift was measured by electrode offset potential after withdrawal from each cell and its evolution tracked throughout the experiment via changes in membrane potentials under control conditions. The direction of drift was consistent and did not suggest a reversible aminergic modulation of the glutamate reversal potential.

RESULTS

Aminergic modulation of the IGluR

1-L-Glutamate (1 mM) was puffed onto isolated stomatogastric neurons under voltage clamp. Evoked peak IGlu cur-
MODULATION OF IGluRs  

ponent processes both within and among the networked neurons, providing clues to the mechanistic interplay that underlies the power and complexity of neuromodulation.

Dopamine reliably suppressed the IGluR-mediated current in our sample of unidentified cultured neurons. In most identified pyloric neurons in situ, glutamate-evoked potentials are enhanced by dopamine (Johnson and Harris-Warrick 1997), perhaps because the increase in neuritic input resistance overpowers the effects of IGluR current suppression. The use of separate and opposing pathways as a mechanism for bidirectional regulation of functional synaptic strength has precedent in the STG (Johnson et al. 1993). Although this

FIG. 1. Current-voltage (I-V) plot of glutamate-evoked current before dopamine application, in the presence of dopamine, and after dopamine was washed out. Application of 100 μM dopamine reduced slope conductance of inhibitory glutamate receptors (IGluRs) by an average of ~35%. Error bars indicate ±SE. Slope conductance under dopamine modulation was significantly different from that under control and wash conditions at the P < 0.001 level, using a 2-tailed Student’s t-test modified for comparison of regression slopes (Zar 1996). Slope conductances under control and wash were not significantly different (P > 0.05, n = 3). CTRL, control; DA, dopamine. Inset: response of 1 cell to 1 mM glutamate under same 3 conditions depicted in I-V plot. Dopamine (100 μM) reversibly reduced glutamate-evoked membrane currents. Holding potentials are −40 mV (top) and −90 mV (bottom); all inset data are derived from the same neuron.

rents were plotted against holding potential to yield the whole cell IGluR slope conductance. Superfusion of 100 μM dopamine reversibly reduced this glutamate-evoked conductance by an average of ~35% (Fig. 1, n = 3); this effect was highly statistically significant (P < 0.001). In contrast, although 10 μM serotonin reliably modulates glutamatergic synaptic strengths in situ (Johnson et al. 1995), its effect on glutamate responses in situ is less robust (Johnson and Harris-Warrick 1997). In cultured neurons, superfusion of 20 μM serotonin did not reversibly affect IGluR slope conductance in any neuron tested, nor was the mean slope under serotonin distinguishable from control (Fig. 2A; P > 0.05, n = 6). Serotonin application clearly modulated other membrane properties in the same cultured neurons (Fig. 2B), indicating the successful activation of serotonergic modulatory pathways.

DISCUSSION

Comparison of data from isolated neurons and intact ganglia

In cultured neurons, the ability to adequately voltage clamp ligand-gated currents enables these currents to be studied in isolation from other components of functional synaptic modulation (such as neuritic input resistances). This advantage complements those of the in situ preparation, in which the intact synapse can be studied functionally and the preservation of cell identities is more assured. To the extent that currents in cultured neurons are valid representatives of those expressed in situ, these techniques together enable the dissection of modulatory responses into their component processes both within and among the networked neurons, providing clues to the mechanistic interplay that underlies the power and complexity of neuromodulation.

Dopamine reliably suppressed the IGluR-mediated current in our sample of unidentified cultured neurons. In most identified pyloric neurons in situ, glutamate-evoked potentials are enhanced by dopamine (Johnson and Harris-Warrick 1997), perhaps because the increase in neuritic input resistance overpowers the effects of IGluR current suppression. The use of separate and opposing pathways as a mechanism for bidirectional regulation of functional synaptic strength has precedent in the STG (Johnson et al. 1993). Although this

FIG. 2. Effects of serotonin. A: in experiments similar to those depicted in Fig. 1, application of 20 μM serotonin did not affect the slope conductance of the IGluR (P > 0.05). Error bars indicate ±SE (n = 6). CTRL, control; 5HT, serotonin. Inset: response of 1 cell to 1 mM glutamate under same 3 conditions depicted in I-V plot. Serotonin (20 μM) had no effect on glutamate-evoked currents. The different kinetics of glutamate-evoked currents in Figs. 1 and 2A are within normal range of variation for these currents in culture (Cleland and Selverston 1995). Holding potentials are −40 mV (top) and −90 mV (bottom); all inset data are derived from the same neuron. B: influence of 20 μM serotonin on resting I-V relationship in a neuron from the data set depicted in Fig. 2A, demonstrating the capacity of these cultured neurons to respond to serotonin. In some of these neurons, as shown, serotonin evoked an N-shaped resting I-V curve, indicative of an intrinsically bistable state. Data were fit with a 4th-order polynomial.
hypothesis is consistent with observed input resistance changes in situ (Johnson and Harris-Warrick 1997), we cannot rule out the possibilities that receptor-effector coupling may be affected by the extraction of STG neurons into primary culture, or that our samples were somehow biased to exclude neurons exhibiting responses inconsistent with those reported here (e.g., because of identity-specific properties). Although the data reported herein do not sample the diversity of identified cells within the STG adequately to generalize these results to all STG neurons, comparison of these data with those of Johnson and Harris-Warrick (1997) tentatively favors a conservative hypothesis that some cell-specific physiological differences may be mediated by different quantitative combinations of similar effector cascades, rather than by entirely novel intracellular pathways. In any event, direct iGluR modulation by dopamine is a likely contributor to the systemic regulation of glutamatergic synaptic strengths within the STG. A more thorough understanding would best be pursued by identifying individual neurons before culturing.

Implications for systemic regulation, motor control

Within the context provided by Johnson and Harris-Warrick (1997) and Johnson et al. (1995), these data describe one component of what is now clearly a highly distributed architecture for network neuromodulation. One implication of this architecture is that the systemic effects of a neuro-modulator can depend on the prior state of the network, e.g., the net effect of dopamine on a given synapse will depend on the separate modulatory states of each functional synaptic component. It is known that individual modulators can have multiple nonlinear effects, that these effects may be functionally opposing, and that multiple modulators can converge onto the same effector pathways (Brezina et al. 1996; Bygrave and Roberts 1995; Harris-Warrick et al. 1995; Hempel et al. 1996; Johnson et al. 1993, 1994; Offermanns and Schultz 1994). If some of the effector pathways influenced by a newly applied modulator were near the ends of their dynamic response ranges because of the effects of the preexisting modulatory state, then the net effects of the new modulator on the system would be dominated by the remaining effector pathways, altering the net response of the network to that modulator. This model adds further complexity to the regulation of network output: in addition to response specificity based on differential cell identities, modulator concentrations, and recurrent network effects, the preexisting chemical (modulatory) environment and its impact on each effector pathway in the system must be taken into account to reliably predict the response of a synaptic or membrane current to an added modulatory agent. It is becoming apparent that the regulation of output from this neural network is profoundly distributed to an even greater level than previously demonstrated; even the isolated responses of the most reduced elements may be dependent on the global state of the entire system.

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