Proc. Natl. Acad. Sci. USA
Vol. 77, No. 6, pp. 3733–3735, June 1980
Neurobiology

Graded synaptic transmission between spiking neurons

(stomatogastric ganglion/chemotonic inhibition/spikeless inhibition/synaptic input–output/presynaptic inhibition)

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Communicated by Theodore H. Bullock, March 6, 1980

ABSTRACT **Graded synaptic transmission occurs between spiking neurons of the lobster stomatogastric ganglion. In addition to eliciting spike-evoked inhibitory potentials in postsynaptic cells, these neurons also release functionally significant amounts of transmitter below the threshold for action potentials. The spikeless postsynaptic potentials grade in amplitude with presynaptic voltage and can be maintained for long periods. Graded synaptic transmission can be modulated by synaptic input to the presynaptic neuron.**

This study demonstrates that graded synaptic transmission occurs between spiking neurons of the lobster stomatogastric ganglion. These neurons appear to use two modes of synaptic transmission within the ganglion: presynaptic action potentials (spikes) evoke monosynaptic postsynaptic potentials and, in addition, transmitter is released as a continuously graded function of presynaptic voltage. This study describes the soma-to-soma input–output properties of graded synaptic transmission between a group of spiking motoneurons in the stomatogastric ganglion.

It has been known for some time that nonspiking neurons exist (1–13; reviewed in ref. 14) and that such neurons release transmitter as a continuously graded function of presynaptic voltage (5, 12, 13). In previous studies, spiking neurons have been forced to release transmitter as a graded function of presynaptic voltage by manipulating the amplitude of the action potential (15–17) or by blocking action potential activity and voltage clamping the presynaptic terminal (18, 19). Until now, there has only been suggestive evidence for functionally significant graded synaptic transmission by spiking neurons (3, 11, 20–22). We now report that many stomatogastric neurons appear to use both spike-evoked and graded modes of chemical synaptic transmission as a part of normal function, that their input–output curves are similar to those of other neurons, but show a low threshold for transmitter release, and that synaptic inputs can modulate the graded synaptic transmission. [A brief report has been previously published (23); see also Raper (24), who has shown that graded interactions are sufficient to maintain much of the normal pyloric patterned activity when spikes are blocked but the oscillator is functioning.]

The stomatogastric ganglion of the spiny lobster *Panulirus interruptus* consists of the cell bodies and neuropil processes of about 30 neurons, most of which are motoneurons innervating the striated muscle of the gut (25). In addition to their excitatory connections onto muscle fibers, these neurons also synaptically inhibit each other (25, 26). A diagram of the relevant inhibitory synaptic connections is shown in Fig. 1*A*.

Within the stomatogastric ganglion, synaptic connections are made between fine cellular processes in the neuropil; many individual synaptic contacts distributed over several pre- and postsynaptic processes probably contribute to a single functional monosynaptic postsynaptic potential (27, 28), as indicated in Fig. 1*C*. Individual processes are both pre- and postsynaptic (27), suggesting that transmitter released onto a cell might modulate release of transmitter by that cell (29) (see diagram, Fig. 2).

Intracellular recordings were made from cell bodies of identified pre- and postsynaptic neurons (Fig. 1*C*). Current was injected into the cell bodies either through an independent microelectrode or occasionally (Fig. 1*D*; and Fig. 2, PL cell only) through the second barrel of a double-barrelled microelectrode.

Fig. 1*B* shows an example of the spontaneous cyclic activity recorded from the cell bodies of three neurons that participate in the pyloric pattern-generator network. Action potentials are small in the soma [although overshooting in the axon (25)], illustrating the filtering properties of the processes in the neuropil; at the presynaptic terminals the spike is of unknown size, but is sufficient to evoke monosynaptic inhibitory postsynaptic potentials (IPSPs) in the postsynaptic neuron. Slower membrane potential oscillations, presumably closer to full size in the soma than spikes, are typically 15–25 mV in amplitude and are associated with slow IPSPs in the absence of spikes (24).

In an oscillating and spiking preparation (Fig. 1*B*), it is hard to control the membrane potential well enough to study graded release. However, in less active preparations it is sometimes possible to demonstrate graded synaptic transmission below the threshold for action potentials. In Fig. 1*D* two superimposed sweeps are shown; the presynaptic neuron is depolarized by the same amount of current in each, but fires an action potential in only one sweep. The postsynaptic cell responds to the action potential with a monosynaptic IPSP, but most of the postsynaptic hyperpolarization is caused by the graded presynaptic depolarization. Thus, in this example, the graded response is larger than the spike-evoked response.

Both the graded and the spike-evoked responses result from chemically mediated synaptic transmission. Graded and spike-evoked responses have similar reversal potentials, both block in a graded fashion when the extracellular Ca^{2+} concentration is reduced, and the connection shown in Fig. 1*D* (LP → PD) is blocked by addition of 10 μM pictrotoxin to the bathing medium (unpublished data).

In order to study the soma-to-soma input–output properties of graded synaptic transmission, both action potentials and spontaneous membrane potential oscillations were blocked by the addition of 0.1–0.2 μM tetrodotoxin (TTX) to the bathing medium. When TTX was applied, the resting membrane po-

Abbreviations: IPSP, inhibitory postsynaptic potential; TTX, tetrodotoxin.

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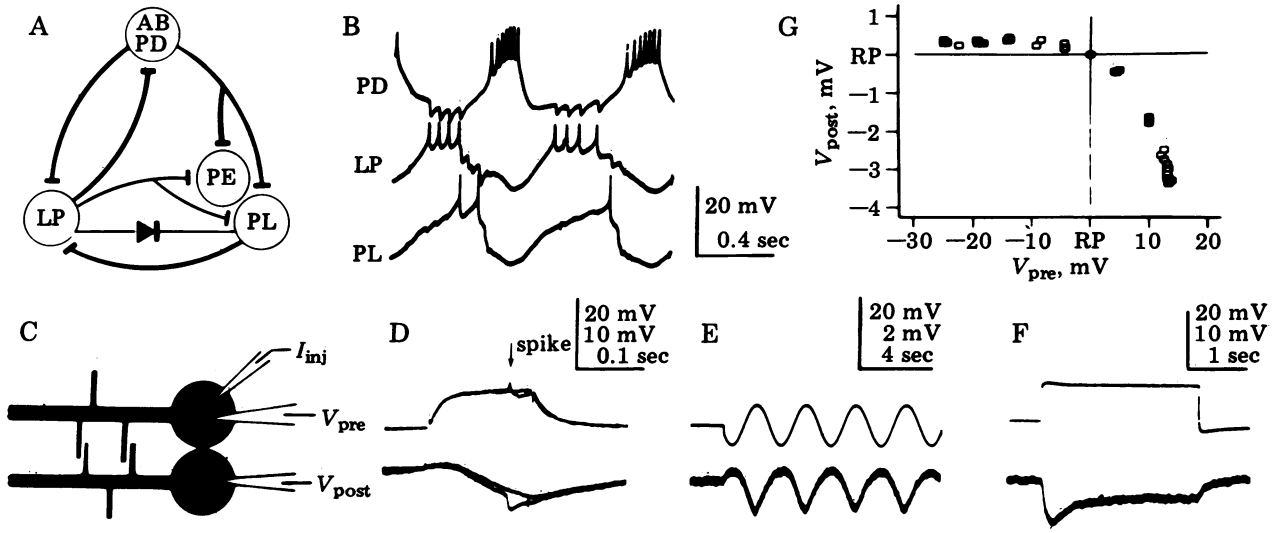

FIG. 1. (*A*) Diagram of the spike-evoked and graded synaptic pathways studied for this report; these connections form a portion of the pyloric synaptic network of the stomatogastric ganglion. All synaptic connections are inhibitory; thin lines indicate weaker connections. The AB and the two PD cells are electrically coupled. PL and PE are groups of three to five cells with similar properties. The LP is a single neuron. ⊣⊢ indicates a rectifying electrical connection. (*B*) Normal oscillatory activity recorded in the cell bodies of neurons representing the three major cell types diagrammed in *A*. Decremented action potentials ride on large membrane potential oscillations. The amplitude and shape of the spike-evoked inhibitory postsynaptic potentials (IPSPs) vary with the cell pair. (*C*) Diagram of stimulation and recording conditions for *D–F*. Electrodes are intrasomatic; chemical synapses are distributed on secondary neurites in the neuropil. (*D*) Comparison of graded and graded plus spike-evoked synaptic transmission. Long current pulses in the presynaptic LP neuron straddle the threshold for causing a spike. Two superimposed sweeps are shown; during one sweep, an action potential occurred in the presynaptic LP neuron. The spike-evoked IPSP is small in the PD cell in comparison to graded release. Such spikeless release is important in the function of many spiking neurons within this ganglion. (*E* and *F*) Records are shown of spiking neurons with spikes blocked with 0.2 μM tetrodotoxin (TTX). (*E*) Sinusoidal current injected into the presynaptic PD neuron mimics its natural oscillation, causing an inverted and rectified oscillatory response in the postsynaptic PL neuron. (*F*) A long presynaptic current pulse (not shown) produces a steplike presynaptic voltage change, as shown in the upper trace. Depolarization of presynaptic PD neuron produces a hyperpolarizing response in the postsynaptic PL neuron. The response has a delay, a peak, and a plateau level of hyperpolarization that lasts for the duration of presynaptic depolarization. Grading the presynaptic voltage causes a grading of both peak and plateau components of postsynaptic response. (*G*) Transneuronal input–output curves derived from experiments like that of *F*. Abcissa: Amplitude of presynaptic soma voltage produced by a current step. Ordinate: Peak response in postsynaptic neuron. Increasing depolarization of the presynaptic PD neuron above the release threshold causes increasing hyperpolarization of the postsynaptic neuron (either PL or PE). In this example, the presynaptic neuron tonically released transmitter at its resting potential in TTX; not all neurons had release thresholds more negative than the TTX resting potential.

tential settled to a value in the lower third of the oscillatory range. The data shown in Fig. 1 *E–G* and in Fig. 2 were obtained with TTX added to the bathing solution.

In Fig. 1*E*, the oscillatory slow waves (see Fig. 1*B*) are mimicked by injecting a sinusoidal current into the presynaptic cell soma, causing a nearly sinusoidal voltage change in the presynaptic neuron. When the presynaptic cell is depolarized, the postsynaptic membrane potential is made more negative; when the presynaptic cell is hyperpolarized, the postsynaptic membrane potential becomes slightly positive with respect to the resting membrane potential in TTX. Thus, this graded chemical interaction acts roughly as an inverting half-wave rectifier.

Most studies of the input–output properties of synaptic transmission use pulses of current or voltage. In Fig. 1*F*, a 2.5-sec depolarizing current pulse is applied to the presynaptic

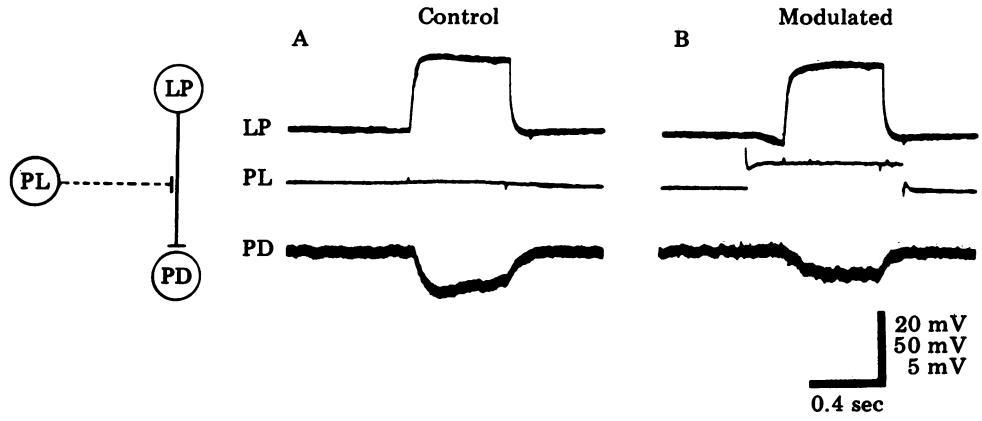

FIG. 2. Synaptic modulation of graded synaptic transmission in the stomatogastric nervous system. (*A*) Step depolarization of the presynaptic LP causes the usual peak-plateau hyperpolarization of the postsynaptic PD. Depolarization of a third cell (PL) will hyperpolarize LP without affecting the PD membrane potential (not shown). (*B*) An LP depolarization of the control amplitude current but in the presence of the PL input produces a much diminished PD response.

cell, producing an approximation of a long voltage pulse. The postsynaptic cell responds after a brief delay with a hyperpolarizing shift to a peak value which then decays to a maintained plateau.

Soma-to-soma input–output properties were examined by the method of Fig. 1*F*; the peak and plateau levels of pre- and postsynaptic voltages were measured during long presynaptic current pulses of varying amplitudes. There was an apparent presynaptic voltage threshold for transmitter release, beyond which both peak and plateau components increased in amplitude with increasing presynaptic depolarizations. An example of the relationship between presynaptic voltage and the peak postsynaptic response is shown in Fig. 1*G*. In this example, the release threshold was 5 mV below the resting potential in TTX; under these conditions, the presynaptic neuron continuously released transmitter at rest. Without TTX in the bath this cell probably released transmitter throughout its entire normal oscillatory range, modulating release as a function of presynaptic voltage.

It is possible to modulate graded synaptic transmission by activating a synaptic input to the presynaptic cell (Fig. 2). [This graded three-cell modulation is comparable to one type of presynaptic inhibition of spike-evoked release (21, 30).] In Fig. 2, the neuronal circuit is diagrammed on the left. Fig. 2*A* shows a normal response to a current step similar to the one seen in Fig. 1*F*. When the presynaptic neuron is itself hyperpolarized by a graded inhibitory input, then the effectiveness of the presynaptic current step is reduced and a smaller response is recorded in the postsynaptic cell (Fig. 2*B*).

We conclude that these stomatogastric neurons can interact by using both graded and spike-evoked synaptic transmissions. Quantitative measurements indicate that many of the cells use both modes of synaptic transmission during normal function; the oscillator will drive graded synaptic transmission, and the spikes atop each depolarizing oscillation will evoke transient postsynaptic potentials. Furthermore, synaptic transmission can be modulated by synaptic input. A previous study (11) showed that injected current can modulate spike-evoked synaptic transmission; we show here that synaptic input can modulate graded synaptic transmission.

The ability of neurons to use both graded and spike-evoked synaptic transmission depends (in part) on the electrical proximity of modulatory inputs to output sites and on the threshold for transmitter release relative to both the normal amplitude of the modulatory inputs and to the spike threshold. Many stomatogastric neurons have both the appropriate geometry and membrane properties to fulfill these criteria. Stomatogastric neurons are structurally typical invertebrate neurons and also resemble those vertebrate local circuit neurons that have both axons and dendro-dendritic synapses. If many of these cells also have favorable membrane properties, then the use of mixed graded and spike-evoked synaptic transmission may prove to be a common and important mode of neuronal computation.

We thank D. Gassie and C. Sirchia for technical assistance. This research was supported by National Institutes of Health Grant NS 13138 (to D.K.H.); K.G. was supported by National Research Service Postdoctoral Award NS 05060.

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