Differential Effects of 4-Aminopyridine, Serotonin, and Phorbol Esters on Facilitation of Sensorimotor Connections in *Aplysia*

SHUZO SUGITA, DOUGLAS A. BAXTER, AND JOHN H. BYRNE

Department of Neurobiology and Anatomy, University of Texas Medical School-Houston, Houston, Texas 77225

Sugita, Shuzo, Douglas A. Baxter, and John H. Byrne. Differential effects of 4-aminopyridine, serotonin, and phorbol esters on facilitation of sensorimotor connections in *Aplysia*. *J. Neurophysiol.* 77: 177–185, 1997. Serotonergic modulation of sensory neurons in *Aplysia* and their synaptic connections with follower cells has been used extensively as a model system with which to study mechanisms underlying neuronal plasticity. Serotonin (5-HT)-induced facilitation of sensorimotor connections is due to at least two processes: a process related to the broadening of presynaptic action potentials and a spike-duration-independent (SDI) process that may involve mobilization of transmitter. We have examined the relationship between spike broadening and synaptic facilitation of relatively nondepressed sensorimotor connections in the intact pleural-pedal ganglia. Previously, 5-HT-induced spike broadening in the sensory neuron was shown to be primarily due to the modulation of a voltage-dependent K⁺ current (I\textsubscript{K, V}). Low concentrations (20–30 µM) of 4-aminopyridine (4-AP) were used to rather selectively block I\textsubscript{K, V}. 4-AP increased spike duration in the sensory neuron and the excitatory postsynaptic potential (EPSP) in the motor neuron. The temporal development of 4-AP-induced spike broadening closely paralleled that of synaptic facilitation. Thus spike broadening via the reduction of I\textsubscript{K, V} can directly contribute to synaptic facilitation. The relationship between spike broadening induced by 5-HT (10 µM) and enhancement of the EPSP was also analyzed. We found that components of 5-HT-induced synaptic facilitation preceded the development of 5-HT-induced spike broadening. The comparison between the results of 4-AP and 5-HT revealed that the SDI processes make an important contribution to the rapid development of 5-HT-induced synaptic facilitation and that spike broadening made an important contribution to its maintenance. The SDI process and a slowly developing component of 5-HT-induced spike broadening are mediated, at least in part, by the activation of protein kinase C (PKC). Application of phorbol 12,13-diacetate (PDAc), an activator of PKC, partially mimicked the effects of 5-HT on spike duration and the EPSP. PDAc-induced enhancement of the EPSP preceded the slower development of PDAc-induced spike broadening. Like 5-HT, PDAc enhanced the EPSP via both spike broadening and the SDI processes. In addition, a 15-min exposure to PDAc occluded 5-HT-induced enhancement of the EPSP, suggesting that PKC and 5-HT engage similar or overlapping mechanisms. On the basis of these results and others, we propose a time-dependent hypothesis for the 5-HT-induced synaptic facilitation of nondepressed synapses, in which multiple second-messenger/protein kinase systems mediate the actions of 5-HT via both spike-duration-dependent and SDI processes.

**Introduction**

Plasticity at the connections between sensory neurons and their follower cells in *Aplysia* has been used extensively as a model system to examine mechanisms of simple forms of learning, such as sensitization (Byrne 1987; Carew and Sah-ley 1986; Hawkins et al. 1993; Kandel and Schwartz 1982). The effects of sensitizing stimuli on sensory neurons are mediated, at least in part, by the transmitter serotonin (5-HT) (e.g., Glanzman et al. 1989; Kandel and Schwartz 1982) and expressed in several forms including spike broadening, enhancement of excitability, and enhancement of transmitter release. Recent work has revealed that the 5-HT-induced increases in spike duration are composed of multiple components expressed in different time domains, which engage different sets of second-messenger/protein kinase systems and modulation of multiple ionic conductances (for summary, see Byrne and Kandel 1996; Sugita et al. 1994a).

A major remaining question in the analysis of plasticity at this synapse is the relationship between spike broadening and transmitter release. Increases in spike duration in the sensory neuron were previously believed to account fully for the 5-HT-induced synaptic facilitation (Kandel and Schwartz 1982; Klein et al. 1980). On the basis of mathematical modeling and computer simulations of the sensorimotor connection, Gingrich and Byrne (1984, 1985, 1987) predicted that spike broadening alone was insufficient to account for presynaptic facilitation. To fully account for facilitation, they hypothesized the presence of mobilization processes, or spike-duration-independent (SDI) processes, that maintained and boosted the pool of releasable transmitter. Experimental evidence supporting this SDI hypothesis was provided by Hochner et al. (1986b) and Pieroni and Byrne (1992), using abdominal sensorimotor connections. In addition, the model of Gingrich and Byrne (1985) suggested that the relative contribution of spike-duration-dependent and SDI processes to synaptic facilitation would vary depending on the state of the synapses (see also Gingrich et al. 1988; Hochner et al. 1986b). For example, sensorimotor connections are known to undergo homosynaptic depression when they are repeatedly stimulated (Kandel and Schwartz 1982; Klein et al. 1980). The SDI processes were predicted to play a key role in the facilitation of depressed synapses, whereas increases in spike duration were predicted to play a dominant role in the facilitation of nondepressed synapses.

Recent evidence, however, suggests that the SDI processes may also be important for the facilitation of nondepressed synapses. For example, Klein (1993) found that adenosine 3',5'-cyclic monophosphate (cAMP), a second messenger activated by 5-HT in the sensory neuron (Bacskaï et al. 1993; Bernier et al. 1982; Occor and Byrne 1985), can facilitate nondepressed sensorimotor connections of cultured...
neurons with no apparent spike broadening in the soma of the sensory neuron. In addition, 5-HT has been shown, under certain conditions, to facilitate nondepressed synapses in the absence of increases in the duration of presynaptic spike (Klein 1994; Stark and Carew 1994). Finally, Dale and Kandel (1990), Eliot et al. (1994), and Ghirardi et al. (1992) found that both 5-HT and/or phorbol esters could increase the rate of spontaneous transmitter release, which was independent of presynaptic action potentials. These results suggest the that SDI processes may play a more significant role in 5-HT-induced facilitation of nondepressed synapses than was previously believed. Thus it is necessary to reevaluate the hypothesis that increases in pre-synaptic spike duration play the primary role in the facilitation of nondepressed synapses. Alternatively, the differences between earlier studies (Hochner et al. 1986a,b; Klein et al. 1980) and recent work (Klein 1994; Stark and Carew 1994) may be simply due to differences in the types of preparations (e.g., intact abdominal ganglion vs. cell culture), the types of synapses (e.g., intact synapse vs. soma-soma synapse), and/or the ages of the animals (adult vs. juvenile). Moreover, temporal factors may also need to be considered to explain different results. For example, the observation that spike broadening played little or no role in 5-HT-induced synaptic facilitation (Klein 1994) may be due to the time at which the synaptic connections were examined (within 2 min after application of 5-HT). Indeed, 5-HT-induced spike broadening develops slowly (Stark et al. 1996; Sugita et al. 1992, 1994a; but see also Goldsmith and Abrams 1992; Hochner and Kandel 1992). Thus the contribution of spike broadening to facilitation may be time dependent, with a small role at early times but with a more important role at later times after the application of 5-HT (see below).

In sensorimotor connections of intact pleural-pedal ganglia, the relationship between spike broadening and facilitation of the synapse has not been examined in detail. In the present study, we used sensorimotor connections in pleural-pedal ganglia and examined how pharmacologically induced broadening of the spike affected the magnitude of the excitatory postsynaptic potential (EPSP). Low concentrations (20–30 μM) of 4-aminoypyridine (4-AP) were used to reduce rather selectively a voltage-dependent potassium current (I_{K,V}). The reduction of I_{K,V} resulted in spike broadening. Previous work suggested that modulation of I_{K,V} plays a key role in 5-HT-induced spike broadening (Baxter and Byrne 1989, 1990a,b; Goldsmith and Abrams 1992; Hochner and Kandel 1992; Sugita et al. 1994a). By simultaneously monitoring the development of spike broadening in the soma and synaptic facilitation following application of 4-AP, we found 1) that 4-AP led to an enhancement of synaptic connections and 2) that the temporal development of 4-AP-induced spike broadening closely paralleled that of synaptic facilitation. Thus spike broadening can directly contribute to facilitation of the synaptic connections in intact pleural-pedal ganglia.

The results of 4-AP were then compared with those of 5-HT and activators of protein kinase C (PKC). PKC is one of the protein kinases activated by 5-HT in the sensory neuron and is known to play a role in somatic spike broadening, enhancement of a nifedipine-sensitive Ca^{2+} current, and, under certain conditions, synaptic facilitation (Braha et al. 1990, 1993; Ghirardi et al. 1992; Goldsmith and Abrams 1992; Hochner and Kandel 1992; Sacktor and Schwartz 1990; Sossin and Schwartz 1992; Sugita et al. 1992, 1994a). These analyses revealed the differential effects of 4-AP, 5-HT, and PKC on synaptic facilitation. Unlike the results obtained with the use of 4-AP, the temporal development of 5-HT-induced synaptic facilitation preceded that of spike broadening, and at early times (1–6 min) after application of 5-HT, facilitation was greater than could be accounted for by spike broadening alone. Synaptic facilitation by PKC was similar although not identical to that by 5-HT. Our results suggest that 1) 5-HT-induced facilitation of synaptic connections has additional components that are different from 4-AP-induced spike broadening and facilitation; 2) SDI processes play important roles in the rapidly developing component of 5-HT-induced facilitation of relatively nondepressed synapses in intact ganglia; 3) spike broadening appears to contribute to the later phases of facilitation, i.e., maintenance; and 4) 5-HT and PKC activate overlapping or common mechanisms for synaptic facilitation. Preliminary results were reported in abstract form (Sugita et al. 1994b).

**METHODS**

**Measurements of sensorimotor connections**

Before dissection, pleural-pedal ganglia (either left or right) were rinsed in artificial seawater and isotonic MgCl₂ (50% vol/vol) containing 0.5% glutaraldehyde (Sigma, St. Louis, MO) for 50 s to reduce 5-HT-induced contractions in the connective tissue sheath (Bryne et al. 1979). In each preparation, a pleural sensory neuron was impaled with a single microelectrode for recording and stimulating. A tail motor neuron in the pedal ganglion was impaled with two microelectrodes, one for recording and one for current injection. Testing, which began 3 min after the sensory neuron was impaled, consisted of eliciting a single action potential in the sensory neuron with a 30-ms suprathreshold depolarizing pulse and recording the monosynaptic EPSP produced in the motor neuron. Connections were tested once every 3 min. This slow rate of stimulation helped to reduce synaptic depression, which develops more rapidly with faster rates of stimulation (e.g., Gingrich and Byrne 1985). Motor neurons were hyperpolarized by ~35 mV during each test stimulus to prevent the EPSP from triggering an action potential. Measurements of input resistance of the motor neuron (while the cell was hyperpolarized) were made by injecting 1 nA hyperpolarizing pulses into the motor neuron. After three baseline trials, 4-AP, 5-HT, or phorbol esters were bath applied. The preparations were maintained at 15 ± 1°C.

**Data analysis**

Action potentials elicited in the sensory neuron and the EPSP recorded in the motor neuron were digitized on-line with a laboratory microcomputer and stored for later display and analysis. In each testing of the connection, 1,500 samples were taken at a sample rate (10 kHz) that was sufficiently fast to retain the details of the original waveform of the action potentials and the EPSPs. The duration of action potentials was measured as the time between the peak of the spike and to the point of the repolarizing phase at which membrane potential was 20% of the peak amplitude of the spike.

In each preparation, data were normalized to the mean of the three baseline measures of spike duration or amplitude of the EPSP
before the application of agents. Two-tailed statistics were used. P values < 0.05 were considered significant.

Chemicals

4-AP (Sigma or Aldrich, Milwaukee, WI) and 5-HT creatine sulfate (Sigma) were dissolved in artificial seawater (ASW) and prepared daily. 4β-phorbol 12,13-diacetate (PDAc) (Sigma) was used to activate PKC. Inactive 4α-phorbols (Sigma) were used as controls. Stock solutions of all phorbols (10 mM) were dissolved in dimethyl sulfoxide and stored at −20°C. The final concentration in the bath was 3 μM for both PDAc and α-phorbol. The final concentration of dimethyl sulfoxide used to dissolve the phorbols did not exceed 0.03% in the bath (vol/vol). The concentrations of phorbol esters used in the present study were the same as those used previously (Sugita et al. 1992, 1994a). Small aliquots (12–18 μl) containing concentrated agents were directly added to the recording chamber (600 μl). The bath was mixed as much as possible during this procedure so as to reduce diffusion times.

RESULTS

Low concentrations of 4-AP induced somatic spike broadening and facilitation of synaptic connections

Previous work indicated that 5-HT-induced somatic spike broadening was mainly due to the modulation of $I_{K,V}$ (Baxter and Byrne 1989, 1990a; Goldsmith and Abrams 1992; Hochner and Kandel 1992; Sugita et al. 1994a). Hodgkin-Huxley type mathematical models and computer simulations of the sensory neurons also supported this hypothesis (Baxter and Byrne 1990b; Belkin et al. 1992; Byrne et al. 1990; Canavier et al. 1991). In the present study, we examined the effects on spike duration and synaptic transmission of a pharmacological agent that rather selectively blocked $I_{K,V}$. Previously, 1 mM of 4-AP was shown to almost completely block $I_{K,V}$ but to have little effect on other membrane currents, including Ca(2+)-dependent $K^+$ current ($I_{K,Ca}$) and serotonin-sensitive $K^+$ current ($I_{K,S}$) (Baxter and Byrne 1989).

[Although 4-AP can inhibit the transient, A-type $K^+$-current, this current is largely inactive at the resting membrane potential of the sensory neuron (Baxter and Byrne 1989, 1990c).] In the present study, low concentrations (20–30 μM) of 4-AP were used to partially block $I_{K,V}$ and to examine the effects of the pharmacologically induced spike broadening on synaptic connections.

We found that these concentrations of 4-AP induced moderate spike broadening and an enhancement of the relatively nondepressed sensorimotor EPSP (Fig. 1A). 4-AP-induced spike broadening developed gradually and reached a peak in ~9 min after application. This broadening was significant when compared with control (n = 8; 4-AP: 205 ± 16% of baseline, control: 102 ± 2%; mean ± SE, independent t-test, $t_{14} = 6.31, P < 0.001$) (Fig. 1B). [The values used for comparison between the experimental (4-AP-treated) group and control group were expressed as percentages of the normalized baseline.] The somewhat slow time course of the development of the effect of 4-AP on action potentials may be explained by the observation that the inhibition of $K^+$ channels by 4-AP, in some cases, requires the opening of the channels (e.g., Arhem and Johansson 1989; Wagoner and Oxford 1990). Thus progressively greater numbers of $K^+$ channels may have been blocked during the repetitive stimulation. The slow effects of 4-AP did not seem to be due to the time of diffusion, because the bath (600 μl) was mixed extensively after the application (see METHODS). In addition, as shown later, some of the effects of 5-HT developed rapidly, within 3 min of bath application. 4-AP-induced facilitation also developed gradually and reached a peak 9 min after application, as did spike broadening (amplitude of the EPSP; 4-AP: 152 ± 18% of baseline, control: 78 ± 9%; $I_{14} = 3.73, P < 0.01$) (Fig. 1B1). Thus the temporal development of 4-AP-induced spike broadening closely paralleled that of facilitation (see below and Fig. 2). In the absence of the application of 4-AP (control experiments),
transmitter release by regulating action potential duration (see also Augustine 1990).

**5-HT-induced facilitation of relatively nondepressed synapses preceded spike broadening in the soma**

The effects of 5-HT (10 μM) on spike duration and synaptic connections were examined. 5-HT-induced facilitation developed rapidly and appeared to reach a peak at 3 min after application. The average amplitude of the EPSP after 3 min in 5-HT was 126 ± 9% (n = 10) of baseline,

**the amplitude of the EPSP gradually decreased (homosynaptic depression), although spike duration remained constant (Fig. 1, B1 and B2) (see also Gingrich and Byrne 1985; Kandel and Schwartz 1982; Klein et al. 1980; Pieroni and Byrne 1992).**

To investigate the time course of 4-AP-induced changes in spike duration and facilitation, the average values of spike duration versus the average amplitudes of the EPSP at each time point (after application of 4-AP) were plotted in Fig. 2. To control for the effects of homosynaptic depression, the amplitudes of the individual EPSPs in 4-AP (and spike durations) were compared with the average values for the control experiments at each time point, rather than with the baseline values. Parallel with the temporal development of spike broadening, an enhancement of the EPSP was observed. As the spike duration continued to increase during the 9 min following application of 4-AP, so the facilitation continued to increase during this time period. Both 4-AP-induced facilitation and spike broadening appeared to saturate after 9 min.

The concentrations of 4-AP that were used did not significantly affect the resting potentials of the sensory neurons (9 min after application; 4-AP: 99 ± 1% of baseline; control: 102 ± 2%; t14 = 1.3) or motor neurons (9 min after application; 4-AP: 98 ± 2% of baseline; control: 98 ± 1%; t14 = 0.1). In addition, 4-AP did not have significant effects on the input resistance of the motor neuron (9 min after application; 4-AP: 104 ± 6% of baseline; control: 101 ± 1%; t14 = 0.47). Thus changes in resting postsynaptic conductances did not contribute to 4-AP-induced synaptic facilitation. In addition, the mean amplitudes of the initial EPSPs for 4-AP and control groups were 6.9 ± 1.4 and 9.0 ± 1.9 mV, respectively. The difference was not statistically significant (t14 = 0.90). Thus pharmacologically induced spike broadening by the reduction of I_{K,V} could facilitate synaptic connections in intact pleural-pedal ganglia. Moreover, these results indicated that I_{K,V} is present in the terminals of sensory neurons and that it plays a critical role in transmitter release by regulating action potential duration (see also Augustine 1990).

**FIG. 2.** Temporal relationship between the magnitude of 4-AP-induced spike broadening and facilitation. The averaged values of spike duration (means ± SE) vs. the averaged amplitudes of the EPSP (means ± SE) at each time point were plotted. Numbers in parentheses: time after application of 4-AP. Letter B in parentheses: normalized baseline value. Arrowhead: direction of the temporal progression (see also Figs. 4 and 6). This relationship provided an estimate of the contribution of spike broadening to increases in the amplitude of the EPSP.

**FIG. 3.** Temporal development of serotonin (5-HT)-induced synaptic facilitation preceded that of spike broadening. 5-HT-induced facilitation developed rapidly, whereas 5-HT-induced spike broadening developed more slowly. A: action potentials elicited in a sensory neuron and the resulting EPSPs recorded in the motor neuron before and 3 min after application of 5-HT (10 μM). Spike broadening was modest at this time point, in contrast to the magnitude of facilitation at the same time. B: time course of the changes in the amplitude of the EPSP (B1) and spike duration (B2). (1 experiment in which 5-HT was used, in which changes in spike duration were not measured accurately because of changes in bridge balance, was excluded in B2.) Arrows: time of application of 5-HT.
groups were $10.9 \pm 1.7$ and $10.6 \pm 2.0$ mV, respectively, and there was no significant difference between the two groups ($t_{17} = 0.09$).

**Activation of PKC had similar effects as 5-HT on the synaptic connections**

The 5-HT-induced SDI processes and a slowly developing component of spike broadening are believed to be mediated, at least in part, via the activation of PKC (e.g., Braha et al. 1990; Ghirardi et al. 1992; Sugita et al. 1992, 1994a). Therefore we examined the effects of PDac ($3 \mu M$), an activator of PKC, on spike duration and facilitation of rela-

**FIG. 4.** Temporal relationship between the magnitude of 5-HT-induced spike broadening and facilitation. The averaged values of spike duration vs. the averaged amplitudes of the EPSP at each time point were plotted. Numbers in parentheses: time after the application of 5-HT. 5-HT rapidly increased the amplitude of the EPSP with little spike broadening and this facilitation reached a plateau after 10% increase in spike duration, despite further broadening of the spike.

whereas the average amplitude of the control EPSP at this time point was $84 \pm 6\%$ ($n = 8$) (Fig. 3, A and B1). This rapid component of 5-HT-induced facilitation was significant ($t_{16} = 3.58, P < 0.01$). In contrast, 5-HT-induced spike broadening developed more slowly (e.g., at 3 min: 108% of baseline; at 9 min: 139%; Fig. 3B2) (see also Sugita et al. 1992). 5-HT-induced spike broadening appeared to reach a peak at 9 min, and this increase in duration was statistically significant when compared with control ($t_{16} = 7.51, P < 0.001$). Thus the peak of the 5-HT-induced facilitation preceded the peak of 5-HT-induced spike broadening by 6 min.

To investigate further the time course of 5-HT-induced changes in spike duration and the EPSP, the averaged value of spike duration versus the averaged amplitude of the EPSP at each time point after application were plotted in Fig. 4. The enhancement of the EPSP developed rapidly with only minor spike broadening. At 3 min, this facilitation was already 40% above control and was associated with only a 10% increase in spike duration. Examination of the data obtained with the use of 4-AP (Fig. 2) suggests that a 10% increase in spike duration should only enhance the EPSP amplitude by 5%. Moreover, despite further development of spike broadening in the presence of 5-HT, the enhancement of the EPSP did not increase further. The discrepancy between the temporal development of 5-HT-induced facilitation and spike broadening was not observed in 4-AP (Fig. 2). These results suggested that 5-HT- and 4-AP-induced facilitation differed and that some factors other than spike broadening, such as the SDI processes, contributed to the rapidly developing phase of 5-HT-induced synaptic facilitation.

5-HT had no significant effect on the input resistance of the motor neuron 3 min after application, at which time a large amount of facilitation was observed (5-HT: $97 \pm 7\%$; control: $101 \pm 4\%, t_{17} = 0.54$). Thus the facilitatory effects of 5-HT on synaptic connections could not be explained readily by changes in resting postsynaptic conductances. The mean amplitudes of the initial EPSPs for 5-HT and control
tively nondepressed synapses. PDAc produced both spike broadening and synaptic facilitation (Fig. 5). Similar to 5-HT, PDAc-induced facilitation developed more rapidly than did spike broadening. PDAc-induced facilitation reached a peak in 6 min (PDAc: 134 ± 15%, n = 8; α-phorbol: 87 ± 4%, n = 7; t15 = 2.75; P < 0.05) (Fig. 5, B1 and B2). In contrast, PDAc-induced spike broadening continued to develop throughout the experiment, and at 15 min after application the average spike duration in PDAc was 133 ± 5%, compared with an average value of 101 ± 3% in α-phorbols (see also Sugita et al. 1992). To investigate the time course of PDAc-induced changes in spike duration and the EPSP, the averaged values of spike duration versus the averaged amplitude of the EPSP at each time point after application were plotted in Fig. 6. Although synaptic facilitation accompanied an increase in spike duration, the relationship between spike broadening and facilitation was not as linear as that observed for 4-AP (see Fig. 2), which suggested that multiple processes, such as the SDI process, contributed to the effects of PDAc.

PDAc produced a slight increase in the input resistance of the motor neuron, but this effect was not significant (6 min after application: PDAc: 114 ± 10%; α-phorbols: 101 ± 2%; t15 = 1.28). Thus the facilitatory effects of PKC could not be explained readily by changes in postsynaptic conductances. The mean amplitudes of the initial EPSPs for PDAc and control groups were 8.4 ± 2.1 and 8.7 ± 2.7 mV, respectively, and there was no significant difference between the two groups (t15 = 0.01).

We also examined the effects of 5-HT when added to the bath already containing PDAc for 15 min. Both 5-HT-induced spike broadening and facilitation were substantially occluded by the preexposure to PDAc (Fig. 5B), particularly at later times. These results support the hypothesis that 5-HT and PKC use overlapping or common mechanisms of spike broadening and facilitation. Alternatively, activation of PKC may inhibit the facilitatory actions of 5-HT, possibly via desensitization of 5-HT receptors (Sugita et al. 1993).

**DISCUSSION**

Previous analyses indicated that 5-HT-induced facilitation of nondepressed synapses was due primarily to spike-duration-dependent mechanisms (Byrne and Kandel 1996; Eliot et al. 1993; Hochner et al. 1986a; Kandel and Schwartz 1982). According to this model, 5-HT-induced facilitation would be expected to be similar to the facilitation induced by some other agent that also produced spike broadening, such as a K⁺ channel blocker (e.g., 4-AP or tetraethylammonium). To examine this possibility, we simultaneously monitored the development of the 5-HT-induced spike broadening in the soma and synaptic facilitation, and then compared the results with those of the experiments with 4-AP. One important assumption of the present study is that 5-HT-induced spike broadening in the soma is comparable with that in the nerve terminal. There is experimental evidence supporting this assumption. Belardetti et al. (1986) and Hammer et al. (1989) attempted to examine spike broadening produced by 5-HT in the synapse, by recording from growth cones of sensory neurons and from intact axons near nerve terminals of cultured sensory neurons, respectively. Both studies concluded that 5-HT-induced spike broadening recorded in the distal site or axon near nerve terminals is comparable with that in the cell body. Thus our recording of spike broadening at the cell body is likely to reflect that in the terminal.

The results of the present study illustrated that facilitation produced by 5-HT and facilitation produced by spike broadening alone (i.e., 4-AP-induced facilitation) differ. For example, after bath application of 5-HT, the temporal development of synaptic facilitation preceded that of spike broadening (Figs. 3B and 4), whereas after bath application of 4-AP, the temporal development of facilitation closely paralleled that of spike broadening (Figs. 1B and 2). In addition, at times ≤6 min after bath application, 5-HT-induced facilitation was far greater than could be accounted for by increases in spike duration alone. These data indicated that 5-HT-induced facilitation was qualitatively different from 4-AP-induced facilitation. Together these data suggest that both spike broadening and the SDI processes contribute to 5-HT-induced facilitation.

The involvement of the SDI processes in synaptic facilitation was first suggested by Gingrich and Byrne (1984, 1985, 1987) on the basis of mathematical modeling and computer simulations of the sensorimotor connection. Experimental support for the SDI hypothesis was obtained by Hochner et al. (1986b) and Pieroni and Byrne (1992) with the use of abdominal sensorimotor connections. Their results also suggested that the SDI processes play a major role for synaptic facilitation when the synapses are depressed (see also below). Our results and Klein (1994) suggest, however, that the SDI processes also play a key role for facilitation of nondepressed sensorimotor synapses, both in intact ganglia and culture.

**Time dependence of mechanisms contributing to 5-HT-induced facilitation**

The relative contribution of spike-duration-dependent and SDI processes to 5-HT-induced facilitation appeared to vary at different times after application of 5-HT. For
example, 3 min after application of 5-HT the EPSP was increased 40% above the control value and the duration of the spike was increased by 10%. The data obtained with the use of 4-AP (Fig. 2) suggest that a 10% increase in spike duration should produce only a 5% increase in EPSP amplitude. Thus the majority of the 5-HT-induced facilitation at 3 min appeared to be related to SDI processes. In contrast, 9 min after application of 5-HT, the EPSP was increased 40% above the control value and the duration of the spike was increased by 40%. The data illustrated in Fig. 2 suggest that a 40% increase in spike duration should increase the EPSP by 25%. Thus the contribution of spike broadening to facilitation appeared to increase at later times, whereas the relative contribution of SDI processes appeared to decrease. Presumably, SDI processes work in concert with spike broadening and the relative contributions of these two sets of mechanisms to facilitate vary with time such that SDI processes make an important contribution to the rapid development of 5-HT-induced facilitation and spike broadening contributes to its maintenance.

Activators of PKC partially mimicked and occluded 5-HT-induced facilitation (Fig. 5). These results suggest that PKC and 5-HT facilitate relatively nondepressed synapses by similar or common mechanisms. There were two differences, however. First, PKC-induced facilitation accompanied more spike broadening than 5-HT. Second, the magnitudes of the PKC-induced SDI processes at 3 min after application were not as large as those induced by 5-HT at similar times. These differences may be explained in part by the fact that activators of PKC need to penetrate the membrane and require more time to induce the facilitatory effects than 5-HT does. In addition, the differential effects of PKC and 5-HT suggested that 5-HT-induced facilitation had components that were not explained fully by the actions of PKC. Possibilities include the likelihood that SDI processes are also mediated via cAMP/protein kinase A (PKA) pathway. Recently it was shown that activation of PKA induced facilitation of the synapse without spike broadening (Klein 1993). In addition, the PKC inhibitors H-7 [1-(5-isouquinolinesulfonyl)-2-methylpiperrazine] and staurosporine did not block a rapidly developing component of the 5-HT-induced facilitation (Ghirardi et al. 1992; Sugita et al. 1992), whereas they appeared to inhibit the facilitation under certain conditions at later times (Sugita et al. 1992). Thus a cAMP/PKA pathway appears to play a role in the 5-HT-induced rapidly developing facilitation.

In the present study, however, prolonged activation of PKC substantially occluded the early phase (as well as the late phase) of facilitatory actions of 5-HT. This result appears to suggest the involvement of PKC in the early phase of 5-HT-induced facilitation as well, and to contradict the hypothesis that the early phase of facilitatory actions is mediated by PKA. The apparently contradictory result (substantial occlusion by PKC of the 5-HT-induced early phase of facilitation) is probably due to the inhibitory actions of PKC on the 5-HT-induced PKA pathway via desensitization of 5-HT receptors. Sugita et al. (1993) showed that prolonged activation of PKC inhibited 5-HT-induced enhancement of excitability and broadening of tetrathyrammonium spikes (well-known cAMP-dependent effects) (Baxter and Byrne 1990a; Hochner et al. 1986b; Kandel and Schwartz 1982). In addition, 5-HT-induced synthesis of cAMP was also partially inhibited by PKC (Sugita et al. 1993). Thus substantial occlusion by PKC of the 5-HT-induced early phase of facilitation does not contradict the hypothesis that PKA plays a major role in the early phase of 5-HT-induced facilitation (Ghirardi et al. 1992; Klein 1993).

State dependence of mechanisms contributing to 5-HT-induced facilitation

The relative contribution of spike-duration-dependent and SDI processes to 5-HT-induced facilitation is also related to the state of the synapses (i.e., depressed vs. nondepressed). Although the results of the present study indicated that SDI processes play an important role in 5-HT-induced facilitation of relatively nondepressed synapses, at early times in particular, several lines of evidence suggest that the facilitation of nondepressed and depressed synapses have some differences. First, different concentrations of 5-HT are necessary to facilitate nondepressed versus depressed synapses (Emp tage et al. 1996). Second, cAMP facilitated nondepressed sensorimotor connections in culture, but failed to facilitate depressed connections (Klein 1993). Third, although PKC activators facilitated both nondepressed and depressed synapses (Brah e et al. 1990; Ghirardi et al. 1992; Sugita et al. 1992), inhibitors of PKC more efficiently inhibited 5-HT-induced facilitation of depressed synapses (Ghirardi et al. 1992). These results suggest that the SDI processes may have two components, one mediated by PKA and acting at one site and another mediated by PKC and acting at another site. For depressed synapses, the PKA-mediated component of SDI is particularly important. One possibility is that the actions of PKC involve mobilization of synaptic vesicles from a nonreleasable (storage) pool to a releasable pool (Gingrich and Byrne 1985; Gingrich et al. 1988). With depression, the releasable pool is depleted and therefore it must first be restored before release can be enhanced. In contrast, the cAMP/PKA cascade may modulate the machinery of transmitter release. Thus, if a sufficient pool of releasable transmitter is available, cAMP can modulate release. If the pool is depleted, this cAMP mechanism would be ineffective. Recently a number of proteins that are associated with mobilization, docking, fusion, and release of transmitter vesicles have been identified (Bennett and Scheller 1993; Südhof and Jahn 1991; Südhof et al. 1993). The phosphorylation of one or more of such proteins by PKA and PKC may play a role in the SDI processes.

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Present address of S. Sugita: Dept. of Molecular Genetics, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235-9046.
Address for reprint requests: J. H. Byrne, Dept. of Neurobiology and Anatomy, University of Texas Medical School-Houston, P.O. Box 20708, Houston, TX 77225.
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REFERENCES


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