Neuroendocrine (Bag) Cells of *Aplysia*: Spike Blockade and a Mechanism for Potentiation

Abstract. *Bag cell activity in* Aplysia *can be recorded intracellularly and extracellularly. Electrical stimulation anywhere along the connective nerves can produce prepotentials which are not synaptic potentials but represent the passive invasion of action potentials blocked in the neurites. Potentiation of these prepotentials results from progressive movement of the site of spike blockade toward the somata. This type of propagation plasticity may occur in many networks of low conduction safety.*

The bag cells in the abdominal ganglion of the marine mollusk *Aplysia* are two bilaterally symmetrical clusters of neuroendocrine cells. A train of electrical stimuli to either connective of an isolated ganglion could activate the bag cells to fire synchronous, long-duration action potentials for many minutes (*1*, *2*). Kupfermann (*3*) showed that similar stimulation could cause the bag cells to release a hormone (or hormones) capable of inducing egg-laying behavior when it was injected into another *Aplysia* (*4*). Each bag cell cluster sends processes (neurites) into the connective tissue sheath surrounding the rostral abdominal ganglion and the ipsilateral pleurovisceral connective (Fig. 1A) to form a neurohemal relationship at vascular spaces (*5*). Stimulation of a connective near the pleural ganglia could activate the bag cells, even though the bag cell neurites extend less than halfway up the connectives; this result suggested an orthodromic connection onto the bag cells (*2*). After an episode of prolonged firing of the bag cells, stimulation of the connective elicited only "all-or-none" prepotentials which showed temporal summation and potentiation. It was not clear whether these responses were synaptic potentials or remote spikes (*2*). We now use simultaneous intra- and extracellular recording from the bag cells of *Aplysia brasiliana* (*6*) to show that (i) the prepotentials result from passive somatic invasion by spikes in bag cell neurites, an event analogous to spike blockade in some dendrites, and (ii) potentiation of these responses is due to progressive movement of the blockade site closer to the soma.

The compound extracellular spikes from the bag cell neurites were recorded at different sites along the connective between an extracellular stimulating electrode near the pleural ganglion (distal) and the intracellular recording electrode (proximal). When the ipsilateral connective was stimulated distally under conditions that elicited a single intracellular bag cell action potential, the temporal sequence of bag cell spikes indicated that they were initiated in the distal neurites and conducted toward the cell bodies (Fig. 1B). The long duration of the slowly propagated bag cell action potential was distinct from the short latency and duration of other axonal spikes from the connectives. As an extracellular recording electrode was moved along the connective away from the abdominal ganglion, the bag cell response became smaller and eventually disappeared (arrow). This supports the hypothesis (*2*) that activation of the bag cells by stimulation of the connective near the pleural ganglion is not antidromic and further suggests a synaptic connection onto the bag cells.

Intrasomatic prepotentials potentiated during a train of stimuli to the ipsilateral connective (Fig. 1C). The extracellular responses rapidly augmented during the first few stimuli in the train. The intracellular prepotentials gradually increased in amplitude until full action potentials were triggered by the last three stimuli. Therefore, the extracellular electrode on the bag cell neurites recorded full spikes before both the action potentials and most of the prepotentials at the soma. When the connective was stimulated at a low rate that evoked prepotentials of constant amplitude, an intracellular subthreshold depolarization could cause full invasion of the soma (Fig. 1D). Hyperpolarizing current injection could reduce the amplitude of the prepotentials (Fig. 1E). Since the current-voltage relations were linear for the bag cells in this range of hyperpolarization, this reduction in amplitude of the prepotentials was not due to a shunting effect of anomalous rectification on a synaptic potential but rather to spike blockade further from the soma. We conclude that the prepotentials are not chemical synaptic potentials, but reflect a passive invasion of

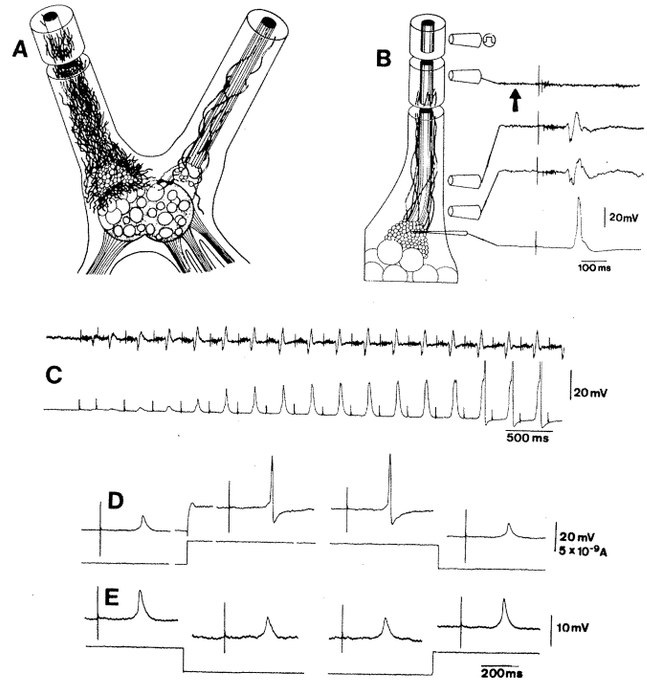

Fig. 1. (A) A diagram (ventral view) of the abdominal ganglion and bag cells of *Aplysia*. The processes of the two bag cell clusters (*1*, *5*) are interconnected and also project over the ganglion and rostrally along the pleurovisceral connectives. (B) A diagram of the upper quadrant of a ganglion shows the position of the stimulating electrode (top), extracellular recording electrodes (middle), and an intrasomatic electrode (bottom). When a stimulus to the connective near the pleural ganglion evoked a long-duration intracellular action potential from a bag cell, the extracellular electrodes recorded rapid, axonal spikes followed by the synchronous bag cell action potential. Conventional axon spikes, but not the bag cell response, could still be recorded when the distal extracellular electrode was moved away (arrow) from the bag cell bodies. Positive is upward for all traces. Stimuli are indicated by the artifacts. (C) A train of stimuli to the connective evoked prepotentials, which progressively potentiated to full soma spikes (last three responses, photographically clipped). Extracellular responses from an electrode on the distal bag cell neurites increased amplitude during the first few responses; but thereafter, constant amplitude extracellular spikes preceded both the somatic prepotentials and action potentials. (D) Subthreshold depolarizing current injection caused the prepotentials to fully invade the soma. Lower trace shows the current monitor. (E) Hyperpolarization (20 mv) resulted in smaller prepotentials by blocking the spike further from the soma.

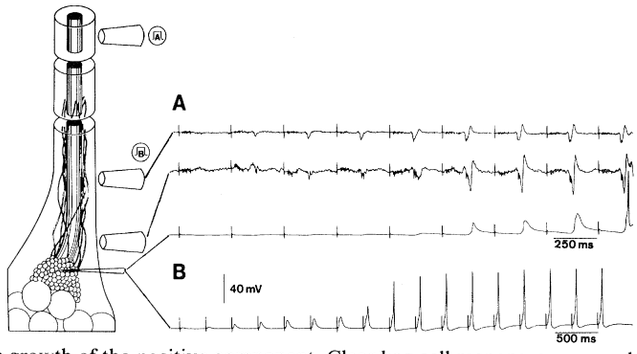

Fig. 2. (A) Intracellular responses (third trace) to a stimulus train from an electrode near the end of the connective (circled stimulus A) rapidly potentiated to a full soma spike (last response). Extracellular recordings (upper two traces) indicated that the initial stages of potentiation were associated with increased spike negativity and then growth of the positive component. Clear bag cell responses appeared first on the extracellular recordings from the bag cell neurites and then from the impaled soma. Full spikes were also recorded from the neurites before the cell bodies. (B) The distal extracellular recording electrode, which recorded extracellular responses (upper trace) in A and could also record bag cell responses further from the ganglion, was used to directly stimulate (circled stimulus B) the bag cell neurites. The intrasomatic bag cell response (lowest trace) still showed a progressive sequence of potentiating prepotentials followed by full action potentials.

the bag cell soma by action potentials that block in bag cell processes.

The most likely explanation for potentiation of the prepotentials is progressive movement of the blockade closer to the soma. At the beginning of the train, the extracellular electrodes recorded a small negativity during the smallest intrasomatic prepotentials (Fig. 1C, and particularly, Fig. 2A). With successive spikes, the negativity increased in amplitude and was later followed by a positivity corresponding to the discharge of bag cell membrane closer to the cell bodies. Potentiation was not caused by temporal summation of the prepotentials (*7*), since they increased amplitude at frequencies too low for response interactions at the soma.

After each response shown in Figs. 1C and 2B, the membrane potential returned to resting baseline level before the onset of the next response; furthermore, stimulation at a frequency one order of magnitude lower (0.3 hertz) could still elicit pronounced potentiation. If this form of potentiation is due to a change in the properties of the bag cell membrane, direct bag cell stimulation should also induce potentiation. Stimulation through an extracellular electrode on the bag cell processes (that is, an electrode that had recorded bag cell spikes and was then moved proximal to the site of bag cell spike initiation) directly evoked typical potentiating responses (Fig. 2B). These experiments suggest (i) that potentiation of the bag cell prepotentials is caused by progressive movement of the locus of spike blockade along the connective toward the bag cell bodies, and (ii) that it is not dependent on input from other cells (*8*). The spike from each successive stimulus within the train propagates beyond the region of blockade for the previous spike, causing a progressively larger electrotonic response at the soma.

The bag cell neurites serve an effector role (neurosecretory) and are functional axons. However, the bag cell neurites around the connectives may be analogous to dendrites (*9*). Neuronal systems with a synaptic input located at a long electrotonic distance from axonal output zones prompted the notion of dendritic spikes. Several lines of evidence indicate that spikes are initiated and blocked in the complex arborizations of some dendrites and, furthermore, that propagation through these neurites can depend on input frequency and modulate neuronal output (*10*). The bag cell processes may be useful as a model for conduction of electrical signals through these kinds of systems. Our experiments directly demonstrate propagation plasticity within a population of neuroendocrine cells. Each blocked spike in the bag cell neurites appears to make the region adjacent to the site of blockade superexcitable (*11*) so that the next spike in a series can propagate further along the neurite. Repetitive action in neural systems with low conduction safety, such as some dendrites, may cause spikes to propagate progressively further through the network and thereby enhance axonal output.

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References and Notes

1. W. T. Frazier, E. R. Kandel, I. Kupfermann, R. Waziri, R. E. Coggeshall, *J. Neurophysiol.* **30**, 1288 (1967).
2. I. Kupfermann and E. R. Kandel, *ibid.* **33**, 865 (1970).
3. I. Kupfermann, *ibid.*, p. 877.
4. ———, *Nature (London)* **216**, 814 (1967); F. Strumwasser, J. W. Jacklet, R. B. Alvarez, *Comp. Biochem. Physiol.* **29**, 197 (1969); L. H. Toevs and R. W. Brackenbury, *ibid.*, p. 207; S. Arch, *J. Gen. Physiol.* **59**, 47 (1972); *ibid.* **60**, 102 (1972).
5. R. E. Coggeshall, *J. Neurophysiol.* **30**, 1263 (1967).
6. F. E. Dudek and J. E. Blankenship, *Soc. Neurosci. Abst. 5th Annual Meeting* (1975), p. 574; comparative relationships between *A. californica* and *A. brasiliana*, plus the basic methods for recording from the preparation have been described (J. E. Blankenship and R. E. Coggeshall, *J. Neurobiol.* in press); F. E. Dudek and J. E. Blankenship, in preparation. The compound extracellular responses of the bag cells were recorded with two suction electrodes next to each other.
7. See figure 6 in (*1*) and figure 6 in (*2*); L. Tauc and G. M. Hughes [*J. Gen. Physiol.* **46**, 533 (1963)] found that spike blockade could facilitate transmission of a subsequent spike in *Aplysia* neurons. Most of the results of Tauc and Hughes are associated with a residual depolarization from the preceding spike; however, their figure 1 shows an example of potentiation that probably did not include electrotonic summation, but they report that "the increase in amplitude is progressive only within narrow limits." Potentiation of the prepotentials from the bag cells is progressive over a wide range of amplitudes.
8. Presynaptic activity could modulate the potentiation. In preliminary experiments a low Ca^{2+} bathing solution could block the bag cell response to stimulation of the connective distal to the terminations of the bag cells. This experiment further supports the hypothesis (*2*) that there is a chemical synapse onto the bag cells, presumably near the site of spike initiation on the connective. The potentiation mechanism apparently does not require the chemical synaptic activation from other cell types, because we have observed potentiation of the prepotentials (like Fig. 2B) in response to direct stimulation of the bag cell processes in the low Ca^{2+} solution.
9. The available evidence indicates that this region of the bag cells is between the putative synaptic input and the soma. The thin, long, and branching processes of the bag cells have ultrastructural characteristics similar to their cell bodies (*1*), which may be a more reasonable basis for assigning them dendritic properties [T. H. Bullock, in *The Neurosciences Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, 1973), p. 343; G. M. Shepherd, *The Synaptic Organization of the Brain* (Oxford Univ. Press, New York, 1974)].
10. R. Lorente de Nó and G. A. Condouris [*Proc. Natl. Acad. Sci. U.S.A.* **45**, 592 (1959)] discussed the potential role in integration of decremental conduction of impulses in dendrites. Early examples of dendritic spikes were found in chromatolyzed spinal motoneurons [J. C. Eccles, B. Libet, R. R. Young, *J. Physiol. (London)* **143**, 11 (1958)], hippocampal neurons [E. R. Kandel and W. A. Spencer, *J. Neurophysiol.* **24**, 272 (1961)], and in Purkinje cells maintained in vitro [W. Hild and I. Tasaki, *ibid.* **25**, 277 (1962)]. Intradendritic action potentials from alligator Purkinje cells have been recorded [R. Llinás and C. Nicholson, *ibid.* **34**, 532 (1971)], and the intrasomatic prepotentials from the bag cells appear similar to these "bumpy" and "notched" intradendritic responses. Dendritic spikes in chromatolyzed motoneurons enhanced synaptic efficacy [M. Kuno and R. Llinás, *J. Physiol. (London)* **210**, 807 (1970)]. Synaptic activation by repetitive stimulation of the perforant path in cats and rabbits enhanced dendritic propagation of spikes and also axonal output in hippocampal pyramidal cells [D. P. Purpura, J. G. McMurtry, C. F. Leonard, A. Malliani, *J. Neurophysiol.* **29**, 954 (1966); P. Andersen and T. Lomo, *Exp. Brain Res.* **2**, 247 (1966)].
11. S. A. Raymond and P. Pangaro [*Q. Progress Report, Research Laboratory of Electronics, M.I.T.* No. 116 (July 1975), pp. 273–281] studied the effects of previous activity on threshold in frog sciatic nerve to generate a model for intermittent conduction which predicts a transient region of superexcitability surrounding the site of spike blockade [see also, E. A. Newman and S. A. Raymond, *ibid.* No. 102 (July 1971), pp. 165–187].

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