Serotonin acts in the synaptic region of sensory neurons in *Aplysia* to enhance transmitter release

M. Hammer², L.J. Cleary¹ and J.H. Byrne¹

¹Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225 (U.S.A.) and ²Institut für Neurobiologie, Freie Universität Berlin, Berlin (F.R.G.)

(Received 3 April 1989; Revised version received 25 May 1989; Accepted 26 May 1989)

Key words: Facilitation; Synaptic release; Sensitization; Serotonin; *Aplysia*; Spike broadening

An important mechanism that contributes to sensitization in *Aplysia* is heterosynaptic facilitation of the synaptic connections between sensory neurons (SNs) and motor neurons (MNs). Heterosynaptic facilitation, in turn, is associated with broadening of the spike in the SN. Spike broadening is readily observed in recordings from somata of SNs, and from growth cones of SNs in culture, but broadening in synaptic terminals has only been inferred. Intracellular recordings were made from somata of SNs and from somata of follower MNs. Additional recordings were made from the axons of SNs as they enter the neuropil in the pedal ganglion. Serotonin (5-HT) broadened action potentials in axons of SNs and enhanced excitatory postsynaptic potentials (EPSPs) in the MNs, even after the axons of SNs were surgically separated from their somata. These results indicate that both heterosynaptic facilitation and spike broadening in the axon are due to the local action of 5-HT and can occur independently of modulation of membrane properties in the soma.

One behavioral modification in *Aplysia* that has been studied in great detail is sensitization, a simple form of non-associative learning. Sensitization appears to be due to the enhanced release of neurotransmitter from sensory neurons (SNs) produced by activation of a network of facilitatory interneurons [18]. Facilitation appears to be due, at least in part, to broadening of spikes in the SNs [2, 14, 16, 17, 21, 22, 31]. Although spike broadening, as a result of either sensitizing stimuli or application of serotonin (5-HT; a transmitter that mimics the effects of sensitizing stimuli), is readily observed in recordings from somata of SNs, and from growth cones of SNs in culture [3a], but broadening in the synaptic terminals has only been inferred. In the pleural ganglion, the somata of SNs that innervate the tail are located 2–3 mm away from their synaptic contacts with motor neurons (MNs) in the pedal ganglion [8]. By recording directly from the axons of SNs near their targets in the pedal ganglion, the kinetics of spikes near synapses can be compared with those far away in the soma.

Correspondence: L. Cleary, Dept. of Neurobiology and Anatomy, University of Texas Medical School at Houston, P.O. Box 20708, Houston, TX 77225, U.S.A
The sensory and motor neurons innervating the tail can be readily identified on the basis of several anatomical and electrophysiological characteristics [30]. The tail SNs are located in a discrete cluster on the ventral surface of the pleural ganglion (Fig. 1). The tail MNs are located along a tract on the dorsomedial surface of the pedal ganglion [30]. Axons of SNs were impaled with an electrode in the neuropil of the pedal ganglion adjacent to the somata of the tail MNs. This region is close to the site of synaptic contacts between tail SNs and MNs as determined histologically [8]. Recordings were also made from the somata of a SN and a follower MN.

Axons that run into the neuropil of the pedal ganglion from the pleural-pedal connective can be readily penetrated with fine-tipped microelectrodes (Fig. 2). Axons from SNs were distinguished from those of other neurons on the basis of several electrophysiological properties characteristic of the SN somata [5, 30]. While these criteria are not absolutely definitive, we are confident that they allowed us to distinguish SN axons since only a small percentage of the impaled axons met these criteria. Moreover, on two occasions we were able to definitively identify SN axons by recording simultaneously from the soma of the same cell in the pleural ganglion (e.g. Fig. 2). In general, the kinetics of axon spikes are quite similar to those of soma spikes, although axon spikes have higher amplitudes and in some cases are slightly broader.

Since the effects of spike broadening are most important at sites of synaptic release, we wanted to establish that the recording sites in the axon were electrotonically close to the terminals. To do this, we took advantage of the fact that the membrane potential of a presynaptic neuron determines the effectiveness of transmitter release (e.g.

![Diagram](image)

Fig. 1. Diagrammatic representation of the experimental preparation. Electrodes were placed in somata of both sensory neurons (SN) in the pleural ganglion and motor neurons (MN) in the pedal ganglion. A separate electrode was used to impale the axon of a SN in the pedal ganglion. In two experiments, simultaneous recordings were made from the axon and soma of the same SN, as shown, but this was not generally the case. To verify the electrophysiological properties of the axon, the posterior pedal nerve (P9) and the pleural-abdominal connective (P1-A) were stimulated. In some experiments, the pleural-pedal connective was cut before the axon was impaled (arrows). Also indicated for orientation is the pleural-cerebral connective (P1-C).
A simple protocol was used to determine the effect of membrane potential on transmitter release. While simultaneously recording EPSPs in the MN, 5 spikes were triggered at intervals of 60 s by passing a brief (1–3 ms) intracellular depolarizing pulse. On alternate cycles, the membrane potential of the axon was hyperpolarized by 10 mV for 5 s, at which time the spike was triggered. A single series of 5 spikes was analyzed as 2 series of 3 spikes. Thus, the effect of hyperpolarization was assessed twice in each series. Because the amplitude of excitatory postsynaptic potentials (EPSPs) decrements when spikes are triggered at regular intervals [6, 7], the effectiveness of hyperpolarization was assessed by comparing it to the amplitude of the following EPSP rather than to the preceding one.

When the current passing electrode was in the axon, the amplitude of the EPSP elicited with the SN at hyperpolarized membrane potentials was reduced to $71 \pm 26\%$ of the first EPSP. The third EPSP recovered to $88 \pm 34\%$ of the first EPSP. The difference between the second and third EPSPs is statistically significant ($t = 2.32, 25 \text{ df}$, $p < 0.05$).

**Fig. 2.** Effects of 5-HT on spike width and EPSP amplitude. Simultaneous recordings were made from the axon and soma of the same SN and from a follower MN. Recordings were made before (solid line) and after (dotted line) addition of 5-HT. The baselines of all traces were aligned. 5-HT increased the spike duration in both the soma and the axon. Spike broadening is better illustrated at the faster time base shown in the inset (arrow). Both the amplitude and the rate of rise of the EPSP were increased after addition of 5-HT. Similar results were obtained in 5 other experiments.

**Fig. 3.** Effects of 5-HT on spike width and EPSP amplitude persist after SN axons have been cut. Simultaneous recordings were made from the axon of a SN and from a follower MN after the pleural-pedal connective had been severed. Recordings were made before (solid line) and after (dotted line) addition of 5-HT. 5-HT increased the duration of the axonal spike and the amplitude of the EPSP. Spike broadening is better illustrated at the faster time base shown in the inset (arrow).
Thus, the recording site is close enough to the synapse to reduce transmitter release to a level below that expected from synaptic depression alone. These results confirm those reported previously [28] in other neurons of *Aplysia*, indicating that hyperpolarization of a presynaptic neuron reduces synaptic transmission.

When the current passing electrode was located in the soma, however, the amplitude of EPSP was unaffected by membrane potential. The amplitude of the EPSP elicited when the soma was hyperpolarized decreased to an average of 92 ± 15% of the first EPSP. The third EPSP, elicited with the soma at the resting membrane potential, decreased further to 88 ± 13% of the first (n = 14). The small reduction in EPSP amplitude was probably due to homosynaptic depression.

The regional effects of 5-HT were examined by recording simultaneously from SN axons and somata. Recordings were usually made from different neurons, but in two cases we were able to record from the same neuron. When recording from different cells, spikes were triggered separately in the somata and axons at intervals of 60 s and offset by 2 s. When recording from the same cell, the spike was triggered at 60 s intervals by firing the soma. After 5 min, small concentrated aliquots (20 μl) of 5-HT (Sigma) were added to the static bath for a final concentration of 50–300 μM. Within 5 min spike broadening was observed. The effects of serotonin on the axon spikes were partially reversed by washing out the 5-HT (n = 4 of 4), but we could not record from the axons long enough to observe complete reversal.

Bath applications of 5-HT enhanced the EPSP in the MN and broadened the spike in both the soma and the axon (Fig. 2). However, the degree of spike broadening is clearly less in the axon than in the soma. Nevertheless, the amount of broadening we observed in the axon is of approximately the same magnitude as that shown in the somata of abdominal SNs [16, 23].

The above experiments demonstrated that spike broadening could be observed in recordings from the axon. Because the somata were electrotonically far, it was unlikely that modulation of membrane properties in the soma contributed to this phenomenon. To test further this hypothesis, we surgically isolated axons from their somata by cutting the pleural-pedal connective (see arrows in Fig. 1).

The protocol analyzing the effects of 5-HT was identical to that described above. Serotonin produced spike broadening in the axon and facilitated the EPSP recorded from the MN (Fig. 3). The spike recorded from a soma in the pleural ganglion proximal to the cut was also broadened (data not shown). The degree of broadening in the axon was not distinguishable from that observed in intact cells. Results similar to those shown in Fig. 3 were obtained in three other experiments. Thus, it appears that 5-HT does have direct effects in the synaptic region of SNs. This indicates that the somata of SNs are not necessary for short-term modulation of spike kinetics and synaptic transmission by 5-HT. Moreover, the axons themselves, which generally function as signal-conducting elements, may also have receptors for 5-HT.

These results do not rule out the possibility that the action of 5-HT in the synaptic terminal itself is more like that in the soma than that in the axon. 5-HT-sensitive channels may be more concentrated in the terminals than the axon, resulting in more
prominent spike broadening in the terminals themselves. Calcium channels which are essential for broadening are also more concentrated in terminals than axons. Alternatively, these results may indicate that the cellular mechanisms active in the soma are less prominent in the terminal and that other mechanisms such as mobilization [13–15, 17] or altered Ca\(^2+\) handling [4] may be critical for the enhancement of synaptic transmission.

Although the broadening of spikes in somata is unlikely to affect synaptic transmission at the sensory-to-motor synapse in the pedal ganglion, it may have significant effects on synaptic transmission between SNs and their followers in the pleural ganglion [9]. In addition, responses in the soma may reflect other functions of modulation. For example, a plexus of serotonergic fibers infiltrates the cluster of pleural SNs, making contacts with their soma [32]. These serotonergic inputs could produce generalized changes in excitability [3, 10, 20]. In addition, serotonergic input to somata could activate mechanisms that are localized to the soma such as those regulating protein synthesis [1, 11, 12] that are likely to contribute to the changes underlying long-term sensitization [10, 24–27].

While our results demonstrate a local effect of 5-HT on heterosynaptic facilitation and show that spike broadening occurs near the release sites, the experiments do not address the extent to which the broadening accounts quantitatively for facilitation of the EPSP. Further experiments are necessary to examine the relative contribution of spike broadening and other processes such as mobilization of neurotransmitter [14, 17] to facilitation at the pleural sensory to pedal motor neuron synapse.