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# Reversal of synaptic depression by serotonin at *Aplysia* sensory neuron synapses involves activation of adenylyl cyclase

(cAMP/synaptic facilitation/learning)

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ABSTRACT **Facilitation of the monosynaptic connection between siphon sensory neurons and gill and siphon motor neurons contributes to sensitization and dishabituation of the gill and siphon withdrawal reflex in *Aplysia*. The facilitatory transmitter serotonin (5-HT) initiates two mechanisms that act in parallel to increase transmitter release from siphon sensory neurons. 5-HT acts, at least partly through cAMP, to broaden the presynaptic action potential. 5-HT also initiates a second process that facilitates depressed sensory neuron synapses by a mechanism independent of changes in action potential duration. Recent experiments indicated that either of two protein kinases, cAMP-dependent protein kinase A and protein kinase C, are capable of effectively activating this second facilitatory mechanism, restoring synaptic transmission in depressed synapses. We have used the adenylyl cyclase inhibitor SQ 22,536 [9-(tetrahydro-2-furyl)adenine or THFA] to explore the contribution of cAMP to the reversal of synaptic depression. THFA effectively inhibited both adenylyl cyclase activity *in vitro* and known cyclase-mediated effects in intact sensory neurons. THFA also completely blocked facilitation of depressed synapses by 5-HT. These results suggest that adenylyl cyclase plays a critical role in the reversal of synaptic depression that contributes to dishabituation in this system.**

Since the work of Sharpless and Jasper (1) and Thompson and Spencer (2), neurobiologists, as well as psychologists, have been interested in the relationship between two forms of nonassociative behavioral plasticity: sensitization and dishabituation. The gill and siphon withdrawal reflex of the marine mollusc *Aplysia californica* undergoes both forms of plasticity. In naive animals, a noxious stimulus, such as a shock to the skin, produces sensitization of the defensive withdrawal reflex, enhancing the withdrawal response elicited by a subsequent siphon touch. The same noxious stimulus can also cause dishabituation, an enhancement of the withdrawal response after it has previously been depressed by repeated siphon stimuli. During both sensitization and dishabituation, there is a parallel enhancement of the synaptic connections between the siphon sensory neurons that initiate the reflex and the postsynaptic motoneurons that produce the reflex; this synaptic facilitation contributes to the behavioral change. Nevertheless, recent behavioral and cellular evidence suggests that these two forms of behavioral enhancement depend, at least partly, upon different cellular mechanisms of synaptic plasticity (3–5).

At the synapse between the siphon sensory neuron and the motoneuron, repeated presynaptic activity results in synaptic depression, which contributes to habituation of the withdrawal response. Noxious stimuli result in facilitation of these same synapses by activating modulatory interneurons that release facilitatory transmitters, including serotonin (5-HT) (6, 7). Klein and Kandel (8) identified one facilitatory mechanism that contributes to the increase in transmitter release from sensory neurons. 5-HT causes a decrease in the S-$K^+$ current in the sensory neurons, which slows the repolarization of the presynaptic action potentials, thereby allowing more time for $Ca^{2+}$ influx during each spike (8, 9). Subsequently, Hochner *et al.* (4) examined sensory neuron synapses that were substantially depressed and found that the prolongation of the presynaptic action potential was incapable of increasing transmitter release. Since 5-HT effectively facilitates these depressed synapses, it must activate a second facilitatory process in the sensory neurons that is independent of modulation of action potential shape (4); recent experiments imply that this process is also independent of modulation of $Ca^{2+}$ influx (10). Such a process could involve either mobilization of vesicles that would replenish a depleted releasable pool of vesicles, as proposed by Gingrich and Byrne (3), or restoration of some mechanism in the release process that has become inactivated. This reversal of synaptic depression restores the sensitivity of release to action potential duration that is seen in nondepressed synapses; thus, spike broadening also contributes to facilitation of release from previously depressed synapses (4).

Recently, efforts have been made to determine which second messenger system initiates reversal of synaptic depression. 5-HT stimulates adenylyl cyclase in siphon sensory neurons (11); the increased cAMP-dependent protein phosphorylation is responsible for closure of the S-$K^+$ channels (12, 13). Brunelli *et al.* (14) reported that cAMP injections into sensory neurons facilitate depressed synaptic connections. Photolysis of caged cAMP, which causes cAMP elevations within the range produced by 5-HT, similarly facilitates previously depressed sensory neuron synapses (15). Further evidence that cAMP can reverse synaptic depression comes from the observation that the neuropeptide small cardioactive peptide B ($SCP_B$), which activates cyclase less powerfully than 5-HT (H. E. Jarrard and T.W.A., unpublished results), can facilitate depressed synapses if applied with a phosphodiesterase inhibitor (16). cAMP is not the only modulatory pathway activated by 5-HT in sensory neurons. Sacktor and Schwartz (17) showed that 5-HT caused translocation of protein kinase C (PKC) from cytosol to membranes, a process that normally accompanies activation of the kinase (18). Phorbol esters, activators of PKC, produced facilitation of depressed sensory neuron synapses, suggesting that PKC might be involved in triggering reversal of synaptic depression (15, 19). This facilitation by phorbol esters occurred in the absence of changes in action potential duration and independent of modulation of ionic currents produced by phorbols (10, 15, 20). Taken together, these results suggest

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Abbreviations: 5-HT, serotonin; PDBu, phorbol 12,13-dibutyrate; PKA, protein kinase A; PKC, protein kinase C; THFA, 9-(tetrahydro-2-furyl)adenine; $SCP_B$, small cardioactive peptide B; EPSP, excitatory postsynaptic potential.

that both PKC and cAMP-dependent protein kinase A (PKA) may contribute to this second process of facilitation.

To test whether the cAMP cascade is required for the reversal of synaptic depression produced by facilitatory transmitter, we inhibited adenylyl cyclase activity by using a cell-permeant adenosine analogue, 9-(tetrahydro-2-furyl)adenine (THFA) or SQ 22,536. Our results suggest that cAMP synthesis is necessary for the reversal of synaptic depression produced by 5-HT. Thus, cAMP appears to play a critical role in this second facilitatory process, as well as in the modulation of the S-$K^+$ current.

## METHODS

*A. californica*, weighing 70–200 g, obtained from Marinus (Long Beach, CA), were anesthetized by injection with isotonic $MgCl_2$. Abdominal or pleural ganglia were removed and partially desheathed in a 1:1 (vol/vol) mixture of $MgCl_2$ and artificial sea water. Ganglia were superfused at room temperature with high divalent culture medium [328 mM NaCl/10 mM KCl/66 mM $CaCl_2$/88 mM $MgCl_2$/10 mM Hepes, pH 7.6/7 mM glucose/minimum essential medium (MEM) essential and nonessential amino acids (0.2× normal concentration, GIBCO/Life Technology)/MEM vitamin solution (0.7× normal concentration, GIBCO/Life Technology)] to reduce both spontaneous synaptic activity and recruitment of interneurons when stimulating sensory neurons. Intracellular recordings were made from sensory neurons and postsynaptic neurons, either LFS motoneurons (21) or other similar neurons in the same region of the abdominal ganglion. Data were digitized with a Modular Instruments interface and analyzed using Spike software (Hilal Associates, Englewood Cliffs, NJ). Spike durations were measured from the peak to 30% maximum amplitude.

Inhibitors and transmitters were delivered through a multibarrel microperfusion system (22). 5-HT and $SCP_B$ were in culture medium with 0.1% fast green to monitor delivery. In dose–response studies with THFA, 2-min exposures to 10 μM 5-HT were separated by intervals of 1 hr in which ganglia were incubated with various concentrations of THFA; a newly penetrated sensory neuron was used for each test; such repeated brief exposures to 5-HT produce no desensitization of the spike-broadening response (23).

[$\alpha$-$^{32}$P]ATP (NEG-003) and [$^3$H]cAMP (NET-275) were purchased from NEN. SQ 22,536 (THFA) was donated by Squibb. $SCP_B$ was purchased from Bachem. Wiptide (Peninsula) and PKC-(19–36) (Bachem) were conjugated to fluorescein 5-isothiocyanate (Molecular Probes) to monitor their diffusion within sensory neurons after injection. Phorbol 12,13-dibutyrate (PDBu) (LC Services, Woburn, MA) was dissolved in 100% EtOH and diluted to a final EtOH concentration of <0.01%.

## RESULTS

Although 5-HT effectively facilitates depressed sensory neuron synapses, Hochner *et al.* (4) demonstrated that broadening of the presynaptic action potential, comparable to that produced by 5-HT, was unable to enhance transmitter release if the synapses were first substantially depressed. We similarly found that increasing the action potential duration by 1.5- to 2-fold by using the $K^+$ channel blocker 3,4-diaminopyridine (100 μM) was ineffective in facilitating siphon-sensory-to-motoneuron synapses that had been depressed to ≈30% of their initial amplitude (data not shown; by comparison maximum broadening with 5-HT in siphon sensory neurons is usually <30%). This loss of sensitivity to the duration of the presynaptic action potential with synaptic depression provides a reliable distinction between depressed and nondepressed synapses. Because we wanted to investigate the facilitatory process involved in reversing synaptic depression, these studies were done on synapses that were >60% depressed.

**THFA Effectively Blocks Both Adenylyl Cyclase Activity *in Vitro* and cAMP-Mediated Effects in Intact Sensory Neurons.** To test whether cAMP mediates the reversal of synaptic depression produced by 5-HT, we wanted to inhibit the cAMP cascade in presynaptic terminals. Injection of specific peptide protein kinase inhibitors [Wiptide (24) and PKC-(19–36) (25)] conjugated to fluorescein indicated poor diffusion to neuropilar processes; effects on synaptic facilitation were quite variable. We therefore explored membrane-permeant inhibitors of adenylyl cyclase, which would penetrate into the presynaptic processes of sensory neurons, as well as into their cell bodies. 2′,5′-Dideoxyadenosine (26), an effective inhibitor of *Aplysia* neural cyclase *in vitro*, had variable efficacy in blocking cAMP-dependent spike broadening in sensory neuron cell bodies, indicating poor penetration into these neurons. We therefore tested a second cyclase inhibitor, THFA (27, 28). THFA reduced cyclase activity in homogenized neural membranes, with 5 mM THFA blocking 80% of cyclase activity (Fig. 1 *A* and *C*). (THFA similarly reduced cyclase activity in membranes from pleural sensory neurons pooled from eight animals in a single experiment.) The cyclase activity remaining in THFA could still be stimulated by 5-HT [2.39 (±0.14)-fold stimulation for neural membranes in THFA vs. 3.14 (±0.35)-fold stimulation in control assays (difference not significant; all data are mean ± SEM)]. However, in THFA the cyclase activity stimulated by 5-HT did not reach control basal levels. Thus, if THFA similarly inhibited cyclase in intact cells, 5-HT would be unable to elevate cAMP above basal levels, producing only modest occupation of cAMP binding sites on the regulatory subunit of PKA (30) and minimal kinase activation. THFA did not interfere with PKA activity assayed *in vitro* (31) [e.g., cAMP stimulation of PKA activity was 63 (±3)-fold in 5 mM THFA vs. 62 (±2)-fold in controls].

To test the ability of THFA to inhibit cyclase in intact sensory neurons, we examined the effect of THFA on modulatory actions of 5-HT known to be mediated by cAMP. Spike broadening produced by 5-HT in sensory neurons is due to both a cAMP-dependent decrease in S-$K^+$ current and a cAMP-independent alteration in the kinetics of the delayed $K^+$ current (32). To examine cAMP-dependent spike broadening, action potentials were recorded either in 50 mM tetraethylammonium, which blocks the delayed $K^+$ current, or during continuous 4-Hz stimulation, which inactivates the delayed $K^+$ current. After incubation with 5 mM THFA for 40–60 min, the spike broadening produced by 5-HT was found to be completely blocked in both siphon and pleural sensory neurons (Fig. 1*B*). (Short exposures to THFA gave incomplete blockade, presumably due to inadequate intracellular penetration.) THFA similarly blocked the spike broadening produced by the peptide $SCP_B$, which also activates cyclase in the sensory neurons (23, 33) [mean broadening with 2 μM $SCP_B$ in tetraethylammonium was 7.77 (±3.05)-fold in controls vs. 1.11 (±0.06)-fold in 10 mM THFA; $n$ = 3]. Blockade of spike broadening by THFA was only partially reversible after 60 min of washout. The concentration of THFA at which inhibition of both cyclase activity in neural membranes and spike broadening occurred were comparable (Fig. 1*C*). Thus, preincubation of ganglia with THFA resulted in effective blockade of cAMP-dependent processes.

**THFA Effectively Blocks Facilitation of Depressed Synapses by 5-HT.** In preliminary experiments, we examined the effect of 10 mM THFA on the basic electrophysiological properties of siphon sensory neurons. Incubation in THFA for 1 hr had no detectable effect on the resting potentials or input resistance of sensory neurons. Initial sensory neuron–motoneuron connections were also not significantly affected [mean amplitude = 11.4 ± 2.7 mV for THFA-treated preparations

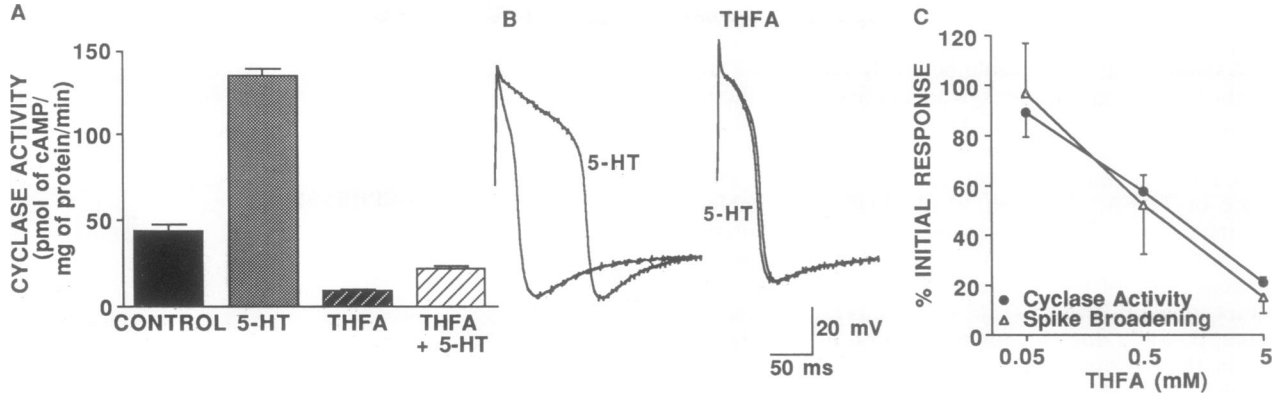


FIG. 1. THFA inhibits adenylate cyclase activity *in vitro* and cAMP-mediated responses *in vivo*. (*A*) THFA inhibits cyclase activity in homogenized *Aplysia* neural membranes. Cyclase activity was assayed for stimulation by 10 $\mu$M 5-HT in the presence of 10 $\mu$M GTP and 2 mM $Mg^{2+}$ (29). (*B*) Effect of THFA on the broadening of action potentials produced by 5-HT. Action potentials were recorded from pleural sensory neurons in 50 mM tetraethylammonium before and during application of 1 $\mu$M 5-HT in normal culture medium (traces on the left) and after a 40-min incubation in 5 mM THFA (traces on the right). (*C*) THFA blockade of spike broadening by 10 $\mu$M 5-HT closely parallels the blockade of cyclase activity in homogenized neural membranes. Spike widths were recorded in pleural sensory neurons during continuous 4-Hz stimulation. Cyclase activity is shown in the absence of 5-HT; cyclase activity in the presence of 5-HT showed similar inhibition at each THFA concentration. In *A* and *C*, data are the mean of three experiments. In each cyclase experiment, cyclase activity was assayed in four replicates. In this and subsequent figures, error bars are SEMs.

($n$ = 10) vs. 13.5 ± 2.8 mV for controls ($n$ = 8)]. The single effect that we did observe after THFA exposure was a broadening of the siphon sensory neuron action potential of ≈75% (mean duration = 3.9 ± 0.5 ms for THFA-treated preparations vs. 2.2 ± 0.1 ms for controls), probably due to a partial blockade of one or more $K^+$ channels. An increase in action potential duration with THFA was also seen in pleural sensory neurons and LFS motoneurons [in addition, LFS motoneurons showed small amounts of depolarization (5–8 mV) after incubation with THFA, perhaps also due to blockade of a $K^+$ channel]. 5-HT effectively facilitates depressed synapses under conditions in which durations of sensory neuron action potentials have been comparably increased by 3,4-diaminopyridine (19) or much more dramatically increased with tetraethylammonium (e.g., to >15 ms) (34). Therefore, the amount of spike broadening produced by THFA should not interfere with facilitation of depressed synapses.

To assess the role of adenylyl cyclase in the facilitation of depressed synapses, we examined the effects of THFA on the facilitatory actions of 5-HT. 5-HT produced significant facilitation of depressed synaptic connections from siphon sensory neurons [mean facilitation = 2.88 (±0.34)-fold increase in excitatory postsynaptic potential (EPSP) amplitude‡; $n$ = 8; $P < 0.002$, two tailed $t$ test]. After incubation with 10 mM THFA, 5-HT was ineffective at reversing synaptic depression [mean facilitation = 1.10 (±0.13)-fold, $n$ = 10]; facilitation in control experiments was significantly greater than in THFA ($P < 0.001$, two tailed $t$ test) (Fig. 2). In these experiments, we compared the effect of 5-HT on depressed synaptic connections from two sensory neurons in a single ganglion with and without THFA. Because THFA effects were not completely reversible, facilitation in the absence of THFA was always tested first. However, in pilot experiments, we found the efficacy of 5-HT to be undiminished when two 5-HT applications were given separated by 1 hr in normal culture medium. In addition, THFA was observed to completely block facilitation of depressed sensory neuron synapses in ganglia not previously exposed to 5-HT ($n$ = 2). These results suggest that the facilitation of depressed synapses produced by 5-HT is mediated by an increase in cAMP levels through activation of adenylyl cyclase.

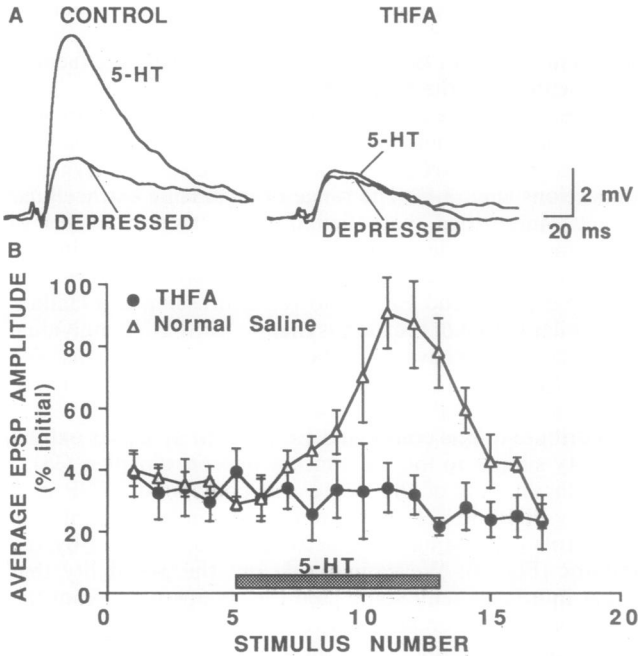


FIG. 2. Effect of THFA on facilitation of depressed sensory neuron synapses by 10 $\mu$M 5-HT. (*A*) Facilitation by 5-HT of a depressed siphon sensory neuron to motoneuron synaptic connection is shown on the left. During the control phase of each experiment, after the EPSP was depressed by repeated sensory neuron stimulation to 28% of initial size, 5-HT was applied for 2 min. Facilitation is blocked by THFA as shown on the right. After the control phase of the experiment, the ganglion was exposed to 10 mM THFA for 1 hr, a second sensory neuron was penetrated, the EPSP was depressed to 39% initial size, and 5-HT was applied for 2 min. (*B*) Comparison of average effects of 5-HT on depressed synapses in the presence and absence of THFA. In each experiment using the protocol in *A*, a sensory neuron synapse was depressed in normal culture medium by stimulating the sensory neuron at 15-s intervals. After the EPSP had declined to <40% of initial size, 5-HT was applied for 2 min. Data were normalized by dividing each EPSP amplitude by the amplitude of the first EPSP elicited. The results shown begin after the initial period of rapid synaptic depression; thus, the first point represents a depressed EPSP.

‡Facilitation was calculated by dividing the mean amplitude of the three consecutive largest EPSPs during a 2-min 5-HT exposure by the mean amplitude of the three depressed EPSPs immediately before 5-HT application.

**THFA Does Not Interfere with PKC-Mediated Synaptic Facilitation.** The blockade by THFA of facilitation of depressed synapses by 5-HT could be due to effects of THFA on another second messenger system involved in facilitation or on the release process itself. To test for possible interference with actions of PKC or with facilitation in general, we examined the facilitatory effects of phorbol esters in the presence of THFA. Although 10 nM PDBu is capable of facilitating synapses between sensory neurons and motoneurons in single-cell coculture (15), we found that substantially higher concentrations were required to produce facilitation in the intact ganglion. Even 500 nM PDBu was not consistently effective, possibly due to poor penetration to synaptic terminals in the neuropil. To ensure we studied some more superficial sensory neuron terminals, which would be more accessible to the phorbols, we activated a population of sensory neurons by stimulating the siphon nerve. Consistent with the conclusion that penetration of phorbols to the synaptic terminals was poor, this procedure resulted in reliable facilitation of the depressed compound EPSP after 30–40 min of PDBu exposure. With this protocol, PDBu actually produced slightly more facilitation than 5-HT; if the PDBu reached only a subset of sensory neuron terminals, this would suggest that phorbols act more effectively than 5-HT in reversing synaptic depression. In principle, the increase in compound EPSP amplitude with PDBu could be explained by a reduction in the threshold of axons causing a larger population of sensory neurons to be activated with each stimulus. However, PDBu exposure did not result in a consistent change in threshold that could account for the large facilitation produced by the phorbols.

In the high divalent culture medium used in these experiments, the compound EPSP recorded in motoneurons appeared to be primarily or exclusively due to monosynaptic connections (Fig. 3*A*). The range of increasing extracellular stimulus intensities that elicited short latency EPSPs of increasing amplitude overlapped with the range of stimulus intensities that activated individual LE siphon sensory neurons. The compound EPSP showed depression and facilitation similar to that of the monosynaptic EPSP from individual LE neurons. Moreover, the facilitation by 5-HT was also blocked by THFA (Fig. 3*B*). Although other groups of siphon sensory neurons with axons in the siphon nerve are also likely to contribute to this compound EPSP, their synapses exhibit plasticity similar to the LE sensory neuron synapses (35).

The facilitation of the depressed compound EPSP produced by PDBu in THFA was not significantly different than in controls suggesting PKC activity is not affected by the inhibitor (Fig. 3). We cannot rule out the possibility that THFA interferes with some step that is upstream from the activation of PKC in addition to inhibiting cyclase. We did not explore effects of THFA on such upstream steps in the PKC pathway since it has not been possible to demonstrate a mechanism for PKC activation in response to 5-HT.§

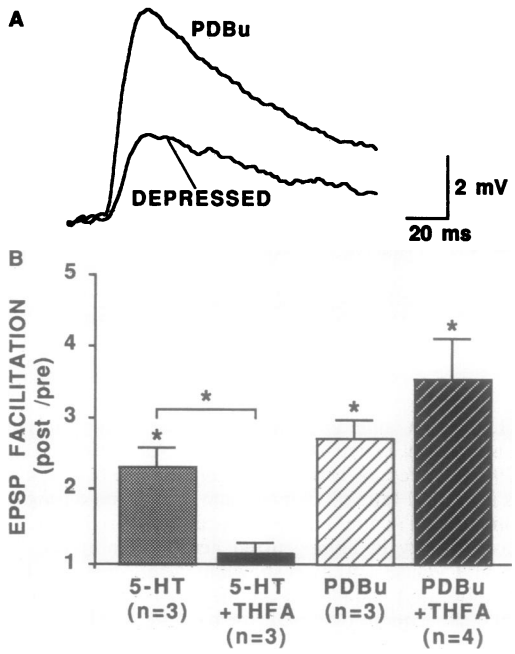


FIG. 3. Phorbol esters in the presence of THFA facilitate depressed compound EPSPs elicited by siphon nerve stimulation. (*A*) After a 60-min preexposure to 10 mM THFA, 500 nM PDBu enhanced compound EPSPs recorded in a motoneuron. Stimulation of the siphon nerve at 40-s intervals was used to activate multiple sensory neurons. (*B*) Effects of THFA on the reversal of synaptic depression produced by 5-HT and PDBu. Compound EPSPs were elicited as in *A*. Facilitation was calculated by averaging the amplitude of three consecutive EPSPs as with the monosynaptic EPSP.‡ Because phorbol esters exerted their facilitatory effect over many minutes, EPSPs were monitored for up to 45 min after the onset of phorbol application and facilitation was measured when EPSP amplitude reached a peak. During 5-HT experiments, facilitation was measured during the 2-min exposure. Despite the presence of THFA, PDBu produced significant facilitation (*) of the complex EPSP ($P < 0.02$, two-tailed $t$ test) comparable to the facilitation in control experiments. In contrast, 5-HT produced significant facilitation of the depressed compound EPSP in normal culture medium ($P < 0.05$, two-tailed $t$ test) but not in THFA. Moreover, the facilitation produced by 5-HT in control experiments was significantly greater (*) than in THFA ($P < 0.02$, two-tailed $t$ test). Control experiments and experiments in THFA were conducted on separate ganglia.

## DISCUSSION

Recently, several lines of evidence have suggested that PKA and PKC may both be involved in the restoration of synaptic transmission from depressed sensory neuron synapses (15, 19). We have used an adenylyl cyclase inhibitor, THFA, to explore the contribution of the cAMP cascade to the reversal of synaptic depression produced by the facilitatory transmitter 5-HT. At twice the concentration that inhibited 80% of cyclase enzyme activity *in vitro*, THFA eliminated virtually all facilitation of depressed sensory neuron synapses by 5-HT.

Several experiments suggest that THFA may be exerting its effects on synaptic facilitation by inhibiting cyclase rather than by acting at other loci: (*i*) Although cyclase activity was greatly reduced by THFA, the fold stimulation of the remaining cyclase activity by 5-HT was not significantly reduced by THFA, suggesting that THFA did not interfere with the 5-HT receptor or the stimulatory guanine nucleotide-binding protein that couples the receptor to the cyclase; thus receptor–enzyme coupling was not substantially affected. (*ii*) The activity of PKC, as measured by the ability of phorbol esters to facilitate depressed synapses, was unaffected by THFA. This same result demonstrates that facilitation of depressed sensory neuron synapses by cyclase-independent mechanisms is possible in the presence of THFA. (*iii*) Consistent with the conclusion that cAMP-dependent phosphorylation is required for reversal of synaptic depression produced by 5-HT, we found in preliminary experiments that when two cell-permeant PKA antagonists, ($R_P$)-adenosine cyclic 3′,5′-phosphorothioate and KT-5720, blocked 5-HT effects on

§Phospholipase C in sensory neurons has not been shown to be activated by 5-HT, since biochemical studies failed to detect liberation of diacylglycerol or inositol phosphates (J. David Sweatt, personal communication). It should be noted that an absence of detectable diacylglycerol or inositol phosphate release might reflect the limited sensitivity of these assays in *Aplysia*; however, fura-2 measurements on intact sensory neurons have failed to detect a substantial increase in intracellular $Ca^{2+}$ (36), such as would be triggered by inositol trisphosphate release. It is possible that 5-HT activates PKC via some other pathway.

sensory neuron excitability, they also blocked facilitation of depressed synapses. In summary, these results suggest that reversal of synaptic depression produced by 5-HT requires activation of adenylyl cyclase and, presumably, phosphorylation by PKA. This conclusion is consistent with the results of Belardetti *et al.* (37) who studied facilitation of a complex synaptic response to siphon nerve stimulation in the motoneuron L7.

If PKC, as well as PKA, participates in reversing synaptic depression, what is the relationship between the two kinases in this process? Our observation that an inhibitor of the cAMP cascade blocks reversal of synaptic depression by 5-HT argues that PKC is not acting in parallel with, and independent of, PKA. The finding that either phorbol ester or cAMP can, alone, initiate reversal of synaptic depression (15) argues against the possibility that parallel activation of both second messenger systems is required to produce facilitation of depressed synapses. This leaves another alternative, that the two kinases are activated in series. Since 5-HT stimulates cyclase directly in homogenized sensory neuron membranes (38) and since phorbol esters do not affect cyclase activity in *Aplysia* neural membranes (T.W.A. and K. A. Karl, unpublished results), we propose that PKA activity may lead to activation of PKC, which in turn could act to reverse synaptic depression.

Alternatively PKC may not contribute to the reversal of synaptic depression produced by 5-HT. Sweatt and Kandel (39) found that 5-HT and cAMP stimulated phosphorylation of an identical array of proteins in the sensory neurons; in contrast, phorbol ester treatment stimulated phosphorylation of a different array of proteins, only two of which overlapped with the 5-HT and cAMP substrates. Although this could indicate that PKC is not activated by 5-HT, it has been found that in sensory neurons, 5-HT translocates one or a limited subset of multiple isoforms of PKC from cytosol to membranes (17, 40); this suggests activation of one or a few PKC isoforms (18). While phorbol esters are capable of activating all isoforms of PKC [since they substitute for diacylglycerol (41)], 5-HT would phosphorylate proteins that include substrates of only a subset of PKC isoforms. Thus, it is unclear whether the species of PKC activated by 5-HT is the same type that enhances transmitter release in *Aplysia* sensory neurons in response to phorbol treatment.

In *Aplysia* mechanosensory neurons, a single second messenger, cAMP, initiates multiple changes that contribute in parallel to enhancement of sensory input to the central nervous system. cAMP-dependent closure of S-$K^+$ channels increases excitability in sensory neurons and broadens the action potential, thereby increasing transmitter release during each spike. The present results indicate that 5-HT acts through cAMP to initiate a restoration of the release process in sensory neurons that have had their synapses depressed by repeated activation. In addition, repeated elevations of cAMP can initiate more persistent changes in sensory neurons that facilitate their synaptic connections over the long term (42, 43).

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