Analysis of Decreased Conductance Serotonergic Response in Aplysia Ink Motor Neurons

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SUMMARY AND CONCLUSIONS

1. Micropressure ejection of serotonin (5-hydroxytryptamine, 5-HT) produced excitatory responses in the L14 ink motor neurons of Aplysia that depended on the site of application. Ejection of 5-HT onto the cell body produced a slow response that showed variability in voltage sensitivity between preparations. In contrast, ejection of 5-HT onto the neuropil underneath the cell body produced a response whose amplitude was consistently a linear function of the holding potential, reversing near the predicted potassium equilibrium potential. Subsequent analyses focused on this second response.

2. The neuropil response induced by 5-HT had a linear current-voltage relationship (reversing at ca. -80 mV), was associated with a decrease in input conductance, and was sensitive to changes in the concentration of extracellular K+. Serotonin application in artificial seawater (ASW) containing 30 mM K+ produced a response that reversed close to the altered Nernst potential for K+.

3. The 5-HT response did not appear to be due to secondary activation of interneurons or to depend primarily on extracellular Ca2+, since ejection of 5-HT onto cells bathed in ASW containing 30 mM Ca2+ produced responses comparable to, although somewhat attenuated from, those observed in ASW.

4. Serotonin responses similar to those produced in ASW were obtained after perfusing the ganglion with ASW containing Co2+, 4-aminopyridine (4-AP), and tetraethylammonium (TEA). This suggests that the 5-HT-sensitive current is separate from the Ca2+-activated, fast, and delayed rectifying K+ currents.

5. The 5-HT response appeared to be mediated by changes in levels of cAMP. Bath application of the phosphodiesterase inhibitors IBMX (3-isobutyl-1-methylxanthine) or Ro 20-1724, or the adenylate cyclase activator forskolin mimicked the 5-HT response by producing a slow inward current associated with a decrease in membrane conductance.

6. Alteration of cellular cAMP metabolism modulated the response to 5-HT. Exposure of the ganglion to low concentrations of either Ro 20-1724 or forskolin potentiated the 5-HT response. Higher concentrations of these agents largely blocked the response to subsequent 5-HT applications.

7. Bath application of the 8-bromo derivative of either cAMP or cGMP produced a slow inward current associated with a decrease in membrane conductance in cells voltage clamped at the resting potential. Responses to 5-HT were blocked, however, after exposure to 8-bromo-cAMP, but not to 8-bromo-cGMP.

8. These results suggest that 5-HT produces a voltage-independent decrease in a steady-state potassium conductance that may be mediated by cAMP. The 5-HT response parallels the excitatory postsynaptic potential (EPSP) produced physiologically by stimulation of the skin, the pleural-abdominal connectives, or cell L31 (8, 18, 40). The slow, decreased conductance EPSP, possibly mediated by 5-HT, is important for modulating the activity of the L14 cells and inking behavior (7, 10–12, 16, 18, 40).

INTRODUCTION

A noxious stimulus delivered to Aplysia causes ink release or an enhanced probability
of eliciting ink release with subsequent test stimuli, as well as sensitization of various other defensive responses (10, 11, 15, 16, 18, 36, 37, 40, 48, 49). In an isolated ganglion preparation, electrophysiological correlates of these effects can be examined by electrical stimulation of the pleural-abdominal connectives. Such stimuli produce slow and long-lasting decreased conductance excitatory postsynaptic potentials (EPSPs) in the sensory neurons innervating the siphon skin and the L14 ink motor neuron (6, 12, 18, 26). Analysis of the neural circuit for inking behavior (8) demonstrated that the slow and long-lasting decreased conductance EPSP produced in L14 was mediated at least in part by cell L31. The slow EPSP in the sensory neuron is associated with presynaptic facilitation, whereas the motor neuron EPSP increases the cell’s excitability. In the siphon sensory neurons serotonin (5-hydroxytryptamine, 5-HT) mimics sensitizing noxious stimuli and acts on a novel potassium conductance distinct from the fast (gKv), delayed outward (gKv), calcium-activated (gCa), and muscarinic-sensitive potassium conductances (14, 25). This 5-HT-modulated current (S current) is mediated by a cAMP cascade that eventually leads to closure of individual potassium channels (41). As a result 5-HT causes a reduction of repolarizing K+ current and subsequent broadening of action potentials. The broadening is believed to allow for enhanced Ca2+ influx during the action potential and a resultant enhancement of transmitter release (26, 27).

Since common sensitizing stimulation produces similar slow EPSPs in the sensory neurons and the L.14 cells, we wondered whether 5-HT could also mimic the slow EPSP in the L14 cells and, if so, whether similar subcellular mechanisms were involved. We found that 5-HT mimics the physiologically produced decreased conductance EPSP in L14 and this response appears to be produced at least in part by a cAMP-mediated decrease in resting K+ conductance. The 5-HT response in the L14 cells differs from the 5-HT response observed in the siphon sensory neurons, however, in its apparent lack of voltage sensitivity. In addition, there are regional differences in 5-HT sensitivity over the surface of L14 cells (i.e., cell body vs. neuropil) that have not been reported in the sensory cells. [Preliminary reports of some aspects of this work have been presented (45, 46).]

METHODS

_Aplysia californica_, weighing 100–350 g were obtained from Marine Specimens Unlimited (Pacific Palisades, CA) and Alacrity Marine Biological (Redondo Beach, CA). They were maintained in an aquarium filled with artificial sea water (ASW; Instant Ocean) at 15°C before use. All experiments were performed at room temperature (20–24°C).

Isolated, desheathed abdominal ganglia were pinned to the Sylgard (Dow-Corning) floor of a 10-ml chamber. The ganglion was perfused with ASW buffered to pH 7.4 with 10 mM HEPES. The L14 ink motor neurons (16) were impaled with 2–5-MΩ microelectrodes filled with either 2 M potassium citrate or 2 M potassium acetate. Previously described voltage- and current-clamp techniques were used (12). Solutions containing elevated K+ and Co2+ were made by adding 20 mM KCl and 30 mM CoCl2 to ASW. Na+-free seawater was made with 10 mM CaCl2, 55 mM MgCl2, 10 mM KCl, 460 mM Choline-Cl, and 10 mM HEPES (pH 7.4). Solutions containing tetraethylammonium chloride (TEA; Eastman Kodak) and 4-aminopyridine (4-AP; Sigma) were made by adding TEA and 4-AP to ASW.

Serotonin creatine sulfate (Sigma; saturated in ASW) was applied by microprobe ejection. A pneumatic pump (Medical Systems Corp.) was attached to blunt-tip microelectrodes (2–6 μm in diameter) filled with the 5-HT solution. Ejection of 5-HT was obtained by application of constant pressures ranging from 10 to 25 lb/in² for 2 s or less. The microejection electrode was positioned above either the cell body or the neuropil located beneath the cell body. The 5-HT was applied at regular intervals of 8–10 min.

Concentrated stock solutions of 3-isobutyl-1-methylxanthine (IBMX; Sigma), Ro 20-1724 (Hoffman-LaRoche), N6,O2-dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP; Sigma), 8-bromoadenosine 3',5'-cyclic monophosphate (8-bromo-cAMP; ICN Pharmaceuticals), 8-bromo-guanosine 3',5'-cyclic monophosphate (8-bromo-cGMP; ICN Pharmaceuticals), and forskolin (Cal Biochem) were added directly to the bath using a Pasteur pipette. In some experiments 5-HT was added to the bath using the above procedures (e.g., Fig. 6A1). Concentrated stock solutions of forskolin were made by dissolving 10 μg of forskolin in 1 ml of dimethylsulphoxide (DMSO; Sigma). Microliter aliquots of this solution were then added to ASW to obtain the desired concentrations.
RESULTS

In an initial series of experiments we examined the regional sensitivity of the ink motor neurons to micropressure ejection of 5-HT using current-clamp techniques. Application of 5-HT to the cell body produced slow and long-lasting depolarizations that varied in apparent voltage sensitivity between preparations. Plots of the amplitude of cell body 5-HT response vs. the membrane potential did not demonstrate a consistent trend \((n = 22)\). The responses appeared to be due to a combination of increased and decreased conductance mechanisms. In several experiments \((n = 3)\) exposing the ganglion to Na"-free seawater caused the 5-HT-induced depolarization to decrease and reverse at ca. \(-80\) mV.

In contrast to the cell body 5-HT response, micropressure ejection of 5-HT onto the neuropil beneath the cell body \((n = 7)\) produced excitatory responses that consistently reversed at ca. \(-80\) mV when the preparation was perfused with ASW. The neuropil response, unlike the soma response, demonstrated little variability between preparations. In this report we focus on the neuropil response since it more closely resembled the response to synaptic input reported previously \((8, 18)\).

**5-HT response has linear current-voltage relationship within range examined**

For each of the experiments described, the 5-HT electrode was lowered to the neuropil region beneath the cell body of L14. The 5-HT was then applied to various points in this region until a response was found that reversed at hyperpolarized levels. The 5-HT electrode then remained at this point throughout the experiment. An example of responses obtained while systematically varying the membrane potential using current-clamp techniques is illustrated in Fig. 1. The 5-HT responses obtained while the cells were at their resting potential were associated with an \(8.5 \pm 1.05\%\) (mean \(\pm\) SE, \(n = 13\)) increase in input resistance. The resistance change did not appear to be due to a nonlinear

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**FIG. 1.** Voltage responses to serotonin (5-hydroxytryptamine, 5-HT) and their sensitivity to changes in membrane holding potential. \(A\): experimental data. In artificial seawater, 5-HT was micropressure ejected while membrane potential of L14 was systematically varied. 5-HT was applied to axon hillock region in 8- to 10-min intervals to obtain stable responses. Depolarizing L14 increased the amplitude of 5-HT response. Hyperpolarizing L14 inverted 5-HT response, indicating that 5-HT response was due to decreased conductance mechanism. Fast upward deflections on traces are spontaneous synaptic potentials. Unless otherwise indicated, in this and subsequent illustrations, arrows below traces indicate brief (1-2 s) microejection of 5-HT. \(B\) calculated reversal potential. Peak amplitudes of 5-HT response in \(A\) were plotted as function of membrane potential in L14. Data were fitted with linear regression analysis \((r = 0.99)\), and calculated reversal potential was \(-81.7\) mV. In 7 experiments calculated reversal was \(-78.2 \pm 1.4\) mV (mean \(\pm\) SE).
current-voltage relationship of the membrane since artificial depolarizations to the same levels produced by 5-HT caused no obvious changes in resistance. The 5-HT responses were slow, with a time to peak of 20–30 s and a duration of 1–3 min.

We next used voltage-clamp techniques to further characterize the ionic and cellular mechanisms underlying the 5-HT response and to eliminate the secondary activation of voltage-dependent membrane conductances at more depolarized holding potentials. Under voltage clamp 5-HT produced an inward current that increased with depolarized holding potentials (Fig. 2A) and reversed at hyperpolarized holding potentials more negative than ca. −80 mV. In 6 experiments the reversal potential ranged from −80.4 to −68.3 mV, with a mean of −77.7 ± 3.7 mV. The early inward current component seen at hyperpolarized holding potentials (Fig. 2A) was observed in five of these six experiments. A similar early component preceding the slow component has also been observed in L14 after stimulation of cell L31 (8). The conductance change produced by 5-HT showed no apparent voltage dependency in the range of membrane potentials examined (−30 to −110 mV; see Fig. 2C).

**5-HT modulates novel K⁺ conductance**

The fact that the 5-HT responses reversed at ca. −80 mV suggested that the responses were due to a decrease in a resting K⁺ conductance. As a first step in testing this hypothesis, the extracellular concentration of potassium was increased and 5-HT was again applied at a series of clamped membrane potentials (Fig. 2B). Increasing extracellular K⁺ from the control level of 10 to 30 mM shifted the reversal potential of the 5-HT response in a depolarizing direction to an

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**FIG. 2.** Changes in membrane currents produced by 5-HT and their sensitivity to membrane holding potential and extracellular K⁺ concentration. *A:* in presence of ASW containing normal levels of extracellular K⁺ (10 mM), 5-HT was applied while L14 was voltage clamped at different holding potentials. Voltage clamping membrane potential at depolarized levels increased inward current produced by 5-HT. At holding potentials more negative than −80 mV, 5-HT response was inverted. *B:* same cell, after exposure to elevated K⁺ (30 mM); membrane potential where 5-HT response reversed was shifted in depolarized direction. *C:* calculated reversal potentials. Peak amplitudes of 5-HT responses in both *A* and *B* were plotted as function of membrane potential. Data were fitted with linear regression analysis. In ASW containing normal level of extracellular K⁺ (10 mM), calculated reversal potential was −77.5 mV (r = 0.98). With elevated K⁺ (30 mM), calculated reversal potential was −54 mV (r = 0.99). In 6 experiments calculated reversal potential for normal level of extracellular K⁺ (10 mM) was −77.7 ± 3.7 mV and for elevated K⁺ (30 mM) it was −57 ± 2.9 mV.
FIG. 3. 5-HT produced a response associated with decrease in input conductance. L14 was voltage clamped at membrane potential of -58 mV while a train of constant voltage (5 mV) hyperpolarizing pulses was applied. 5-HT was applied by microejection to axon hillock region. At peak of 5-HT response there was a 12% decrease in input conductance.

average value of $-57 \pm 2.9$ mV ($n = 6$). The change in the reversal potential for the 5-HT response corresponded roughly to the calculated Nernst equilibrium potential of -52 mV (assuming intracellular K$^+$ concentration of 236 mM, similar to that reported in cell R2 of Aplysia; see Ref. 4). The 5-HT response was consistently enhanced in the presence of 30 mM K$^+$, as illustrated in Fig. 2C by the increase in the slope over the control 5-HT current-voltage plot. The change in input conductance produced by 5-HT was also directly measured by applying constant voltage hyperpolarizing pulses while clamping the cell at its resting membrane potential (Fig. 3; see also Fig. 6A).

The possible contribution of various ionic conductances to the 5-HT response was examined further by adding a series of agents believed to block various ion channels to the bath. To examine the possibility of the direct modulation of a Ca$^{2+}$ conductance or a Ca$^{2+}$-activated K$^+$ conductance by 5-HT, the ganglion was bathed in ASW containing 30 mM Co$^{2+}$. This solution also minimized the possibility that the 5-HT response in L14 was secondary to the activation of 5-HT-sensitive interneurons. While 30 mM Co$^{2+}$ reduced the 5-HT response by $42 \pm 5\%$ ($n = 8$) it was not blocked (Fig. 4). Application of 30 mM Co$^{2+}$ also produced a $7.2 \pm 0.8$ mV ($n = 8$) depolarization of the membrane potential. This change in membrane potential was returned to the original holding potential with reapplication of the voltage clamp (Fig. 4B).

To examine whether 5-HT was modulating the fast ($I_A$) or delayed ($I_{KV}$) K$^+$ currents, 40 mM TEA and 5 mM 4-AP were added to ASW already containing 30 mM Co$^{2+}$. These concentrations dissolved in Co$^{2+}$-ASW have been shown previously to be effective in blocking $I_A$ and $I_{KV}$ in the L14 cells (6, 12). The K$^+$-channel block with aminopyridines can be voltage dependent (51) but this complication should be minimal in the voltage range used in our experiments (51, 43). In the presence of these agents 5-HT produced a response that was comparable to, although somewhat smaller (reduced by 19 ± 2%; $n$
Elevation of cellular cAMP mimics 5-HT response

Cellular cAMP levels were artificially elevated by bath addition of agents known to cross cell membranes and alter cyclic nucleotide levels. In some experiments agents were added to an ASW solution containing 30 mM Co\(^{2+}\) to block synaptic transmission and therefore minimize secondary effects on interneurons. Concentrations previously demonstrated to reliably generate large cellular responses in other preparations were used (cf. Refs. 23, 26, 31, 33, 42). The effect of lower concentrations of some of these agents on the 5-HT response is described in the next section. The cells were voltage clamped at the resting potential while constant voltage hyperpolarizing pulses were applied to monitor changes in input conductance. In separate experiments, the addition of the phosphodiesterase-resistant analogue of cAMP, 8-bromo-cAMP, or the adenylate cyclase activator, forskolin (39), produced a slow inward current associated with a decrease in input conductance (Fig. 6). Serotonin was also applied in the same fashion for comparison (Fig. 6A). Addition of the phosphodiesterase inhibitor, IBMX (1 mM), and db cAMP (1 mM) also produced similar changes in mem-

A CONTROL (ASW + Co\(^{2+}\))

B 40mM TEA + 5mM 4-AP + Co\(^{2+}\)

FIG. 5. 5-HT response was not blocked in presence of 30 mM Co\(^{2+}\), 40 mM tetraethylammonium (TEA), and 5 mM 4-aminopyridine (4-AP). Responses to microljection of 5-HT were obtained in ASW containing 30 mM Co\(^{2+}\) (A) and containing 30 mM Co\(^{2+}\), 40 mM TEA, and 5 mM 4-AP (B). Both traces are from same cell, voltage clamped at membrane potential of \(-50\) mV. Application of TEA and 4-AP led to small reduction in 5-HT response.

This was 4), than that obtained in ASW with Co\(^{2+}\) alone (Fig. 5).

In contrast to the response to bath application of 5-HT, the responses to agents altering cAMP levels were associated with a variable delay between application and onset of the response (cf. Fig. 6, A\(_1\) and A\(_2\)). The delay was presumably due to the time necessary for these agents to pass across the cell membrane.

To examine the specificity of the cAMP effects, we applied the phosphodiesterase-resistant analogue of cGMP, 8-bromo-cGMP, to ganglia bathed in ASW. The response to 8-bromo-cAMP was tested initially (Fig. 7A).
FIG. 7. 8-Bromo derivatives of cAMP and cGMP produced similar responses. A: in ASW, bath application of 10^{-3} M 8-bromo-cAMP produced inward current associated with 22% decrease in input conductance. B: after 45-min ASW wash, addition of 8-bromo-cGMP produced an inward current associated with 23% decrease in input conductance.

Then, after a 45-min wash period, 8-bromo-cGMP was added (Fig. 7B). Both 8-bromo-cAMP and 8-bromo-cGMP produced similar inward currents associated with decreases in input conductance. The 5-HT response, however, appears to be mediated by cAMP and not cGMP (see next section and DISCUSSION).

Modulation of 5-HT response by agents that alter cellular cAMP metabolism

If 5-HT is acting through a cyclic nucleotide second-messenger system, then agents that alter cyclic nucleotide metabolism should modulate the 5-HT response. To test this hypothesis, 5-HT was applied by micropressure ejection onto cells bathed in ASW containing either the phosphodiesterase inhibitor Ro 20-1724, the adenylate cyclase activator forskolin, or the 8-bromo-derivatives of cAMP and cGMP. These responses were then compared with responses obtained previously from identical 5-HT applications to the same cell bathed in ASW alone.

In the presence of low concentrations of forskolin (10^{-6} M) the response to 5-HT was

![Diagram of A and B with current traces and concentrations](attachment:image_url)

FIG. 8. Alteration of cellular cAMP metabolism modulated the response to 5-HT. 5-HT was applied by microejection once every 10 min while an L14 cell was voltage clamped at its resting membrane potential. After 3 stable control responses, different concentrations of each agent were added to bathing media. A1: control response elicited by microejection of 5-HT in presence of ASW alone; A2: potentiated response to 5-HT after 15 min of exposure to 10^{-6} M forskolin; and A3: response to microejection of 5-HT was largely blocked after 15 min of exposure to 5 x 10^{-4} M forskolin. B1: in separate experiment, control response elicited by microejection of 5-HT in presence of ASW alone; B2: response to microejection of 5-HT was potentiated after 15 min of exposure to 10^{-4} Ro 20-1724; B3: in same cell, response to microejection of 5-HT was largely blocked after 15 min of exposure to 10^{-3} M Ro 20-1724.
FIG. 9. 5-HT response is sensitive to changes in cellular cAMP and not cGMP. 5-HT was applied by microejection once every 10 min with cell voltage clamped at its resting potential. After 3 stable control responses, either 8-bromo-cAMP or 8-bromo-cGMP was added to bathing media. 5-HT responses were then tested in presence of each agent. $A_1$: control 5-HT response elicited by microejection of 5-HT in presence of ASW alone; $A_2$: after 30 min of exposure to $10^{-3}$ M 8-bromo-cGMP, response to 5-HT had not changed as compared with control. 8-bromo-cGMP frequently caused an increase in frequency and decrease in amplitude of spontaneous synaptic input received by L14. $B_1$: in same cell as $A$, after 1-h wash with ASW, 5-HT produced inward current similar to that produced in $A_1$. $B_2$: after 15 min of exposure to $10^{-3}$ M 8-bromo-cAMP, 5-HT response is completely blocked. Fast-inward, slow-outward current transients seen in $B$ were occasionally observed and were presumably due to activation of interneurons by 5-HT application.

potentiated by 25 ± 7% ($n = 5$), whereas the addition of higher concentrations of forskolin ($5 \times 10^{-4}$ M) produced an inward current and attenuated subsequent 5-HT responses by 54 ± 8% ($n = 5$; Fig. 8A). The addition of Ro 20-1724 produced similar results (Fig. 8B). In the presence of $10^{-4}$ M Ro 20-1724, the response to 5-HT was potentiated by 42 ± 11% ($n = 3$), whereas $10^{-3}$ M Ro 20-1724 produced an inward current and attenuated subsequent 5-HT responses by 70 ± 10% ($n = 3$).

Although both 8-bromo-cAMP and 8-bromo-cGMP produced an inward current associated with a decreased conductance in the L14 cells (see Figs. 6 and 7), only 8-bromo-cAMP significantly blocked responses to 5-HT (Fig. 9). After three control responses to 5-HT, the ganglion was exposed to 1 mM 8-bromo-cGMP. After 30 min of cGMP exposure, the 5-HT response showed no sign of blockade (Fig. 9B). The ganglion was then washed for 1 h with ASW. Three control 5-HT responses (Fig. 9B) were again obtained in the same cell followed by exposure to 8-bromo-cAMP. In contrast to treatment with 8-bromo-cGMP, after 15 min of cAMP exposure the 5-HT response was completely blocked (Fig. 9B). We obtained the same results when the order of 8-bromo cyclic nucleotide addition was reversed. The fast-inward, slow-outward current complexes seen in Fig. 9B were probably not due to cyclic nucleotide addition, but rather to activation of interneurons by the 5-HT application (e.g., cell L32, Ref. 8), since similar effects in response to 5-HT have been observed occasionally in other preparations perfused with ASW. Whereas $10^{-3}$ M 8-bromo-cAMP and 8-bromo-cGMP had different effects on modulating the 5-HT response, both agents produced equivalent depolarizations ($5.0 \pm 0.7$ and $5.8 \pm 0.9$ mV, $n = 4$, respectively; see also Fig. 7).

**DISCUSSION**

The ink motor neurons have unusual biophysical properties underlying their high threshold for generating action potentials (6, 7, 11, 12, 17). Inking responses are relatively insensitive to brief stimuli but are highly sensitive to long duration stimuli of identical intensity (40), due to both the activation of
a transient fast K⁺ current \( (I_A) \) that opposes initial synaptic current, and the slow buildup of a decreased conductance EPSP in the L14 motor neurons (7, 11). Some of the neural network involved in the fast excitatory input as well as the slow decreased conductance input to the L14 cells has been described (8). The results presented here extend the analysis of the inking system by demonstrating the similarity between the 5-HT response and the previously reported slow EPSP. Although much additional evidence is needed to establish that 5-HT is the natural neurotransmitter mediating the physiological response, our results provide interesting insights into the possible cellular mechanisms responsible for the generation of slow synaptic responses in these cells and document further the diverse effects that 5-HT has on the membrane properties of neurons in *Aplysia*.

**Site-specific 5-HT application mimics synaptic activity**

The L14 cells exhibited differential sensitivity in response to 5-HT depending on the site of application. Pressure ejection of 5-HT onto the neuropil region located beneath the L14 cell body produced a response that closely paralleled the time course of the response produced by electrical stimulation of the pleural-abdominal connectives (6, 12, 18) or activation of cell L31 (cf. Fig. 7 of Ref. 8). In addition, the neuropil 5-HT response and the L31 response have similar reversal potentials.

By contrast, 5-HT application to the cell body appeared to produce a mixed conductance response with no clear reversal potential. Perfusion of the preparation with Na⁺-free seawater resulted in a somatic 5-HT response that was much smaller than either the cell body or neuropil response seen in normal ASW. This latter response, however, showed a voltage dependency identical to the neuropil responses reversing near the predicted potassium equilibrium potential. Such a relationship would exist if a smaller number of the K⁺ channels sensitive to 5-HT were located in the cell body than in the neuropil. The variability of the cell body 5-HT response may reflect the degree of activation of multiple conductance mechanisms sensitive to 5-HT (24).

At least one component of 5-HT response is due to decrease in resting conductance to K⁺

The current modulated by 5-HT in L14 appears to be distinct from the S current, M current, anomalous rectifier current, Ca²⁺ current, and Ca²⁺-activated potassium currents modulated by neurotransmitters in *Aplysia* and other systems (see Refs. 1, 5, 20, 21, 25, 33, 35). The 5-HT response in L14 more closely resembles the α-response to 5-HT described by Gerschenfeld and Pau-pardin-Tritsch (24). In contrast to the apparent voltage dependence of the S current and M current (5, 14, 25), the membrane current produced by 5-HT in L14 is linear with respect to voltage within the range examined (−30 to −110 mV), reversing at ca. −80 mV (Fig. 2A). Both the input conductance measurements and the reversal potential of −80 mV are consistent with a response produced by a decreased conductance to K⁺. In addition, perfusion of the preparation with ASW containing 30 mM K⁺ produced a depolarizing shift of the reversal potential for the response to a level that was in fair agreement with the altered Nemst K⁺ equilibrium potential [Fig. 2; (4)].

The 5-HT response is not due primarily to secondary activation of interneurons or the direct modulation of a Ca²⁺ conductance, since similar responses can be obtained while bathing the preparation in ASW containing 30 mM Co²⁺. Adding Co²⁺ also eliminates 5-HT modulation of a K⁺ conductance activated by Ca²⁺ entering across the plasma membrane but does not rule out the possibility that 5-HT may modulate a Ca²⁺-activated K⁺ current by changes in uptake or release of Ca²⁺ from intracellular stores. Although 5-HT responses in the L14 cells could still be obtained in the presence of 30 mM Co²⁺, they were attenuated by an average of 42%. Similar attenuation of 5-HT-modulated K⁺ conductances by Co²⁺ have been observed previously in *Aplysia* (25) and in *Helix* (21). Possible explanations for this effect include a partial contribution of \( I_{K,Ca} \) to the response (33), changes in levels of intracellular Ca²⁺ that may alter the sensitivity of Ca²⁺-modulated adenylate cyclase (3, 47), or a partial block of the 5-HT-sensitive K⁺ channel by Co²⁺.
As is the case for the siphon sensory neurons in Ap\textit{lysia} (25) the 5-HT-sensitive potassium current appears to be separate from the fast ($I_a$) or delayed rectifying ($I_{Kv}$) potassium currents, since comparable responses to 5-HT were obtained after exposure of the preparation to ASW containing TEA and 4-AP. The small attenuation of the 5-HT response observed in the presence of TEA and 4-AP may be due to a partial block of the 5-HT-sensitive $K^+$ channel by these agents, but we cannot exclude the possibility that the 5-HT also partially modulates $I_a$ and $I_{Kv}$. A large contribution seems unlikely, however, due to the marked voltage dependency of $I_a$ and $I_{Kv}$ (6, 12) compared with the linear current-voltage relationship of the 5-HT response (Fig. 2A).

5-HT response appears to be mediated by cAMP

Cyclic nucleotides appear to mediate the response to biogenic amines in many neural systems (2, 20–23, 26, 30, 33, 34, 38, 42; see Ref. 29 for review). In Ap\textit{lysia}, the ionic conductances mediated by 5-HT and cAMP differ between various identified cells. Serotonin has been shown to elevate cellular cAMP levels and to generate a hyperpolarization associated with an increase in conductance to potassium in cell R15 of Ap\textit{lysia} (22, 30). In contrast, while both the tail sensory neurons and siphon sensory neurons also show increases in cellular cAMP in response to 5-HT (2, 34, 38), elevating cAMP in these neurons produces a depolarization associated with a decrease in input conductance (13, 26, 38, 41, 46a). The decreased conductance response to 5-HT reported here also appears to be mediated by cAMP. Bath application of the phosphodiesterase inhibitors IBMX and Ro 20-1724, the adenylyl cyclase activator forskolin, and the phosphodiesterase-resistant analogues of cAMP (db cAMP and 8-bromo-cAMP) all mimicked the action of 5-HT, with each generating inward currents associated with a decrease in input conductance (Fig. 6). In addition, the 8-bromo-derivative of cGMP also produced an inward current associated with a decrease in input conductance (Fig. 7). Levitan and Norman (31) have shown that high concentrations of either of the eight-position substituted derivatives of cAMP or cGMP block cAMP and cGMP phosphodiesterase equally when applied individually. The 8-bromo-cGMP response we observed could therefore be explained by an indirect elevation of cellular cAMP by blockade of the cAMP phosphodiesterase. Arguing against the role of phosphodiesterase inhibition is our finding that, in the presence of high concentrations of 8-bromo-cAMP, the 5-HT response was blocked, while in the same preparation 8-bromo-cGMP had little or no effect on the 5-HT response (Fig. 9), even though both 8-bromo-cAMP and 8-bromo-cGMP produced similar changes in membrane current and conductance (cf. Fig. 7). If 5-HT is acting through a cAMP second-messenger system and the 8-bromo-cGMP response is due to an indirect elevation of cAMP, one would expect responses to 5-HT to be altered subsequent to addition of the cGMP derivative. Further support for a role of cAMP in mediating the 5-HT response was demonstrated when low concentrations of both the phosphodiesterase inhibitor Ro 20-1724 and the adenylyl cyclase activator forskolin potentiated the 5-HT response. In addition, high concentrations of these agents blocked subsequent 5-HT responses. We have not examined how low concentrations of 8-bromo-cAMP and cGMP might alter 5-HT responses.

Although 8-bromo-cGMP had no effect on the 5-HT response, the similarity in current and conductance change to that produced by both 5-HT and 8-bromo-cAMP suggests that different internal mechanisms can generate parallel forms of cellular response. Such a redundancy of response expression would allow for an additive integration of afferent information, provided each internal messenger was increased through separate extracellular signals. This type of mechanism could produce summation of decreased conductance excitatory responses while still maintaining autonomy for each signal, since the intracellular mechanism for generating each response appears to be different. The separation of cellular mechanisms might also allow for differences in the sensitivity and/or ability of $Ca^{2+}$ to modulate each mechanism (3, 47). These possibilities are
speculative at this point, but should provide a fruitful direction for further research.

Functional significance of 5-HT response

Much of the behavioral repertoire seen in *Aplysia* has been shown to be modified by aversive stimulation (49). For example, a noxious stimulus to the tail produces sensitization of both the tail and gill withdrawal reflexes and a reduction in the threshold for inking (15, 19, 48; and our unpublished observations). Using the isolated abdominal ganglion preparation to analyze the electrophysiological correlates of sensitization of the gill withdrawal reflex and inking behavior, it has been demonstrated that stimulation of the connectives produces a slow and long-lasting depolarization in the ink motor neurons and the siphon sensory neurons (12, 18, 26). In addition, the cellular changes and resultant facilitation of the connections between the sensory neurons and their follower motor neurons produced physiologically can be mimicked by application of 5-HT (26). Serotonin has also been implicated in the facilitation of excitation-contraction coupling of the buccal musculature and tachycardia associated with food arousal (28, 32, 50), and mimics the sensitization of the tail withdrawal reflex produced by tail shock in *Aplysia* (48). The 5-HT response reported here further demonstrates the ability of 5-HTT to mimic the synaptic actions occurring during generalized behavioral arousal in *Aplysia*.

The same siphon sensory neurons that show presynaptic facilitation in response to both electrical stimulation of the connectives and 5-HT application also make synaptic connections with the ink motor neurons (8, 9). The ink motor neurons therefore not only are more excitable after noxious stimulation, but also have their sensory input facilitated presynaptically. Thus release of the neurotransmitter would produce larger postsynaptic currents in L14 due to its presynaptic action on the sensory neurons, and this enhanced postsynaptic current would flow across a larger input resistance. In each case the effects appear to be mediated by cAMP through a common mechanism (decreased K+ conductance). The net result of these parallel cellular changes would be a powerful facilitation of synaptic input in the L14 cells during behavioral arousal. An additional extension of this functional redundancy, yet to be investigated, is that 5-HT may act peripherally to enhance the excitability of the sensory neurons or may modulate the musculature of the ink gland as described in the buccal musculature (50) and in the opaline gland (44).

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