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Serotonin and Cyclic Adenosine 3′:5′-Monophosphate Modulate the Potassium Current in Tail Sensory Neurons in the Pleural Ganglion of *Aplysia*[1]

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Abstract

Tail sensory neurons in the pleural ganglion that mediate the afferent portion of the tail withdrawal reflex in *Aplysia californica* undergo heterosynaptic facilitation of transmitter release during sensitization. As in the siphon sensory neurons, the transmitter serotonin produces facilitation and also elicits a slow, decreased conductance excitatory postsynaptic potential (EPSP) in these neurons. Using voltage clamp and biochemical analyses, we have found that the slow EPSP in the pleural sensory neurons is due to a decrease in a potassium conductance identical to the S potassium current characterized in siphon sensory neurons. Like the S current, the current modulated by serotonin in the pleural sensory neurons is a non-inactivating potassium current, and it contributes to both the resting and action potentials. The current reverses in 120 mM external K^+ at −20 mV, close to the predicted Nernst equilibrium potential. Intracellular cesium blocks the serotonin response, but the current is not blocked by equimolar substitution of barium for calcium, nor by 50 mM tetraethylammonium chloride. The effect of serotonin is cAMP dependent, since serotonin elevates cAMP and both cAMP injection and forskolin mimic the serotonin response.

These results indicate that the mechanism associated with sensitization of the siphon-gill withdrawal reflex, a slow decreased potassium conductance, is also a component of the neuronal circuitry underlying modulation of another reflex, the tail withdrawal reflex. Therefore, two distinct populations of neurons subserving similar behavioral functions have related biophysical and biochemical properties.

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In sensory neurons of the abdominal ganglion of *Aplysia californica*, which mediate the siphon-gill withdrawal reflex, serotonin elicits a slow, decreased conductance postsynaptic potential (slow PSP) by decreasing a specific potassium current (Klein et al., 1982; Siegelbaum et al., 1982). This potassium current, called the S current, is distinct from the fast potassium current (Connor and Stevens, 1971; Neher, 1971), the calcium-activated potassium current (Meech and Standen, 1975), and the delayed potassium current (Hodgkin and Huxley, 1952; Thompson, 1977). The current is non-inactivating, and it participates in the resting and action potentials of the sensory neurons. Modulation of the S current by serotonin via a cAMP-dependent phosphorylation cascade (Brunelli et al., 1976; Klein and Kandel, 1978; Castellucci et al., 1980, 1982; Bernier et al., 1982) has profound effects upon the electrophysiological behavior of these cells. One of these effects is that repolarization of the action potential is delayed and the duration of calcium influx is prolonged. Since transmitter release depends on intracellular calcium (Llinas and Heuser, 1977), more transmitter is released by a sensory neuron exposed to serotonin. This is postulated to be the mechanism for presynaptic facilitation of transmitter release (Klein and Kandel, 1978). Presynaptic facilitation in turn is thought to be the cellular basis of sensitization, a simple form of learning, in the siphon-gill withdrawal reflex (Castellucci and Kandel, 1976).

To establish the slow PSP as a general mechanism for short-term sensitization, the mechanism must be shown to operate in other reflexes that manifest sensitization. Recently, Walters et al. (1983a, b) found that the tail withdrawal reflex is enhanced by nonspecific aversive stimuli. Sensitization of this reflex is mediated by presynaptic facilitation of sensory neurons in the ventral caudal cluster of the two pleural ganglia that innervate the tail. Furthermore, serotonin produces identical effects in both the sensory neurons of the left pleural ganglion and the sensory neurons of the abdominal ganglion: it increases the size of the postsynaptic potential of sensory neuron follower cells, it broadens the width of the action potential in the presence of 4-amino-pyridine and tetraethylammonium chloride, and, in the isolated cell body, it produces a depolarization and an increase in input resistance.

These observations raised several questions. What membrane currents are involved in presynaptic facilitation in this system? Does serotonin modulate the same current in different groups of cells showing similar synaptic and behavioral changes? Is the same ion channel present in these cells? Is channel modulation accomplished in the same way? To answer these questions, we used the same techniques to analyze the serotonin effect on the pleural sensory cells as were used in the abdominal sensory neurons. We report here that serotonin modulates a potassium current in the pleural cells with properties identical to those of the S current and that this modulation occurs by means of the intracellular messenger, cAMP.

A preliminary report of these results has been made (Pollock et al., 1982).

Materials and Methods

Aplysia californica (obtained from Pacific Biomarine, Sea Life Supply, or raised from eggs at the Marine Biological Laboratory, Woods Hole, MA) between 100 and 200 gm in weight were used. The animals were kept in tanks containing Instant Ocean (Instant Ocean, Aquarium System, Mentor, OH) at 15°C on a 12-hr day/12-hr night cycle.

Electrophysiology. The animals were anesthetized with isotonic $MgCl_2$ in a volume in milliliters equal to one-half the body weight injected into the body cavity. The left pleural-pedal ganglia were removed and pinned out in a 5-ml chamber, made of Silgard (Dow Corning, Midland, MI). The experiments were done at room temperature (22 to 25°C).

The sensory neurons of the ventral caudal cluster of the pleural ganglion were impaled with 3 M KCl electrodes with resistances of 10 to 20 megohms. In the cesium experiments 3 M CsCl electrodes were used. These neurons, located along the lateral edge of the ganglion contiguous with the pleural-pedal connective, were identified by their position in the ganglion and by their typical electrophysiological characteristics of being silent, accommodating to sustained depolarization, and having a resting potential between −40 and −50 mV. A cell was accepted after impalement if the input resistance was greater than 10 megohms, the resting potential was at least −40 mV, and the cell fired an action potential to less than 2 nA of depolarizing current. In some experiments the cell bodies were isolated to improve the space-clamp conditions by tying the axon off with a strand of dental floss.

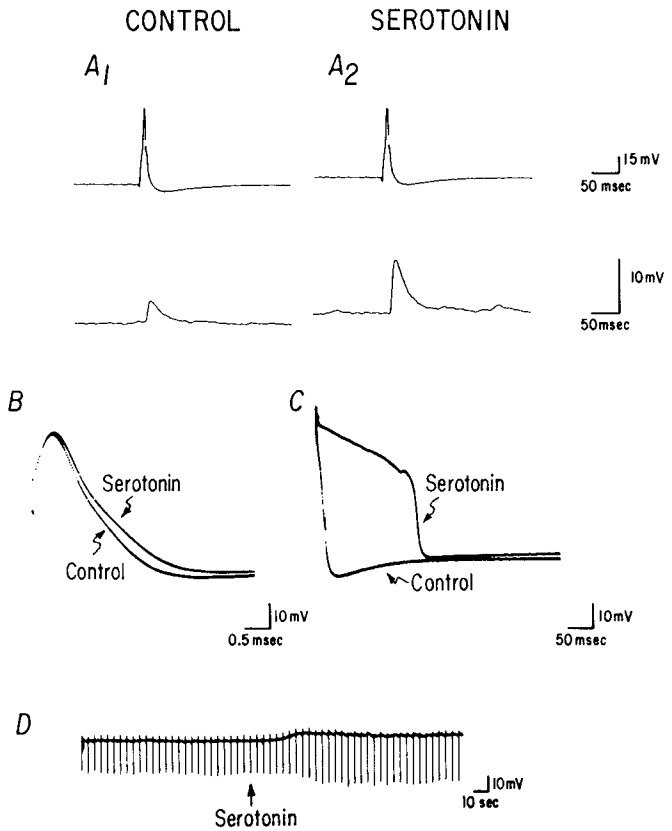

Figure 1. Serotonin enhances transmitter release and produces a slow EPSP. *A*, A pleural sensory and tail motor neuron were each impaled with a 3 M KCl electrode. The motor neuron was hyperpolarized to −60 mV below the resting potential of −40 mV. PSPs in the tail motor neuron were elicited by firing an action potential in the sensory neuron with a constant current depolarizing pulse once every 10 sec. A_1, A PSP elicited by an action potential in normal seawater. A_2, Enhancement of the PSP by serotonin. *B*, The duration of the action potential in the same neuron before and after serotonin ($1 \times 10\ M^{-4}$) in seawater. *C*, The effect of 1×10^{-4} M serotonin on action potential duration in seawater with 50 mM TEA. *D*, The effect of 1×10^{-4} M serotonin on input resistance and membrane potential measured with a constant current hyperpolarizing pulse in 50 μM TTX. The increase in resistance is not secondary to depolarization.

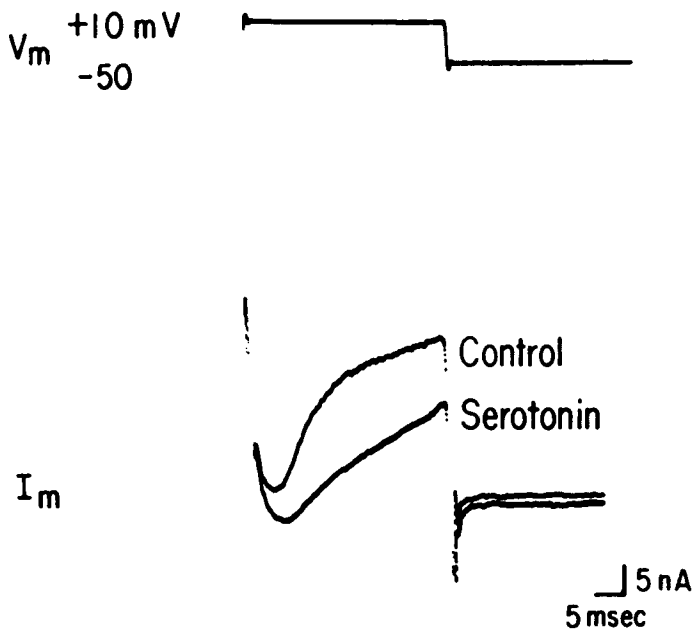

Figure 2. Serotonin decreases the net outward current in voltage-clamped left pleural sensory neurons in TTX. The cell was held at −50 mV and stepped to +10 mV for 250 msec (*top trace* shows voltage step) (1×10^{-4} M). The control and serotonin current records are superimposed. Note that the outward current is decreased as early as can be detected and remains decreased throughout the voltage step.

Voltage clamp experiments were done using a Dagan 8500 intracellular preamp clamp and standard shielding technique.

The ionic composition for normal seawater used in most experiments was 460 mM NaCl, 10 mM KCl, 11 mM $CaCl_2$, 55 mM $MgCl_2$, 2.5 mM $NaHCO_3$, and 10 mM Tris-HCl buffer (pH 7.6). In barium-substituted seawater 11 mM $CaCl_2$ was replaced by 11 mM $BaCl_2$. When the KCl concentration was increased, or tetraethylammonium chloride (TEA; Eastman Kodak, Rochester, NY) was added, an equivalent osmolarity of NaCl was removed. In one experiment chloride was replaced by using acetate salts in the same concentration as in normal seawater, and in another, NaCl was replaced with Tris-Cl, pH 7.6.

The other drugs used in this study were tetrodotoxin (TTX; Sigma Chemical Co., St. Louis, MO, and Calbiochem, San Diego, CA), serotonin creatinine sulfate (Sigma), adenosine 3′:5′-monophosphate (Sigma), and forskolin (Calbiochem, La Jolla, CA). Serotonin was dissolved in normal seawater or distilled water and applied to the bath so that the final concentration was 1×10^{-4} M. Forskolin was also applied to the bath to a final concentration of 1×10^{-4} M. cAMP was injected intracellularly by adjusting positive braking current to prevent leakage into the cell; injection was controlled by releasing and reapplying braking current.

Biochemistry. The method used to measure cAMP has been described by Bernier et al. (1982). Five *Aplysia* weighing between 80 and 150 gm were used. The contralateral pleural sensory neuron cluster served as a control. Before the addition of serotonin (2×10^{-4} M) to the right or left pleural sensory neuron cluster, the ganglia were incubated in $^{32}P_i$ for 3 hr and then washed. The ganglia were then kept in artificial seawater for an additional 23 hr so that the α-phosphate of ATP became maximally labeled. After incubating the ganglion in serotonin or vehicle for 5 min, the cells were frozen, dissected, and assayed for cAMP. cAMP was determined by thin layer chromatography on polyethylene amine sheets. The portion of the chromatogram containing the cAMP was scraped and extracted. A liquid scintillation counter was used to count the radioactivity in the supernatant.

Results

Application of serotonin mimics heterosynaptic facilitation. The first series of experiments replicates the findings of Walters et al. (1983b). As seen in Figure 1*A*, serotonin increased the size of the PSP in a tail motor neuron from 1 mV to 8.5 mV. This was accompanied by a 16% increase in action potential duration at half-peak from 2.1 msec to 2.4 msec (Fig. 1*B*).

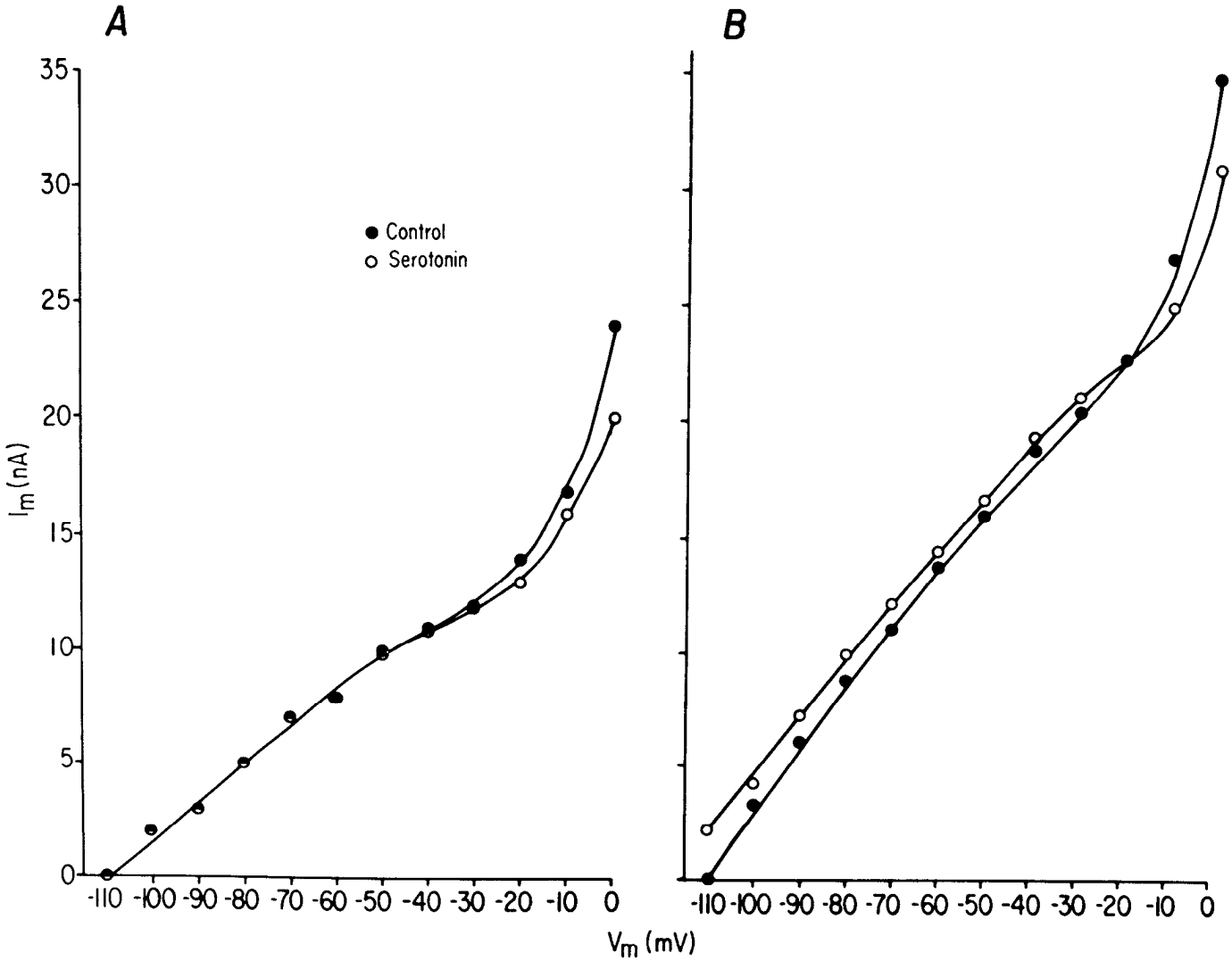

Figure 3. Steady-state current-voltage relation in normal (10 mM K^+) (*A*) and 120 mM K^+ seawater (*B*). A voltage-clamped sensory neuron was held at −110 mV and steady depolarizing ramp commands to +10 mV were given. The cell bodies were tied off. Serotonin (1×10^{-4} M, final concentration) decreased outward current at potentials less than −45 mv in normal seawater and had no effect at more negative potentials. There was no reversal. In 120 mM K^+, the current moves inward at potentials less than −20 mV, and outward at potentials greater than −20 mV. This reversal potential corresponds to that predicted by the Nernst equation, within the range of experimental error.

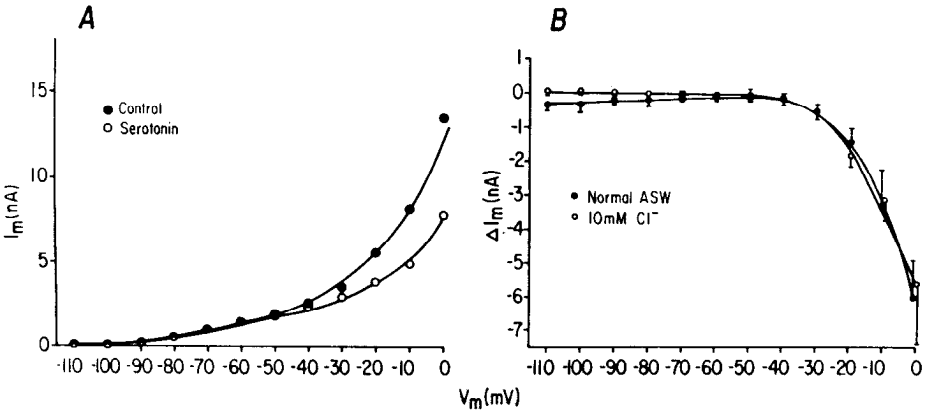

Figure 4. Substitution of chloride with acetate did not block the serotonin response. The usual Cl concentration is 612 mM; this was substituted with 602 mM acetate, and five tied-off sensory neurons were voltage clamped in the presence of TTX. The cells were held at −110 mV, and steady, depolarizing, 10-sec ramps to +10 mV were given. *A*, The average steady-state I-V curve ($n = 5$) was plotted before and after serotonin. *B*, the average synaptic current (obtained by subtracting the current in serotonin from that in the control) is plotted as a function of voltage in normal seawater (*ASW*) ($n = 5$), or 10 mM Cl ($n = 5$). The average synaptic current was unchanged, despite the absence of chloride.

The effect of serotonin on spike duration was greatly enhanced in the presence of 50 mM TEA (Fig. 1C). Here, serotonin increased the action potential duration from 17.0 to 194.0 msec at half-peak. Furthermore, in the presence of 50 μM TTX, serotonin produced a 5-mV slow depolarization from resting potential accompanied by an increase in input resistance of 25%. A hyperpolarizing component was occasionally observed when serotonin was bath applied, but TTX eliminated this component of the response, as did isolation of the cell body.

We have not explored the mechanism of the hyperpolarizing response that seems to be mediated through interneurons that serotonin excites, but have concentrated on the mechanism of the

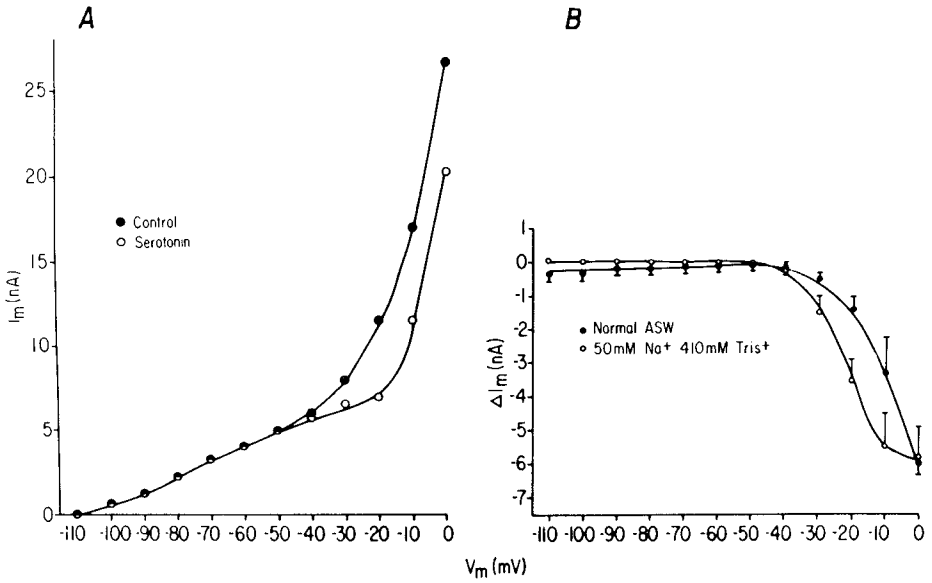

Figure 5. Serotonin response was unaffected by removal of sodium. The usual Na concentration of 460 mM was substituted with 410 mM Tris-Cl. Sensory neurons were voltage clamped to −110 mV, and steady, depolarizing, 10-sec ramps to 0 mV were given. *A*, Effect of serotonin on the I-V relation in 50 mM Na^+. The outward current was decreased. *B*, The I-V relation of the serotonin-induced current was compared jn normal seawater (*ASW*), and low-sodium seawater. The synaptic current was not reduced despite the reduction of Na^+.

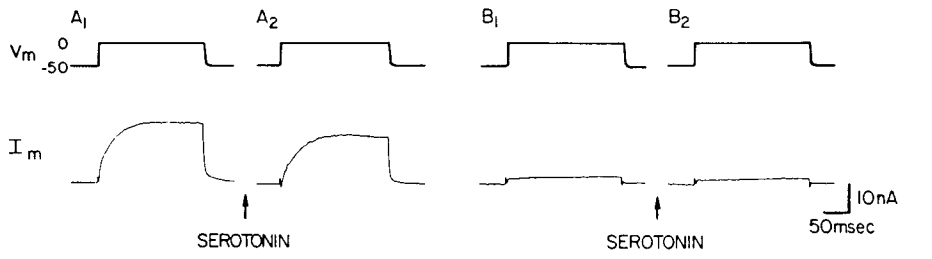

Figure 6. Iontophoretic injection of CsCl blocked the decrease in outward current. Voltage commands to 0 mV for 250 msec from a holding potential of −50 mV were given in normal seawater with 50 μM TTX. *A*, Serotonin response of a cell impaled with two 3 M KCl electrodes. *B*, Response of a cell to serotonin, iontophoretically injected with CsCl. Both the current and voltage electrodes were filled with 3 M CsCl. This significantly reduced the effect of serotonin.

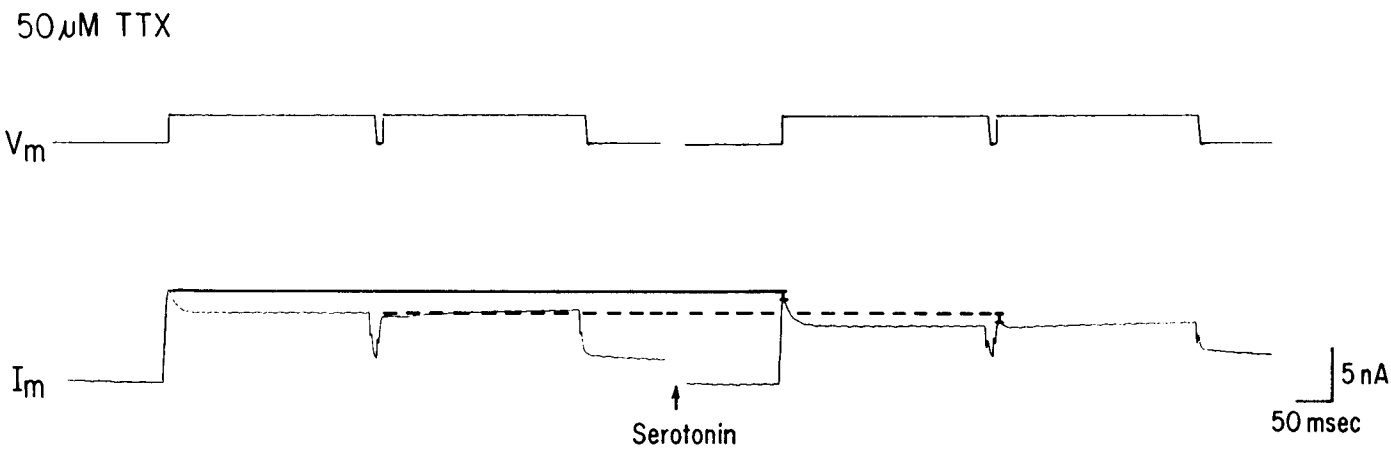

Figure 7. Serotonin did not modulate the inactivating fast potassium current. A sensory neuron was voltage clamped in normal seawater in 50 μM TTX. Double pulse commands to −15 mV of 300 msec duration each, separated by an interval of 20 msec, were given from a holding potential of −90 mV. Partial inactivation of the fast potassium current by pulse I is apparent in pulse II, but this did not reduce the response to serotonin. The decrease in current of pulse I is shown by the *solid line*; for pulse II it is shown by the *dashed line*.

slow, decreased conductance excitatory PSP. Figure 2 shows the effect of serotonin on a voltage-clamped sensory neuron in 50 μM TTX. (Unless otherwise specified, all voltage clamp experiments were done in 50 μM TTX.) Serotonin decreased the net outward current in a 25-msec step to +10 mV from a holding potential of −50 mV; this decrease occurred within the first 5 msec of the step and was maintained throughout the step. The decrease in outward current could result from an increase in conductance to sodium or calcium, a decrease in the conductance to potassium or chloride, or a combination of these. We used voltage clamp and pharmacological experiments to examine these possibilities.

Serotonin modulates a potassium conductance. To investigate the possible role of different conductances in the serotonin response, the concentration of ions in the bathing solution was varied to see whether a reversal potential for the serotonin-sensitive current could be obtained. A decrease in K^+ conductance is the most likely explanation for the decreased conductance PSP; therefore, the effect of serotonin on the current-voltage (I-V) relation was first examined in tied-off pleural sensory neurons in different potassium concentrations. Figure 3*A* shows the effect of serotonin in a sensory neuron in which a 10-sec ramp depolarization to 0 mV from a holding potential of −110 mV was given in normal seawater. The decrease in the net outward current began at −45 mV; at potentials more negative than −45 mV, serotonin had no effect. Therefore, the effect appeared to be voltage dependent, and no reversal of the current was seen. At an intermediate concentration of 70 mM K^+, the I-V relation was shifted but no reversal of the current occurred (data not shown). However, at an external potassium concentration of

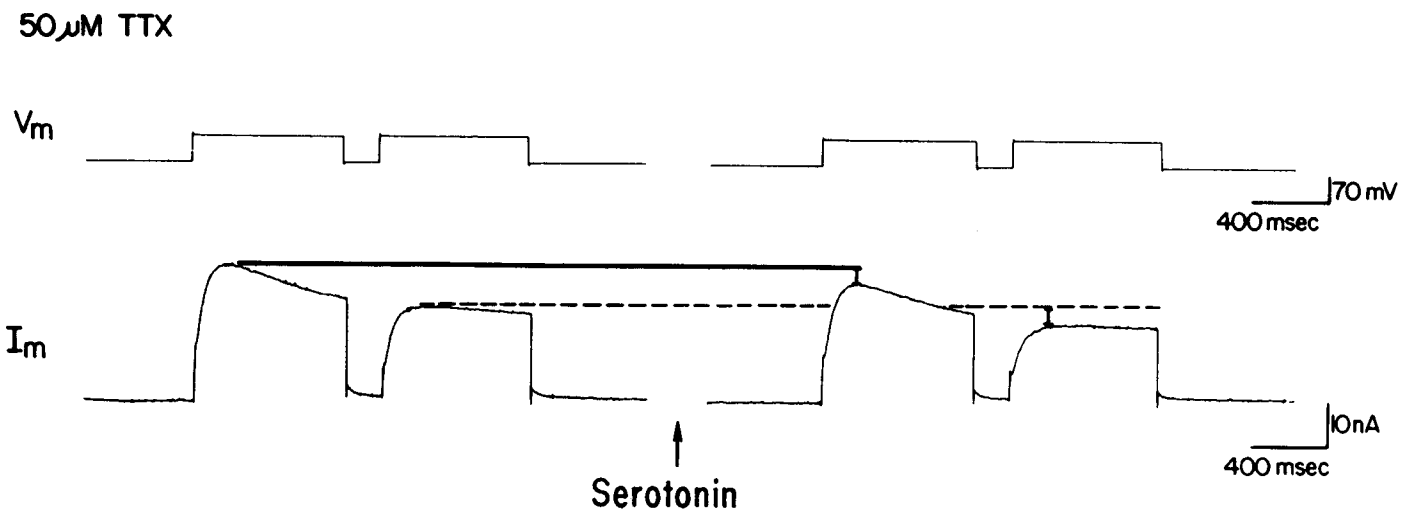

Figure 8. Serotonin did not decrease the delayed potassium current. The delayed rectifier potassium current was elicited in normal seawater in 50 μM TTX by pairs of 800-msec voltage clamp steps to +10 from −70, separated by 200 msec. In the second step of each pair, the delayed current was partially inactivated by the pre-pulse, but this did not reduce the response to serotonin. *Solid line*, pulse I response; *dashed line*, pulse II response.

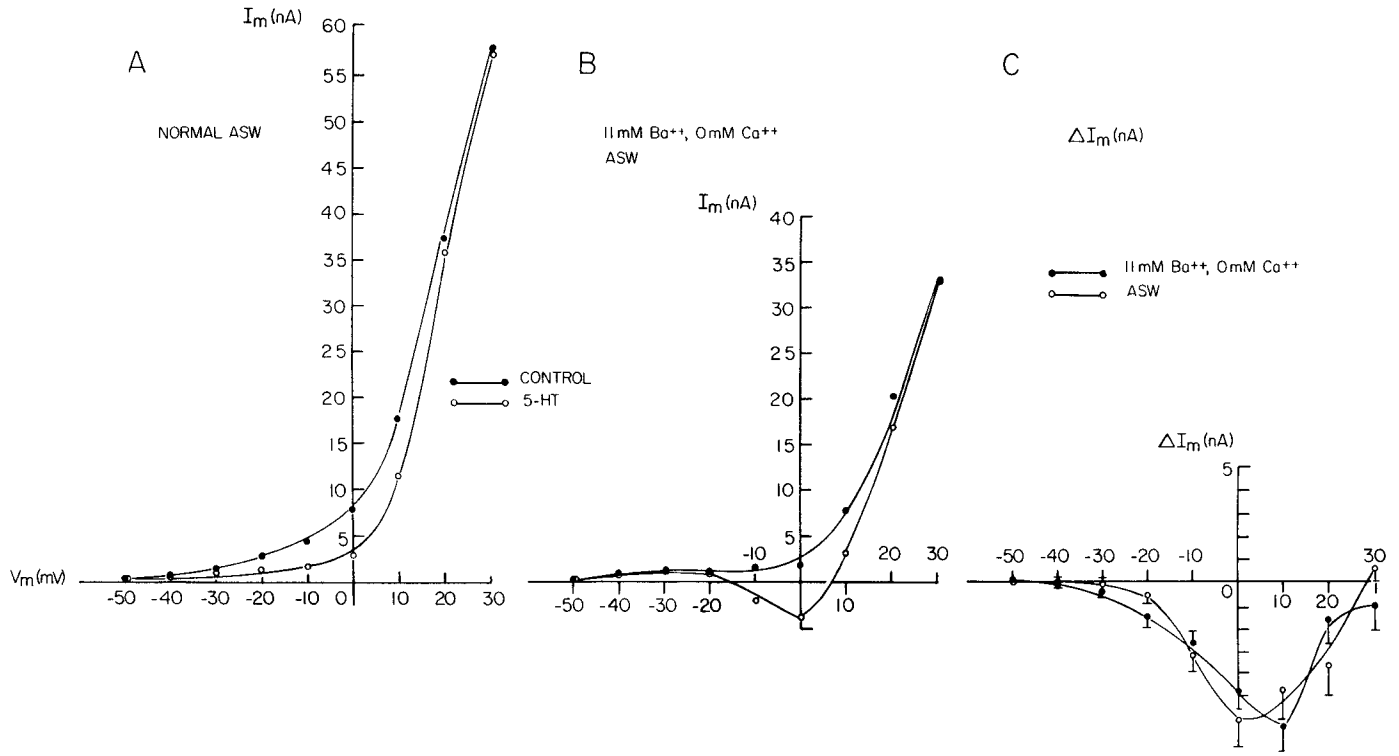

Figure 9. Serotonin did not modulate the Ba^{2+}-sensitive, Ca^{2+}-activated current. Sensory neurons were voltage clamped to −50 mV and commands of 25 msec to depolarized potentials were given. *A*, The I-V relation of the response to serotonin (1×10^{-4} M) in normal seawater (*ASW*) in 50 μM TTX ($n = 5$). *B*, The average effect of 1×10^{-4} M serotonin in calcium-free 11 mM barium-substituted seawater ($n = 5$). *C*, The I-V relation of the average synaptic current (average difference between control and calcium-free 11 mM barium-substituted seawater).

120 mM potassium (Fig. 3*B*), the current reversed at −20 mV (average of five experiments) and the steady-state current was changed by serotonin at all potentials. The reversal at −20 mV was close to the Nernst potential of −17.5 mV for 120 mM extracellular potassium, assuming an internal concentration of potassium of 240 mM (Ascher et al., 1976).

In varying the external potassium concentration, the chloride equilibrium potential also changes since chloride passively distributes according to the resting potential, which is depolarized in high potassium. To control for this and to rule out the participation of chloride current in the slow PSP, external chloride was replaced with acetate, an ion impermeant to the chloride channel (Yarowsky and Carpenter, 1978). Under these conditions, serotonin still reduced the net outward current (Fig. 4*A*), and the reduction of external chloride (Fig. 4*B*) had no effect on the I-V relation of the synaptic current modulated by serotonin.

We also addressed the possibility that sodium contributed to the inward movement of current by replacing 420 mM Na with 420 mM Tris-Cl. This experiment also controlled for the replacement of Na^+ with K^+ when the K^+ concentration was increased. As illustrated in Figure 5, the I-V relation of the serotonin current was unaffected by removal of almost 90% of the external Na^+. The only ion we found to have a concentration which affected the I-V relation of the serotonin-sensitive current was potassium.

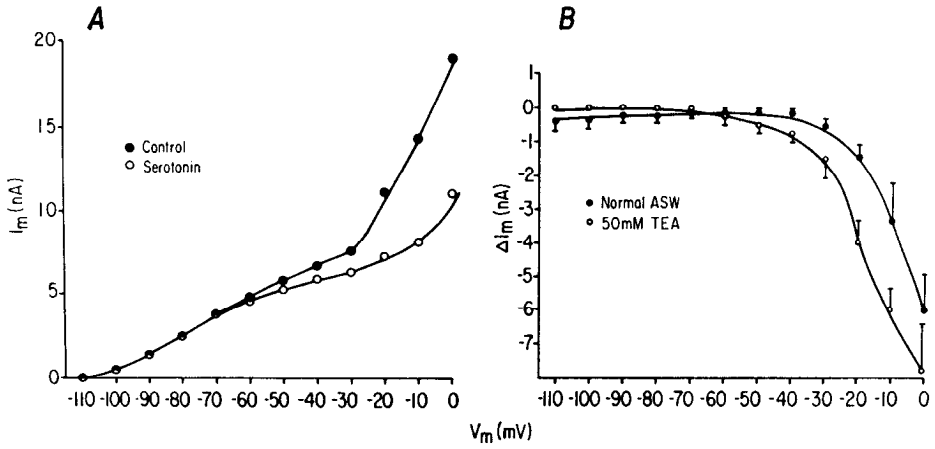

Figure 10. TEA (50 mM) did not block the serotonin response. Four sensory neurons were voltage clamped in 50 mM TEA, 410 mM Na^+ seawater with 50 μM TTX. Depolarizing ramp commands to +10 mV from a holding potential of −110 mV were given for 10 sec, and the I-V curve was plotted. The outward current was decreased by serotonin, despite the presence of TEA. *B*, The average synaptic current in normal seawater (*ASW*) ($n = 4$) was compared to that in 50 mM TEA. The currents did not differ significantly.

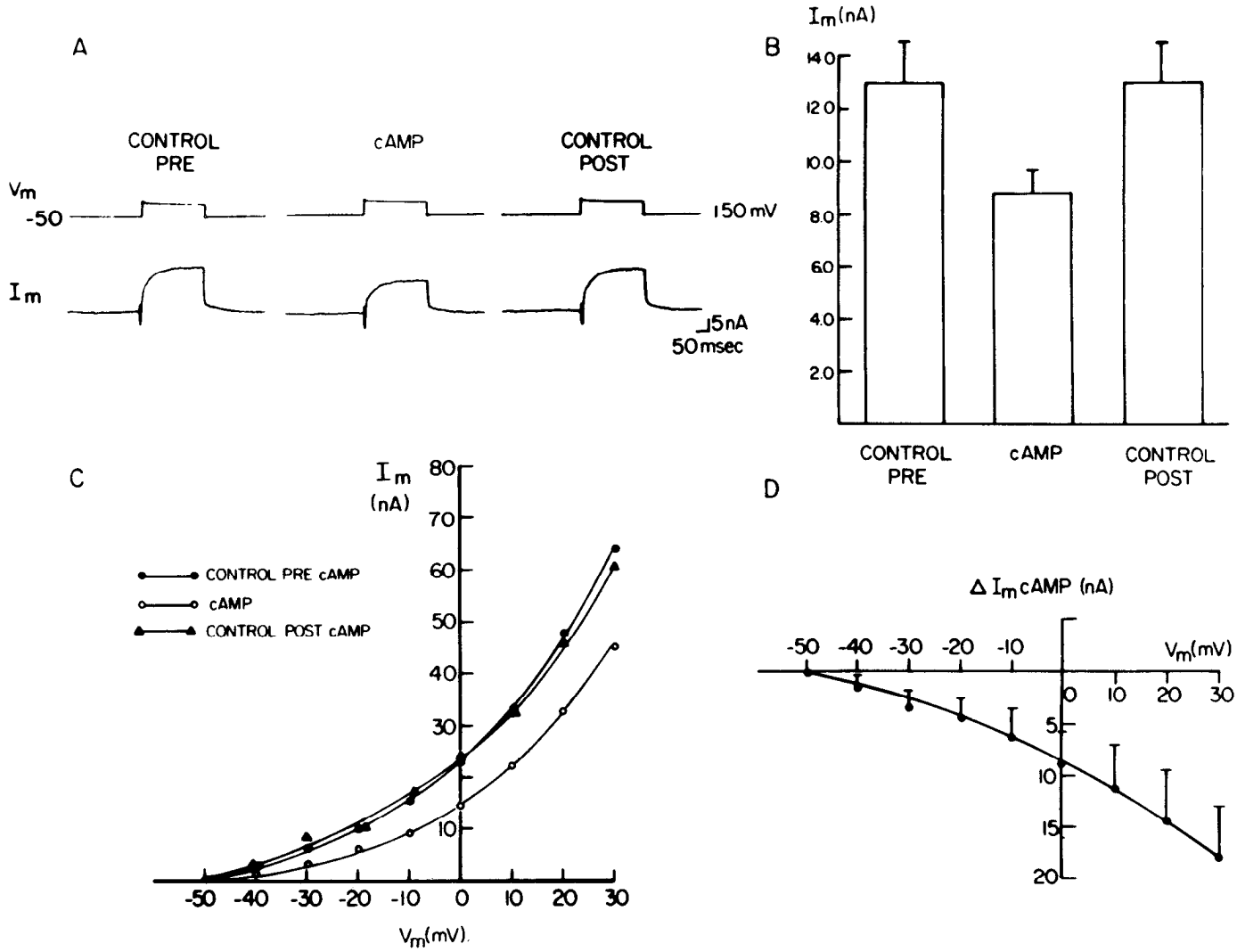

Figure 11. Intracellular injections of cAMP mimic the serotonin response. *A*, cAMP, dissolved in distilled water, was injected into a voltage-clamped sensory neuron stepped to 0 mV for 250 msec, in seawater without TTX. The cAMP injection was terminated by reapplying the braking current. The average latency to a decrease in current was less than 10 sec from the start of the cAMP injection. *B*, The average of seven cells for the experiment shown in *A*. *C*, Mean effect of cAMP on steady-state current-voltage relation. Six sensory neurons were voltage clamped, and steady 10-sec depolarizing ramps to 30 mV from a holding potential of −50 mV were given. *D*, The average synaptic current (mean ± SE; $n = 6$) for cells injected with cAMP for the experiment shown in *C*.

Intracellular injection of cesium blocks the serotonin response. To test further the hypothesis that serotonin modulates a potassium current, five left pleural sensory neurons were impaled with 3 M CsCl electrodes and injected with cesium, an ion impermeant to all known K^+ channels. If serotonin modulates a K^+ current, this modulation should be reduced by blocking K^+ permeability. Five sensory neurons impaled with 3 M KCl electrodes served as controls (Fig. 6). Cesium effectively blocked the outward current. The average peak outward current for a 250-msec voltage step to 0 mV from a holding potential of −50 mV for controls was 21.6 ± 1.24 nA ($\bar{X}$ ± SE). For the CsCl-injected cells, the mean outward current at 0 mV for a 250-msec step from −50 mV was 4.3 ± 0.58 nA. Cesium injections reduced the mean serotonin response by 80% from −5.4 ± 1.02 nA to −1.3 ± 0.67 nA (Student's $t = 3.83$, $p < 0.005$, $df = 8$). The reduction of the serotonin response was proportional to the reduction of outward current by Cs^+. These results, together with the reversal of the current in 120 mM K^+, indicate that serotonin modulates a potassium current and that neither chloride nor sodium participate in this response.

Serotonin modulates a non-inactivating potassium current. We next analyzed the biophysical properties of the potassium current modulated by serotonin and compared it with three other currents known to exist in molluscan neurons, i.e., the fast potassium current (I_A), the delayed potassium current (I_K), and the calcium-activated potassium current (I_C). These currents have particular voltage and pharmacologic sensitivities that permit their separation under voltage clamp and allowed us to assess their possible contribution to the serotonin response. The fast potassium current activates at potentials more depolarized than −50 mV (Connor and Stevens, 1971). Steady-state inactivation is complete at potentials more depolarized than −40 mV, and inactivation is removed by hyperpolarization. Depolarizing voltage clamp steps from hyperpolarized potentials therefore elicit a fast transient current that inactivates rapidly. The delayed potassium current begins to activate at −30 mV (Thompson, 1977; Aldrich et al., 1979) and becomes maximally inactivated with sustained depolarization to potentials between +10 and +20 mV. We therefore examined the effect of serotonin on cells in which these currents were alternately activated and inactivated. If either of these K^+ currents contribute to the serotonin response, the effect of serotonin should be diminished by inactivation of these currents.

To test whether serotonin modulates the fast potassium current, 300-msec voltage steps to −15 mV from a holding potential of −90 mV were given in four pleural sensory neurons. This elicited a clearly defined fast K^+ current. A pre-pulse protocol was used to inactivate I_A (Fig. 7). Double pulses were elicited before and after the addition of serotonin. Pulse I (pre-pulse) produced a 1.2-nA reduction in the fast outward transient elicited by pulse II. (The average amount of inactivation produced by the pre-pulse was 1.65 ± 0.38 nA, $n = 4$.) The reduction of current by serotonin was compared for the peak of the fast K^+ current, in pulses I (fully activated) and II (partly inactivated) as shown in Figure 7; serotonin produced an equivalent decrease of 0.71 nA in both pulses. The mean reduction of the outward transient current produced by serotonin in four cells for pulse I was 0.65 ± 0.05 nA and for pulse II was 0.65 ± 0.05 nA.

In a subsequent experiment the effect of serotonin on the delayed potassium current was tested. The pre-pulse protocol was similar to that used to examine I_A. Sensory neurons were held at −70 mV and pairs of voltage clamp commands were elicited to 10 mV for 800 msec. Although a considerable amount of the peak outward current is inactivated by the pre-pulse, the serotonin response at the peak was not diminished (Fig. 8). Instead, the serotonin response was larger for the inactivated current in pulse II (3.8 ± 0.4 nA; $\bar{X}$ ± SE; $n = 8$) than for the non-inactivated current in step I (2.1 ± 0.5 nA). Since partial inactivation of the fast and delayed K^+ current did not diminish the response to serotonin, these two voltage-dependent K^+ currents did not contribute to the serotonin response.

Pharmacologic blockers of the calcium-activated potassium current do not affect the serotonin response. The previous experiments do not exclude the possibility that the calcium-activated potassium current (Meech and Standen, 1975; Meech, 1978, 1980), which does not inactivate with sustained depolarization (Aldrich et al., 1979), is modulated by serotonin. Several investigators have found slow decrease conductance PSPs in molluscan neurons to be mediated by a decrease in the calcium-activated potassium conductance (Aldenhoff et al., 1979; Cottrell, 1982). We tested this possibility in experiments with barium and TEA, both of which block the Ca^{2+}-activated current (Connor, 1979; Gorman and Hermann, 1979; Hermann and Gorman, 1979, 1981).

We compared the effect of serotonin on the outward current in normal seawater and calcium-free 11 mM barium-substituted seawater. If serotonin modulates the calcium-activated current, blockade of this current should antagonize the serotonin response. Figure 9*A* shows the effect of serotonin on the current elicited by 25-msec depolarizing commands from a holding potential of −50 mV in normal seawater. Figure 9*B* shows that barium reduced the outward current but serotonin decreased the outward current even further. Figure 9*C* shows the synaptic current before and after application of serotonin to the bath; barium does not affect the I-V relation of the current modulated by serotonin.

Further evidence against the possible modulation of the calcium-activated current was obtained by adding 50 mM TEA to the bath. At this concentration, the Ca^{2+}-activated current is nearly completely blocked, as is the delayed K^+ current (Hermann and Gorman, 1981). Figure 10*A* shows the effect of serotonin on the current in the presence of 50 mM TEA and 50 μM TTX when the cell is held at −110 mV and depolarizing ramp commands to +10 mV are given. Figure 10*B* compares the synaptic current for serotonin in normal seawater obtained in a previous experiment with the synaptic current modulated by serotonin in the presence of 50 mM TEA. TEA did not block the response. Thus, the serotonin-sensitive current was unaffected by two pharmacological agents that block the calcium-activated potassium current.

Serotonin elevates cAMP levels and the outward current is decreased by forskolin and cAMP injection. We next examined the role of cAMP as the second messenger for the decreased conductance to potassium. Serotonin (1×10^{-4} M) produced an average 3.7-fold increase in cAMP levels of the pleural sensory neurons from 0.127 ± 0.023% of [^{32}P]ATP to 0.452 ± 0.10% of [^{32}P]ATP ($p < 0.05$, t test for correlated means, $t = 3.71$, $n = 5$). To test whether the elevation of cAMP was related to the decrease in the outward current, cAMP was injected into the cell under voltage clamp. cAMP caused an average decrease of 4.2 nA in the outward current in seven cells stepped to 0 mV for 250 msec (Fig. 11, *A* and *B*). When the injection of cAMP was terminated, the outward current returned to control levels within 30 sec. cAMP decreased the net outward current at all potentials when a depolarizing ramp to 30 mV was given from a holding potential of −50 mV (Fig. 11, *C* and *D*). The current modulated by cAMP was non-inactivating, and the I-V relation was similar to that produced by serotonin.

If the decrease in the outward current is cAMP dependent and not an artifact of injection, then forskolin, an indirect agonist of the catalytic subunit of adenylate cyclase (Daly et al., 1982), should reduce the current. Forskolin in dimethylsulfoxide (DMSO) vehicle reduced the net outward current on average by 10.8 ± 1.4 nA ($\bar{X}$ ± SE, $n = 4$). DMSO alone had no effect on the outward current ($n = 5$). These data, together with the elevation of cAMP and the decrease in outward current by cAMP, support the hypothesis that the decreased conductance to K^+ is mediated by the second messenger, cAMP.

Discussion

We conclude from these experiments that the current modulated by serotonin in the pleural sensory neurons is identical to the S potassium current of the abdominal ganglion. Like the S current (Klein et al., 1982), the current in the pleural sensory neurons has

the following properties. (*1*) It is a K^+ current since it reverses in high potassium concentration and is blocked by intracellular cesium. (*2*) It is active at the resting potential and contributes to repolarization of the action potential. (*3*) It is not blocked by the substitution of barium for calcium or by external TEA. (*4*) It does not inactivate with prolonged depolarization. (*5*) It is decreased by cAMP. Our results further suggest that serotonin does not modulate a sodium or chloride conductance, since removal or substitution of these ions has no effect on the serotonin-modulated current. Neither does serotonin affect the three other K^+ currents in these cells. These findings confirm and extend the observations of Pollock et al. (1982), Walsh and Byrne (1984), and Occor et al. (1983), who reported that, in the pleural sensory neurons, serotonin decreases the potassium conductance through a cAMP-dependent cascade.

Our experiments address two features of the S current in more detail than does previous work: the reversal potential and voltage dependence of the current. We were unable to obtain a reversal potential at external potassium concentrations less than 120 mM K^+, and Walsh and Byrne (1984) report reversal only at 75 mM K^+. The difficulty that we and others have encountered can be explained by the striking outward rectification of the K^+ channel that carries this current. The rectification was observed in the abdominal sensory neurons by single-channel recording (Siegelbaum et al., 1982), and our results using patch clamp (Pollock and Camardo, 1984) indicate that it occurs in the pleural sensory neurons as well. The conductance of the channel is strikingly nonlinear in asymmetric K^+ concentration. Indeed, reversal of current flow through isolated patches of membrane in the siphon sensory cells could not be resolved with external potassium of 360 mM until the internal concentration was greater than 40 mM (Camardo et al., 1983), and, with such a concentration gradient, reversal current remained small even with a large driving force. With the two-electrode voltage clamp, we cannot measure the small amount of current that may reverse at 40 or even 70 mM external potassium in the face of high intracellular potassium concentration—not until 120 mM external potassium is inward current through this channel large enough to resolve.

Our data also address certain aspects of the voltage dependence of the serotonin-sensitive current. Initially it had been thought that the S current is not active at potentials more hyperpolarized than the resting potential and that it had a true threshold of activation. However, this also occurs because current will not reverse in normal K^+ and therefore cannot be observed at hyperpolarized potentials. In 120 mM K^+ the serotonin-sensitive current is active and can be decreased even at −110 mV (see Fig. 3). Much of the apparent voltage sensitivity of the I-V relation appears to be simply a result of the outwardly rectifying properties of the single-channel current. Our voltage step data indicate, however, that some increase in S current does occur during depolarization that may be independent of the single-channel conductance (see Fig. 2). This suggests some moderate time dependence to the current but is not inconsistent with our conclusion that there is no threshold of activation and no potential at which the current is turned off. The S channel in abdominal sensory neurons has the same characteristics.

These experiments also indicate that serotonin exerts its effect on a specific current and does not modulate other K^+ currents known to exist in these cells. This is precisely what happens in the abdominal sensory cells. Since it is non-inactivating, the current described here cannot be the fast or delayed potassium current, and our experiments show that these currents are unaffected by serotonin. This current is also different from the calcium-activated potassium current reported to be decreased by transmitters in molluscan neurons (Aldenhoff et al., 1979; Cottrell, 1982), in hippocampal pyramidal neurons (Madison and Nicoll, 1982; Haas and Konnerth, 1983), and in the myenteric plexus (Grafe et al., 1980), since replacement of calcium by barium did not block the response. The S current is unlike the potassium current decreased by muscarine (the M current) in bullfrog sympathetic ganglia (Brown and Adams, 1980; Akasu, 1981; Adams et al., 1982), spinal cord (Nowak and MacDonald, 1983), cortex, and hippocampus (Adams et al., 1981), since the M current is voltage dependent, activating between −60 and −30 mV, and barium sensitive. The current described here is most like the steady-state potassium current in *Helix* described by Paupardin-Tritsch et al. (1981) and Deterre et al. (1981, 1982).

Although our data clearly support a role for K^+ current modulation, we cannot rule out the possibility that serotonin increases a calcium current in addition to decreasing the S potassium current. Two observations support the possibility that calcium current is modulated. First, cesium did not block the serotonin response completely (Fig. 6). This could be due simply to residual K^+ current not blocked by cesium, but increased Ca^{++} current cannot be excluded. Second, we have observed that the calcium channel blockers cadmium and cobalt can interfere with the effect of serotonin in some experiments. Although this does not occur consistently, it nevertheless suggests a role for either a calcium current component of the response or a requirement for Ca^{2+} in the cAMP cascade that decreases the S current. The latter is known to exist (Rasmussen, 1981), and we favor it as an explanation for the effect of Ca^{2+} blockers. Although direct modulation of calcium current in neurons by cAMP-dependent phosphorylation has been observed by others (Pellmar and Carpenter, 1980; Doroshenko et al., 1982; Kononenko et al., 1983; Pellmar, 1984), indirect modulation of calcium influx by decreased conductance to potassium may be functionally more advantageous to strengthening synaptic transmission. Not only would transmitter release be enhanced, but neuronal excitability would be increased, thereby increasing the capacity for spatial and temporal summation. At the same time, the threshold for firing an action potential and the rate of adaptation are decreased; all of these are critical features of the response of a sensory neuron to a given stimulus. It is conceivable that an increase in Ca^{2+} current and a decrease in K^+ current occur simultaneously, but at present, evidence in the pleural neurons does not support a role for calcium current.

The present findings are significant because they show that a decrease in the S current through cAMP-dependent phosphorylation is an important feature of the neural substrates for sensitization and classical conditioning (Carew et al., 1983; Hawkins et al., 1983; Walters and Byrne, 1983) in two distinct but related reflexes in *Aplysia*. That is, neurons that undergo presynaptic facilitation have similar biophysical properties. In this case, they share the same membrane protein that behaves in a specific way. The expression of the genes that code for these proteins may therefore be similar in different cells with related function. Moreover, the role of K^+ channels in modulation of neuronal excitability may in fact be ubiquitous, although different kinds of K^+ channels may be involved in different species. Experiments by Belardetti et al. (1982) have shown that other synapses (in leech and *Aplysia*) are heterosynaptically facilitated by serotonin and cAMP. Also, Alkon et al. (1982a, b) have implicated a decreased conductance to K^+ via phosphorylation in the conditioning of the phototaxic response in *Hermissenda*. The role of M current in regulating neuronal excitability has also been well established (Adams et al. 1982).

These findings are also important from a methodological standpoint. The identity of the serotonin current in the pleural ganglion to that in the abdominal ganglion will facilitate biochemical studies of the role of ion channels in sensitization and classical conditioning. These studies in the gill withdrawal reflex are virtually impossible because there are only 48 sensory neurons in the entire abdominal ganglion that average 60 μm in diameter. In contrast, there are more than 200 sensory neurons, somewhat larger in size, in each pleural ganglion. The sensory cells of the pleural ganglia are numerous enough to be treated as a relatively homogeneous neural preparation from which material for sophisticated biochemical analysis can be more easily obtained.

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