The Journal of Neuroscience, April 1988, 8(4): 1181–1193

Egg-Laying Hormone, Serotonin, and Cyclic Nucleotide Modulation of Ionic Currents in the Identified Motoneuron B16 of *Aplysia*

Mark D. Kirk,[1,2] Ronald Taussig,[2] and Richard H. Scheller[2]

[1]Department of Biology, Boston University, Boston, Massachusetts 02215, and [2]Department of Biological Sciences, Stanford University, Stanford, California 94305

We have used the 2-electrode voltage-clamp technique to analyze the effects of the neuropeptide, egg-laying hormone (ELH), and the biogenic amine 5-HT on ionic currents in the buccal motoneuron B16 of *Aplysia*. When B16 is voltage-clamped near resting membrane potential, bath-applied ELH induces a prolonged inward shift in holding current. The ELH-induced inward current is not due to a decrease in the transient, the delayed, or the calcium-activated potassium currents. Current–voltage measurements, along with ion substitution and channel-blocking experiments, indicate that ELH primarily induces or increases a voltage-dependent slow inward current carried by sodium.

Serotonin also causes a prolonged inward shift in holding current in B16. Like ELH, 5-HT induces or enhances the voltage-dependent inward current carried by sodium. In addition, 5-HT increases an inwardly rectifying potassium current, and, in some preparations, decreases an outward current that is activated when the cell is depolarized to −40 mV and above. None of the currents modulated by ELH or by 5-HT are affected by 200 μM ouabain or by reducing extracellular chloride concentration.

Extracellular application of isobutylmethylxanthine (IBMX), forskolin, 8-bromo-cAMP and 8-bromo-cGMP, and intracellular injection of cAMP elicits the slow inward current carried by sodium. The inward current response to ELH is blocked by prior application of 8-bromo-cAMP, while, under these conditions, 5-HT continues to elicit the increase in inward-rectifier potassium current, but decreases the slow inward sodium current. Serotonin also reduces the slow inward sodium current when applied after ELH. These results suggest that the modulation of B16 by ELH may be mediated entirely by either cAMP or cGMP, while at least a portion of the response to 5-HT may involve an unidentified second messenger in addition to cyclic nucleotides.

Neuropeptides form a class of bioactive chemical messengers that is ubiquitous in the animal kingdom. They can act at a distance as hormones modulating the activity of neurons and/or organ systems. Peptides can also function in a fashion similar to that of conventional transmitters, with their effects restricted to a small subset of synapses or neurons (Jan and Jan, 1982, 1983; Lundberg and Hökfelt, 1983; Mayeri et al., 1985). The influences of neuropeptides on behavior range from the modulation of neuromuscular responses and initiation of fixed action patterns to the control of pain and effects on learning (Snyder and Innis, 1979; Snyder, 1980; Krieger, 1983; Belardetti et al., 1987).

The purified neuropeptide, egg-laying hormone (ELH) of *Aplysia,* when injected into a sexually mature animal, can initiate egg-laying behavior (Kupfermann, 1970; Stuart and Strumwasser, 1980). Egg-laying behavior in *Aplysia* is a fixed action pattern that consists of a cessation of locomotion, head-waving movements (to clean the substrate and deposit the eggs), and an inhibition of feeding (Arch and Smock, 1977). ELH causes a prolonged excitation of buccal ganglion motoneuron B16 (Stuart and Strumwasser, 1980; Ram, 1983). B16 is one of 2 identified cholinergic motoneurons that innervate the accessory radula closer (ARC) muscle, which closes and retracts the radula and is used to grasp food (Cohen et al., 1978). The prolonged firing of B16 induced by ELH and the consequent retraction or closing of the radula might aid in preventing ingestion of the egg string, and therefore may contribute to the inhibition of feeding behavior during egg laying. Accordingly, the direct excitation of B16 by ELH may represent an important part of the egg-laying fixed action pattern.

The biogenic amine 5-HT modulates a variety of currents in many central neurons of *Aplysia* and related mollusks (Gerschenfeld and Paupardin-Tritsch, 1974a; Klein and Kandel, 1980; Deterre et al., 1982; Benson and Levitan, 1983; Pellmar, 1984; Walsh and Byrne, 1985; Paupardin-Tritsch et al., 1986a, b) It is also involved in the control of many aspects of behavior in *Aplysia.* When released by the metacerebral cell (MCC), 5-HT mediates certain aspects of food-induced arousal (Weiss et al., 1980; Rosen et al., 1983). In addition, 5-HT can initiate locomotion when injected into the hemocele (Mackey and Carew, 1983), and it appears to be a transmitter responsible for sensitization and associative conditioning of gill, siphon, and tail withdrawal (Klein and Kandel, 1980; Kandel et al., 1983; Byrne, 1985; Carew and Sahley, 1986).

Several cells of the buccal ganglion, including B16, receive synaptic input from the MCC (Gerschenfeld and Paupardin-Tritsch, 1974b; Weiss et al., 1978). Furthermore, B16 is directly excited by 5-HT (Kirk et al., 1985; Kirk and Scheller, 1986b; Ram et al., 1986; Sossin et al., 1987) and by agents that increase intracellular cAMP (Kirk and Scheller, 1986b; Ram et al., 1986).

Received Feb. 18, 1987; revised Nov. 27, 1987; accepted Nov. 27, 1987.

We wish to thank Wayne Sossin for expert computer assistance and helpful discussions, and Dr. Stuart Thompson for help in constructing the voltage clamp, and for useful discussions and data analysis in the early phases of this project. We thank Drs. Matt Lee, Richard Aldrich, and Stuart Thompson for reading an early draft of this manuscript. This work was supported by a grant to R.H.S. from the NIMH. R.H.S. is a PEW scholar and the recipient of a Presidential Young Investigator Award from the NSF.

Correspondence should be addressed to Dr. Scheller at the above address.

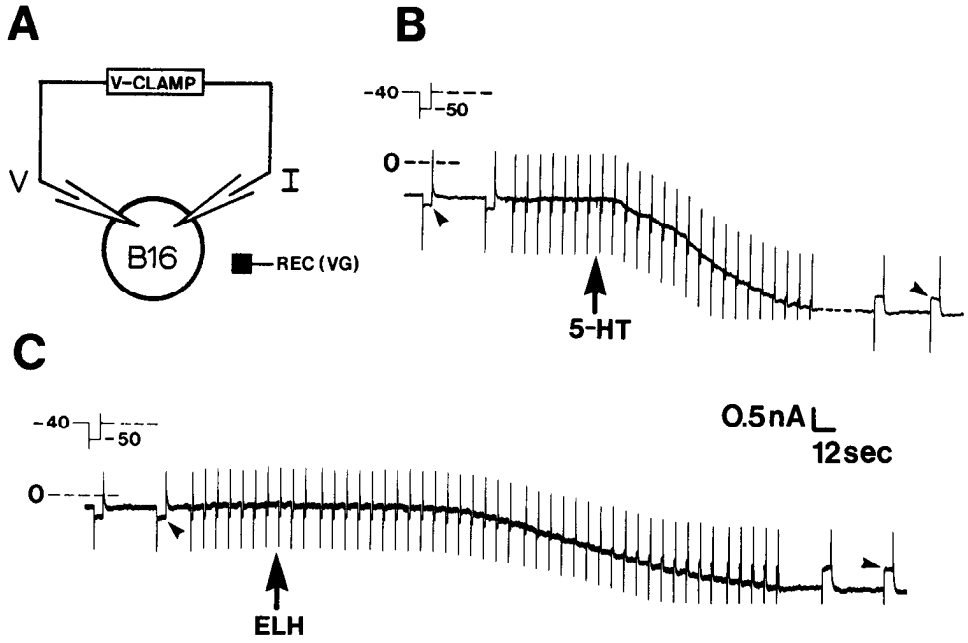


Figure 1. Responses to 5-HT and ELH recorded in voltage clamp. *A*, Schematic showing the voltage clamp of B16. Current was recorded (*REC*) with a virtual ground (*VG*). *B*, 5-HT was applied to the bath at the *arrow*; note the inward shift in the holding current and the reversal of the step current (compare step currents indicated by *arrowheads*). *C*, Different preparation where ELH was bath-applied at the *arrow* and caused an inward shift in holding current and reversal of the step current (*arrowheads*). (Reproduced from Kirk and Scheller, 1986a.)

Therefore, B16 provides an excellent neuron with which to study the molecular mechanisms of peptide action as well as the interaction of a neuropeptide with the more conventional transmitter 5-HT. In this report, the ionic currents in B16 that are modulated by ELH and 5-HT were analyzed by voltage-clamp techniques.

Materials and Methods

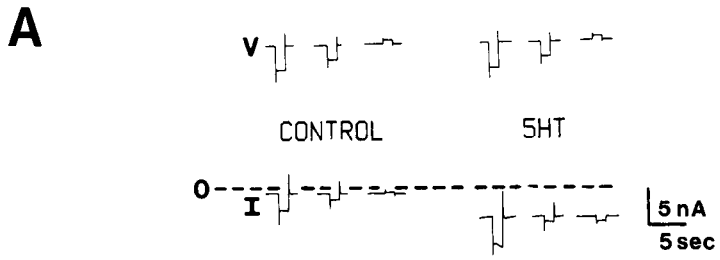
All experiments were performed on *Aplysia californica* (75–200 gm) purchased from Sea Life Supply (Seaside, CA). Generally, before dissection the animals were anesthetized by injection with isotonic $MgCl_2$ equivalent to 50% of their body weight. The physiological experiments were performed at room temperature (22–25°C). The mean data presented are given ±SD. Buccal ganglia were removed and B16 was identified and prepared for voltage clamp according to the procedure of Kirk and Scheller (1986a). Prior to intracellular recording, the sheaths overlying the rostral surfaces of the buccal ganglia were removed. In experiments not involving ELH, trypsin (Sigma, type IX) was usually applied at a concentration of 10% wt/vol in normal artificial seawater (ASW; see below) for a period of 5–10 min to facilitate the desheathing process. The trypsin treatment was reduced or omitted when using ELH because it interfered with the response to ELH even after the preparation was rinsed repeatedly with fresh ASW. Cells were used only if they had a resting membrane potential of at least −35 mV after axotomy. B16 was voltage-clamped with 2 microelectrodes (Fig. 1*A*), using a voltage clamp similar to that described by Barish and Thompson (1983).

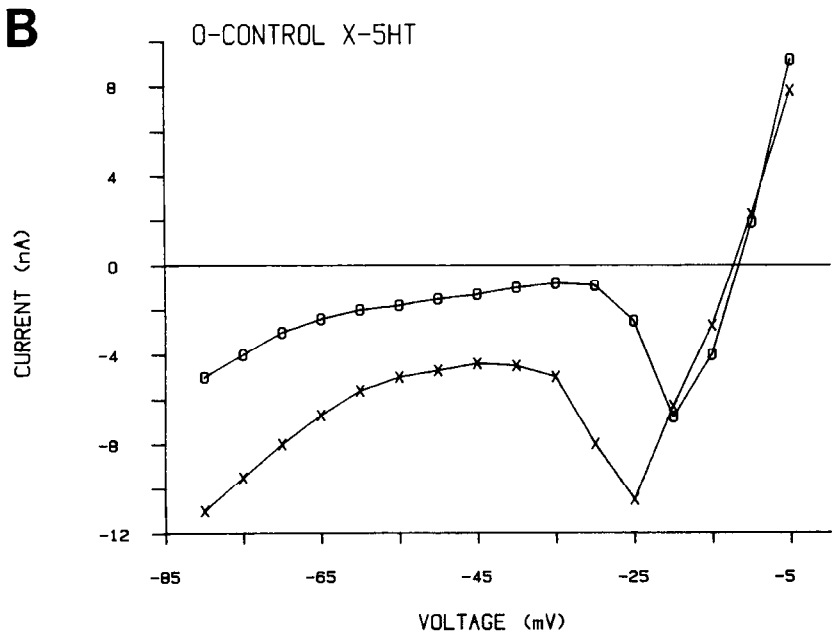
The cell's membrane potential was recorded differentially between the voltage-sensing microelectrode filled with 3 M KCl (3–8 MΩ) and a saline–agar bridge placed in the bath. The current-passing electrode was filled with 3 M potassium acetate (5–10 MΩ), and current was monitored with a virtual-ground circuit (Fig. 1*A*). The signals from the virtual ground were low-pass-filtered with an 8-pole Bessel filter (Frequency Development). Cutoff frequencies were 10–100 Hz for measurements of currents during 1 sec (or longer) voltage pulses and 1 kHz for all other current measurements. The currents obtained during long voltage steps (e.g., 1 sec) were plotted with a pen recorder (Gould). Recordings of transient potassium current (I_a) and delayed potassium current (I_k) were digitized at 1 kHz and stored on diskettes. Delivery of voltage pulses and data acquisition and analysis were performed with an IBM PC XT and software purchased from Axon Instruments (pCLAMP). Stored current traces were also reproduced with an HP plotter (7470A).

A single-barreled electrode filled with 0.2 M cAMP (sodium salt) was used to inject cAMP intracellularly by passing currents between this electrode and the current-injecting electrode of the voltage clamp. First, a voltage was applied to the cAMP-containing electrode while its tip was placed in the bath, and its current-passing ability was monitored using the virtual ground (currents between 270 and 300 nA were used). Then the tip was inserted into B16 and the same voltage was applied. Finally, after the intracellular injection, the electrode was removed and again tested for its ability to pass current. If the electrode had become blocked during this procedure, the results were discarded.

Solutions. Normal ASW solution had the following composition: 490 mM NaCl, 11 mM KCl, 19 mM $MgCl_2$, 30 mM $MgSO_4$, 11 mM $CaCl_2$, and 10 mM Tris-HCl, pH 7.6. In salines with reduced sodium, sodium was replaced with equimolar amounts of either Trizma-7.8 (pH 7.8; Sigma) or *N*-methyl-D-glucamine (Sigma; pH adjusted to 7.6 with concentrated hydrochloric, acetic, or sulfuric acid). For salines low in Cl^-, the NaCl was replaced with 490 mM sodium acetate (final Cl^- concentration, 51 mM). When used, TEA (100 mM), 4-aminopyridine (4 mM), and tetrodotoxin (60 μM) (all from Sigma) were added hypertonically to the saline. When CsCl (10–20 mM) was used, it either replaced potassium or was added hypertonically. To block chemical synaptic transmission, most of the salines contained either (1) zero Ca^{2+} with 10 mM Co^{2+}, or (2) normal Ca^{2+} with 30 mM Co^{2+} added hypertonically. Synaptic transmission was not blocked when sodium was replaced with Trizma-7.8 because high concentrations of Tris and Co^{2+} formed a precipitate. The solutions containing Co^{2+} effectively blocked inward calcium currents (and the calcium-activated potassium current; data not shown), and blocked all chemically mediated synaptic transmission, ensuring that the results observed were due to direct effects. Concentrated stock solutions of the following substances were made in the appropriately buffered saline solutions and diluted into the bath at the concentrations indicated: isobutylmethylxanthine (IBMX; 1 mM), forskolin (100 μM), 8-bromo-adenosine-3′:5′ cyclic monophosphate (8-bromo-cAMP; 250–500 μM), 8-bromo-guanosine-3′:5′ cyclic monophosphate (8-bromo-cGMP; 93–156 μM), 8-bromo-adenosine-5′ monophosphate (8-bromo-5′ AMP; 500 μM), 8-bromo-guanosine-5′ monophosphate (8-bromo-5′ GMP; 500 μM), and 5-hydroxytryptamine creatine sulfate complex (5-HT; 50–250 μM).

Aplysia ELH was purified from crude preparations of synthetic peptide (Peninsula Labs) or from bag cells of large (0.5–2 kg) *A. californica* collected at Moss Landing, CA. Bag cell clusters from these animals were isolated and ELH was purified according to the procedures of Chiu et al. (1979). The ELH was tested for purity using high-performance liquid chromatography (HPLC) and electrophoresis in acrylamide gels containing $NaDodSO_4$ and urea. ELH was dialyzed into the appropriate saline solutions and stored at −70°C until used. Aliquots (5–25 μl) of these ELH stock solutions (20 μM) were brought to room temperature and were applied in the bath with final concentrations between 100 and 500 nM.





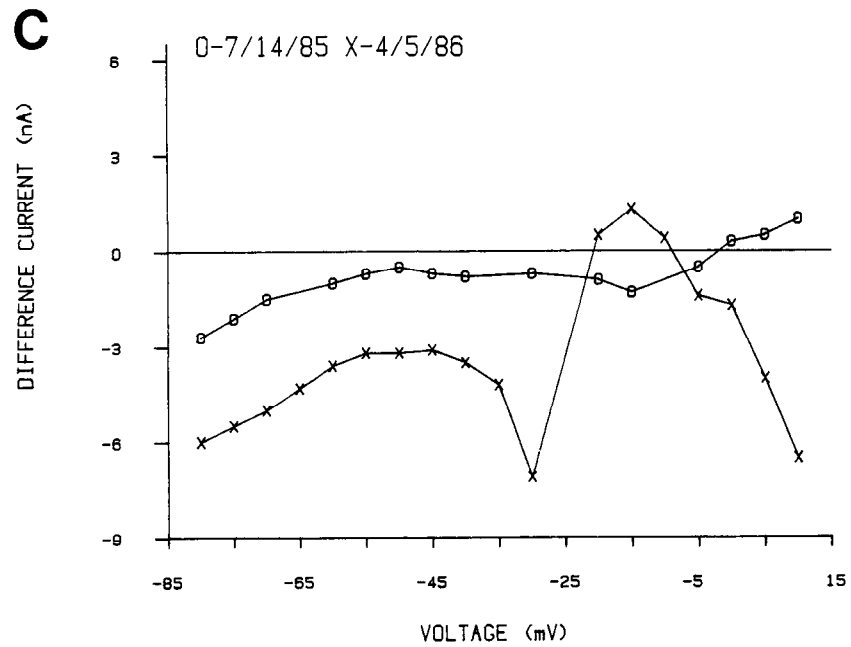


Figure 2. Effects of 5-HT on the I–V relationships of B16 (normal Na^+). *A*, Selected current records from voltage steps to −75, −55, and −30 mV showing the increases in inward-rectifier current (at −75 mV) and in the slow inward current (at −30 mV). *B*, I–V curves taken in control condition and during the response to 5-HT. *C*, Difference I–V curves from 2 different preparations showing the 5-HT-induced currents. In both cases, increased currents at negative voltages were observed, indicating the increased inward rectifier. Both cases also showed some evidence for an increased I_{in} (for ×, from −40 to −30 mV; for 0, from −30 to −10 mV). However, they differed for currents recorded at depolarized levels (−5 mV and above). In one case (O), there was no evidence for any decrease in outward currents, while in the other case (×), there was a clear decrease in outward currents at depolarized potentials.

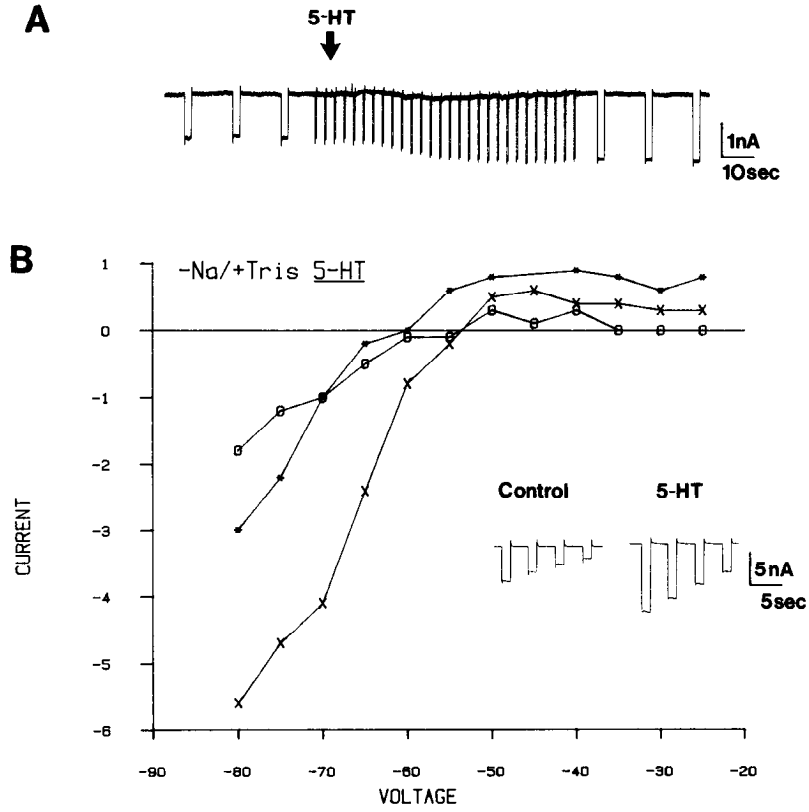


Figure 3. 5-HT increases an inward-rectifier current. *A,* The preparation was bathed in saline lacking Na^+ and 5-HT was applied at the *arrow.* Note the large increase in step-current amplitude, indicating an increased conductance (holding potential, −50 mV). *B,* Difference I–V curves from 3 different preparations, where Tris replaced Na^+, showing the 5-HT-induced current. Note the increased magnitude at negative voltages and the rectification at depolarized levels. *Inset,* Voltage-clamp current recordings in response to voltage steps to −80, −75, −70, and −65 mV, both in the control condition and in the presence of 5-HT.

Results

ELH and 5-HT increase a voltage-dependent slow inward current

When B16 is voltage-clamped near resting membrane potential in normal saline, and ELH or 5-HT is applied to the bath, a slow inward shift in the holding current is observed (Fig. 1, *B,C*). The mean latency for the initial response for 5-HT was 31 ± 12 sec (n = 7), as compared to 98 ± 90 sec for that of ELH (Kirk and Scheller, 1986a). Although substantial time for diffusion through the bath is required, the latencies to inward shifts of holding currents of similar magnitude were consistently shorter for 5-HT than for ELH. For the range of concentrations used in these studies (ELH, 100–500 nM; 5-HT, 50–250 μM), the mean peak amplitudes of the slow inward shifts in holding currents were −1.9 ± 1.0 nA (n = 21) for ELH (Kirk and Scheller, 1986a) and −2.9 ± 0.9 nA (n = 9) for 5-HT.

To monitor input resistance changes of B16, hyperpolarizing steps were applied from a holding potential of −40 or −50 mV during application of the transmitters (all step currents were obtained from 10 mV hyperpolarizations 1 sec in duration). As illustrated in Figure 1, *B, C,* the response to a hyperpolarizing voltage step was an inward current step (step current; see arrowheads at beginning of traces in Fig. 1). During the inward shift in holding current, the step current decreased and then actually reversed direction and became outward with respect to the new holding current level (see arrowheads at the end of traces in Fig. 1). The outwardly directed step current in response to a hyperpolarizing voltage step indicated that a negative slope resistance had appeared at this membrane potential. These results suggested that ELH and 5-HT induced a voltage-dependent slow inward current that partially deactivated during the hyperpolarizing voltage steps. This was observed in 14 out of 19 cases for ELH, and in 3 out of 12 cases for 5-HT. For ELH, in those cases where a reversal in the step current was not observed, the step current decreased in magnitude. The fewer observations of step-current reversal in response to 5-HT likely were due to the overlapping activation of several currents (see below). However, in all cases in which current–voltage curves were constructed (from a holding potential of −40 mV) before, during, and after the response to either transmitter, the induction of a negative slope-resistance region was confirmed (see below).

The I–V curves constructed before, during, and after the response to 5-HT in normal ASW (Fig. 2) were more complex than those for ELH (see Fig. 3; Kirk and Scheller, 1986a). These data, and other experiments with ion substitution and addition of channel-blocking agents (see below), revealed several different currents that were affected. First, as stated above, the reversal of the step current indicated an increase in a slow, noninactivating inward current similar to that affected by ELH. Accordingly, in normal ASW, the I–V curves revealed an increase in the negative resistance region where the slow inward sodium current was maximal (−45 to −25 mV) (Fig. 2, *B, C*).

The reversal potential for the 5-HT effect was determined from difference I–V curves. These curves were constructed by subtracting the I–V curves in the control conditions from the I–V curves obtained during the respective responses. The reversal potential is taken as the point where the difference current curve crosses the zero-current axis. The mean reversal potential obtained in normal ASW for 8 preparations in which the difference current crossed the zero-current axis was −14 ± 10 mV, very similar to that observed for ELH (−14 ± 8 mV) (Kirk and Scheller, 1986a). As was found for the response to ELH, the inward shift in holding current and the increase in the slow inward current by 5-HT were eliminated in salines lacking so-

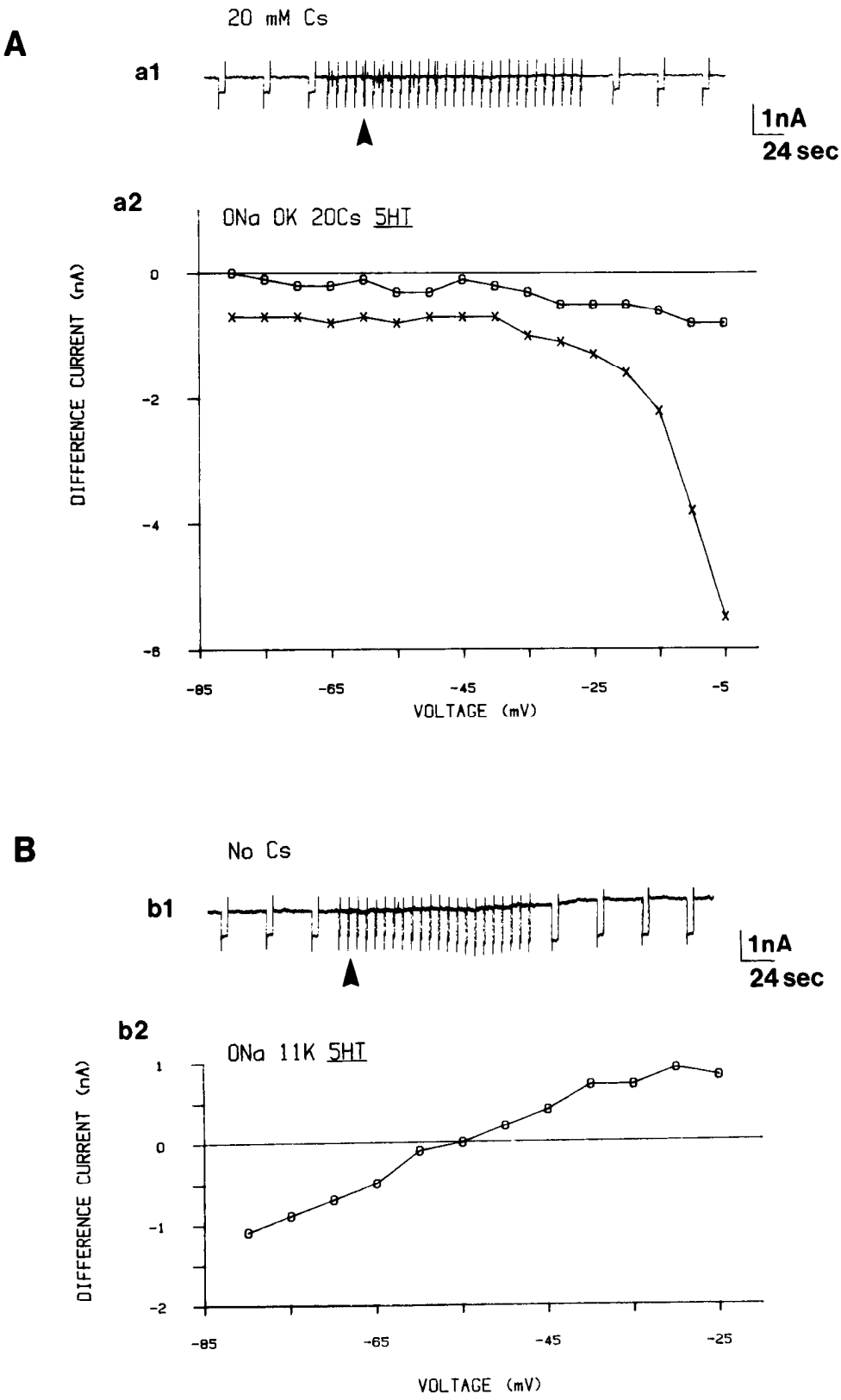


Figure 4. Cesium (Cs^+) blocks the inward-rectifier response to 5-HT (*A*), which recovers when Cs^+ is replaced with K^+ (*B*). *a1*, In saline lacking sodium and with 20 mM Cs^+ replacing K^+, the increased inward rectifier is blocked and no change in holding or step current is seen following application of 5-HT at the *arrowhead* (holding potential, −50 mV). *a2*, Difference I–V curves showing 5-HT-induced currents from 2 different preparations (○, same as preparation in *a1*) perfused with 0 Na^+, 0 K^+, 20 mM Cs^+ ASW. The inward-rectifier current was blocked, but outward currents seen above −40 mV were decreased. *B*, The same preparation as in *a1* was washed extensively with saline without Cs^+ and with normal K^+, and the inward-rectifier response to 5-HT was restored.

dium ions (Fig. 3) and were unaffected by ouabain (data not shown). Therefore, one current modulated by 5-HT is I_{in}.

5-HT increases an inward-rectifier potassium current

When 5-HT was applied to a preparation in which the sodium had been replaced with equimolar Tris or *N*-methyl-D-glucamine, and 10 mV hyperpolarizing voltage steps were applied from a holding potential of −50 mV, the holding current shifted slightly in the outward direction and the step current increased dramatically ($n = 9$) (Fig. 3). Difference I–V curves demonstrated that this 5-HT-induced current increased with hyperpolarization and decreased (or rectified) at depolarized levels (Fig.

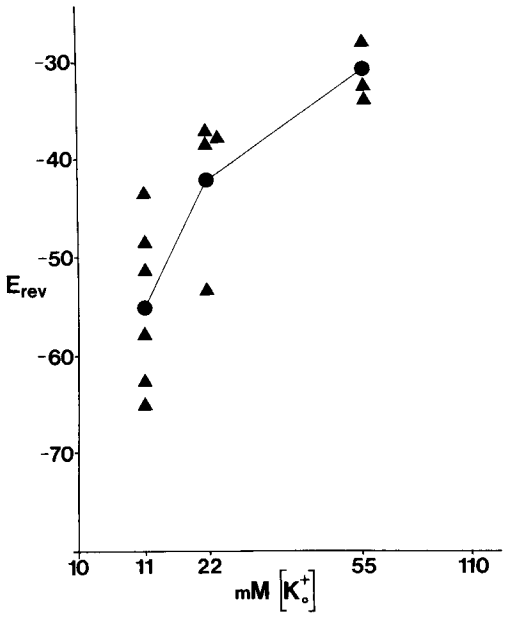


Figure 5. Elevated extracellular K^+ shifts the reversal potential (E_{rev}) of the 5-HT-induced inward rectifier. The reversal potential for the 5-HT effect on the inward rectifier was derived from difference I–V curves at the point where the difference current curve crossed the zero-current axis. The reversal potentials were determined in sodium-free ASW at 3 different potassium concentrations: 11 mM (1 time), 22 mM (2 times), and 55 mM (5 times). *Triangles* represent data from separate preparations; *round dots* are the means for these data.

3*B*). The mean reversal potential of this 5-HT response in 11 mM K^+ (normal ASW) was -57 ± 9 mV ($n = 6$). Although this value is more positive than the expected potassium equilibrium potential (-75 mV; Brown and Kunze, 1974), this current was blocked entirely by substituting 20 mM Cs^+ for K^+ ($n = 6$) (Fig. 4), the reversal potential was increased with elevated extracellular potassium (Fig. 5), and the voltage dependence of the current was altered by changing extracellular potassium concentration (data not shown). These results suggest that 5-HT increases an inward-rectifier potassium current (Benson and Levitan, 1983; Hille, 1984).

Although the reversal potential for the inwardly rectifying current in 11 mM K^+ was more positive than the expected potassium equilibrium potential (and reversal potentials for other potassium currents in *Aplysia* neurons; Thompson, 1977; Benson and Levitan, 1983), the reversal potential obtained in 55 mM K^+ (mean $= -33 \pm 2$ mV, $n = 3$; Fig. 5) was very close to that predicted by the Nernst relation. Using an activity coefficient of 0.6 for both intracellular and extracellular potassium (Brown and Kunze, 1974), the predicted equilibrium potential for potassium (at 20°C) with an extracellular concentration of 55 mM (5 times) is -37 mV.

In 3 preparations, we found evidence for a decrease in an outward current in the presence of 5-HT. To reveal this current, we eliminated I_{in} by replacing Na^+ with an impermeant ion (*N*-methyl-D-glucamine) and blocked the inward-rectifier potassium current with Cs^+ (Fig. 4). Although we did not investigate the remaining current in detail, the shape of the difference I–V curve (decreased outward currents at levels more depolarized than about -40 mV) resembles that of the S-current found in sensory neurons of *Aplysia,* which is also decreased by 5-HT (Klein et al., 1982; Siegelbaum et al., 1982; Pollock et al., 1985). The decrease in outward current caused by 5-HT was not always found (see Fig. 2*C*, 7/14/85); evidence for this effect was seen in only 50% of the experiments in low Na^+ saline with Cs^+ added. The effects of 5-HT on other potassium currents were examined; 5-HT had no effect on the delayed outward current (I_k) or the transient K^+ current (I_a) (data not shown).

Second-messenger effects

We have also investigated the effects on B16 of bath-applied membrane-permeable analogs of cAMP and cGMP, cAMP injected directly into B16, and applied agents that increase the intracellular concentration of cAMP. Addition of 8-bromo-cAMP induced the voltage-dependent slow inward current carried by Na^+ ($n = 15$; Fig. 6). In normal ASW containing Co^{2+}, the mean inward shift in holding current induced by 8-bromo-cAMP was -2.9 ± 2.3 nA ($n = 7$), and the mean reversal potential was -8 ± 14 mV ($n = 6$). The difference I–V curves for 2 preparations, shown in Figure 6*C*, illustrate the increase in the negative slope-resistance region that was particularly prominent in the range from -45 to -30 mV. Occasionally, there were also increases in the difference current found at very negative (-60 to -80 mV) and at positive voltages in the presence of 8-bromo-cAMP. In some cases, these increases resulted from drifts in the recorded currents that were not reversed even 2–4 hr after removal of the cAMP analogs.

Replacing sodium with the impermeant ion *N*-methyl-D-glucamine reduced or eliminated the inward shift in holding current and any persistent change in step current produced by 8-bromo-cAMP (Fig. 7*A*). When the same preparation was subsequently perfused with saline containing a normal sodium concentration, application of 8-bromo-cAMP elicited a large inward shift in holding current (Fig. 7*B*) associated with an increase in I_{in} (I–V data not shown). As was the case for the ELH and 5-HT responses, in 8-bromo-cAMP the step current often either became less inward during the response (Fig. 7*B*) or reversed direction and became outward with respect to the new holding current (see Fig. 6*A*). These data, combined with the I–V curves generated before and during the responses, indicated that 8-bromo-cAMP induced I_{in}, which partially deactivated during the hyperpolarizing voltage steps, resulting in the decrease in step-current amplitude observed in Figure 7*B* as the outwardly directed step currents illustrated in Figure 6*A* (for a more detailed explanation concerning deactivation of voltage-dependent currents, see Kramer and Zucker, 1985). Therefore, the only current that was consistently and reversibly increased by 8-bromo-cAMP was the slow inward current carried by sodium.

Intracellular cAMP injections ($n = 4$) gave results similar to those observed with the extracellular application of 8-bromo-cAMP. The introduction of cAMP into B16 induced a slow inward shift in holding current whose magnitude and duration depended on the magnitude and duration of the injection (Fig. 8). The response to cAMP outlasted the time of injection by several minutes in some cases. In addition, the step current (from a holding potential of -40 mV) reversed direction during the response to cAMP, indicating the induction of I_{in} (Fig. 8*C*). Current–voltage curves confirmed the increase in I_{in} throughout the voltage range tested (-80 to -30 mV), and in one experiment the response to cAMP injection was eliminated by perfusing the ganglion with saline low in sodium (data not shown). Similar results have been observed in other neurons of *Aplysia*

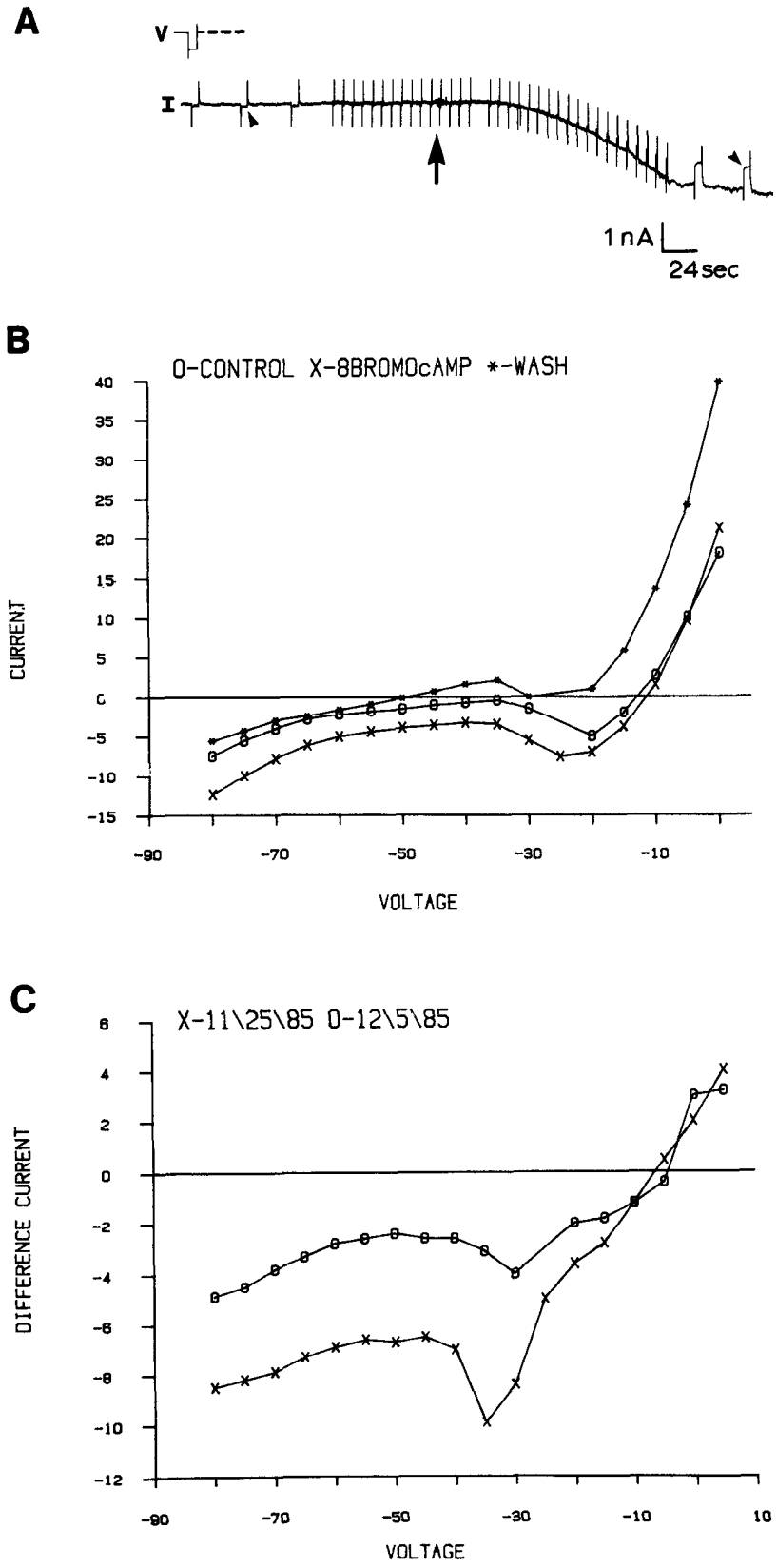


Figure 6. 8-Bromo-cAMP increases I_{in}. *A,* Bath application of 8-bromo-cAMP at the *arrow* caused the inward shift in holding current and reversal in step current indicative of an increase in I_{in} (holding potential, −40 mV). *B,* I–V curves for the preparation shown in *A*. *C,* Difference I–V curves for 2 preparations, showing the currents induced by 8-bromo-cAMP. Note the increased inward current throughout the voltage range from −80 to −10 mV, the increased inward current in the range where I_{in} is maximal (−40 to −25 mV), and the reversal potentials between −10 and −5 mV, similar to the response to ELH.

and related mollusks by Connor and Hockberger (1984). Therefore, the direct injection of cAMP into B16 induces the slow inward current carried by sodium.

In addition, the adenylate cyclase activator forskolin ($n = 2$), and the phosphodiesterase inhibitor IBMX ($n = 2$) increased I_{in}. However, the search for possible effects on outward potassium currents activated at levels above −10 mV was confounded by the fact that ethanol (the carrier solution for both agents),

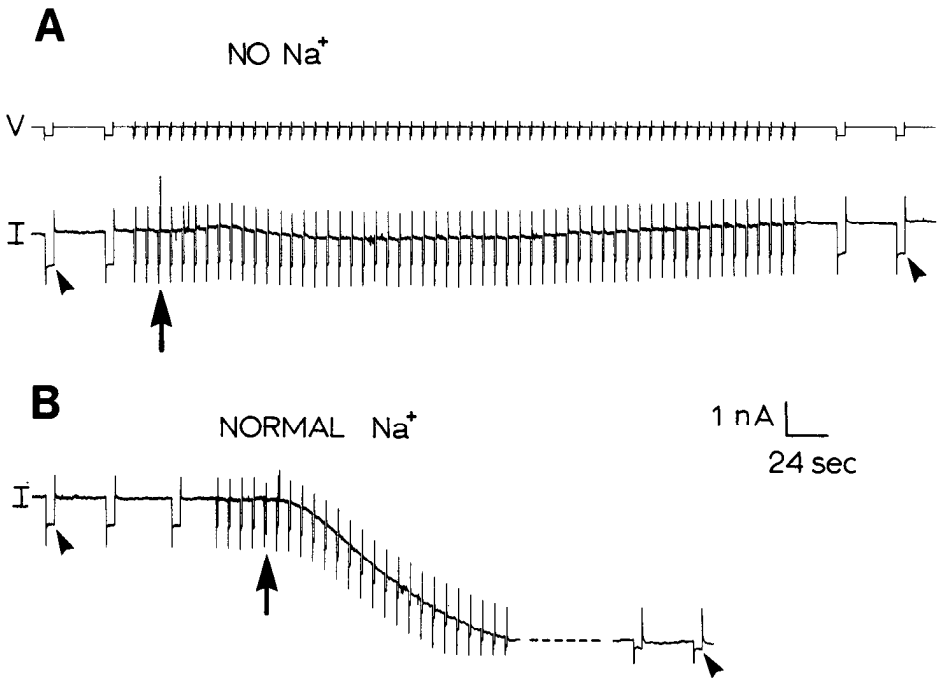


Figure 7. The inward current response to 8-bromo-cAMP is Na^+-dependent. *A*, In the absence of Na^+, bath application of 8-bromo-cAMP at the *arrow* causes little change in holding current or step-current amplitude (*arrowheads*) (holding potential, −40 mV). *B*, The preparation was extensively perfused with saline containing normal Na^+, and 8-bromo-cAMP was reapplied (*arrow*). Note the large inward shift in holding current and the dramatic decrease in step current (*arrowheads*). The increase in I_{in} was confirmed from I–V curves (not shown).

at equivalent concentrations, strongly inhibited outward currents at these potentials.

We found that the membrane-permeable analog of cGMP, 8-bromo-cGMP, also induced the voltage-dependent slow inward current carried by sodium (n = 4; Fig. 9*A*); the I–V and difference I–V curves for one experiment are shown in Figure 9, *B* and *C*, respectively. The activation of an inward sodium current in molluscan neurons by both cAMP and cGMP was first reported by Connor and Hockberger (1984). The current responses described above were specific to cAMP and cGMP because bath applications of the noncyclic nucleotides 8-bromo-5′ AMP and 8-bromo-5′ GMP did not alter the holding current or I_{in} (see also Connor and Hockberger, 1984).

Additivity experiments

ELH, 5-HT, cAMP, and 8-bromo-cAMP all induce or enhance a slow inward Na^+ current, I_{in}, in B16. To determine whether cAMP or cGMP might be acting as second messengers for ELH or 5-HT responses, we applied these agents sequentially in various combinations and observed their summed effects. Figure 10*A* gives an example of I–V relationships from an experiment in which ELH was applied following 5-HT. In 4 of 6 such cases, ELH caused a further increase in the slow inward sodium current, although the inward shift in holding current was slightly attenuated (the response to ELH was only partially occluded). However, when the transmitters were applied in reverse order,

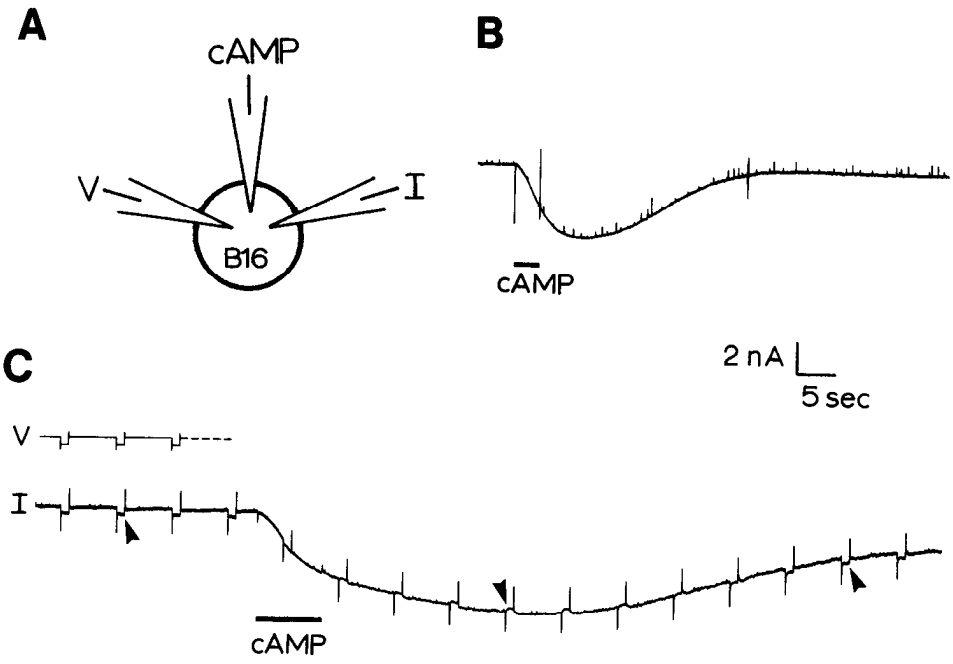


Figure 8. Intracellular injection of cAMP induces I_{in}. *A*, Schematic of the preparation showing the voltage (*V*) and current (*I*) electrodes of the voltage clamp and the injection electrode containing cAMP. *B*, cAMP, injected as marked by the *black bar*, caused a prolonged inward shift in the holding current. *C*, A more prolonged injection of cAMP was applied while monitoring step currents at a holding potential of −40 mV. Note that the step currents reversed at the peak of the response to cAMP and slowly recovered (*arrowheads*). The effect on I_{in} was confirmed from I–V curves obtained during other injections of the same B16 (data not shown).

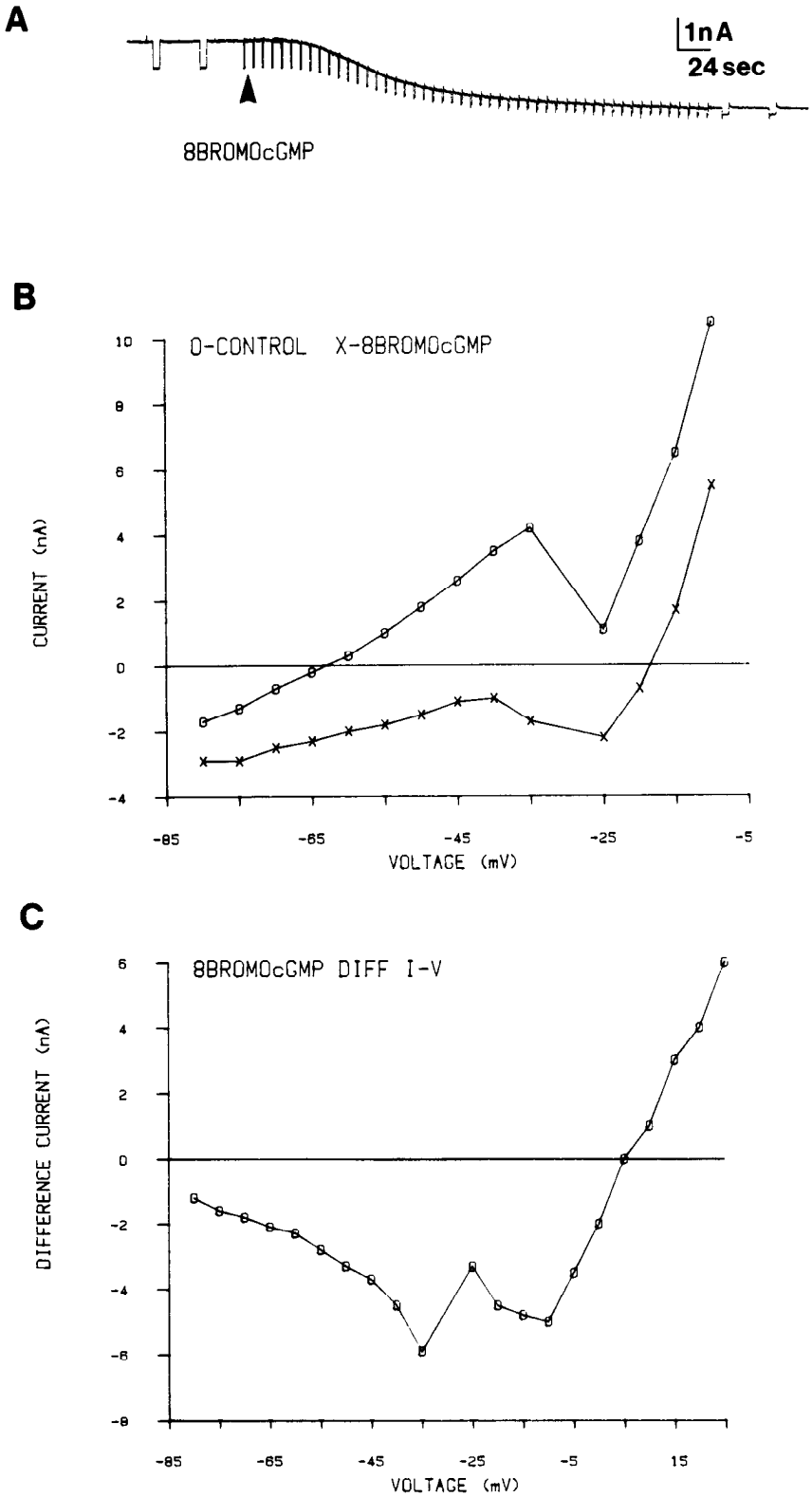


Figure 9. 8-Bromo-cGMP induces I_{in}. *A*, 8-Bromo-cGMP was bath-applied at the *arrowhead*, and caused a large inward shift in holding current and decrease in step-current amplitude. *B*, I–V curves from the preparation shown in *A*. *C*, Difference I–V curve constructed from *B*, showing the current induced by 8-bromo-cGMP. Note the increased inward current from −80 to −5 mV, which is maximal between −40 and −10 mV, where I_{in} is maximal. In addition, the reversal potential is near 0 mV, consistent with the response to ELH, 5-HT (in normal Na^+), and 8-bromo-cAMP.

the result was quite different. When ELH was applied first (and I_{in} enhanced), the subsequent application of 5-HT showed a reduced inward shift in holding current, actually causing a marked decrease in I_{in} between −25 and 0 mV (6 of 6 cases, Fig. 10*B*). In these experiments, 5-HT also increased the inward-rectifier current (4 of 6 cases), consistent with the lack of effect of ELH on this current.

Bath applications of high concentrations (500 μM) of 8-bromo-cAMP resulted in the inward shift in holding current and increased I_{in} (holding potential, −40 mV). Subsequent application of ELH showed no further increase in holding current or I_{in}, suggesting that the ELH-induced increase in I_{in} may be mediated by cAMP. The same was true for 5-HT following 8-bromo-cAMP; that is, the inward current response to 5-HT was oc-

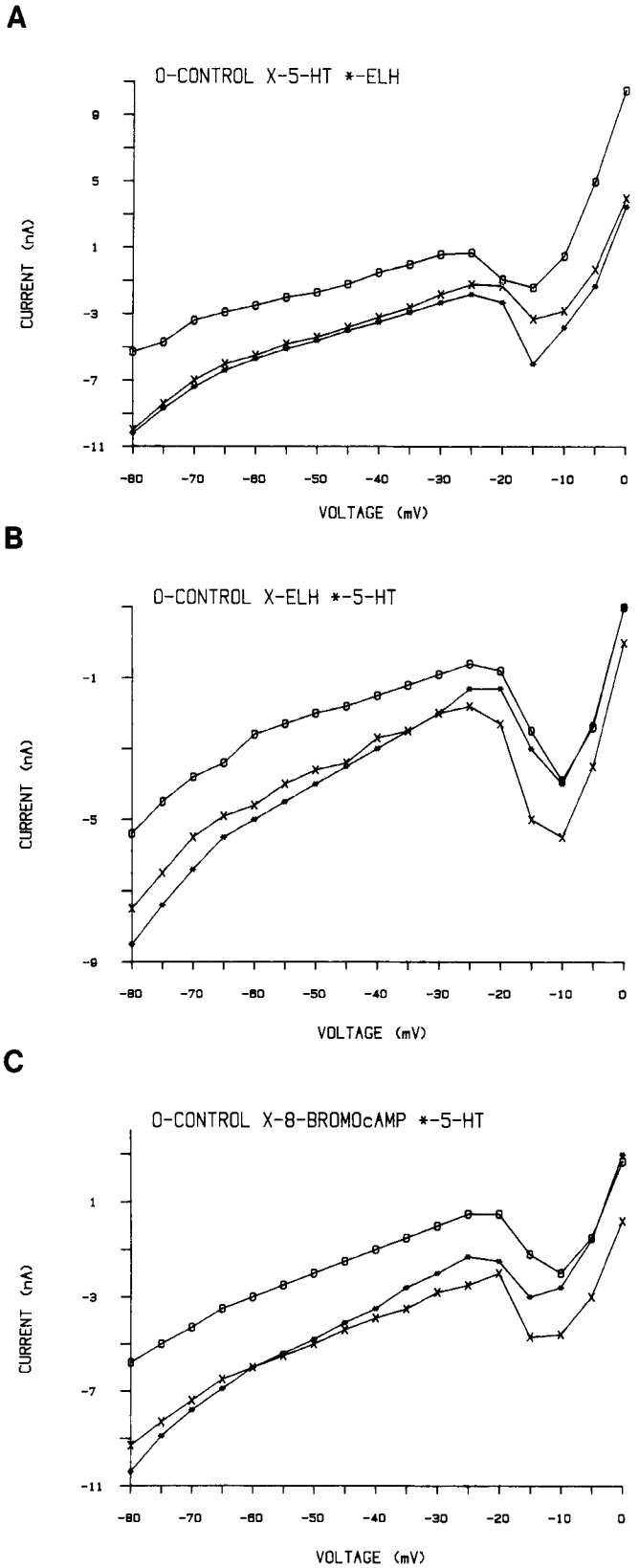


Figure 10. Additivity experiments (normal Na^+). *A*, 5-HT was followed by ELH. 5-HT increased I_{in} and ELH increased it slightly more, mostly between −35 and −5 mV. *B*, ELH was followed by 5-HT. ELH caused a large increase in I_{in} and 5-HT decreased I_{in}, especially between −25 and 0 mV. *C*, 8-Bromo-cAMP was followed by 5-HT. Note that 5-HT decreased the previously induced I_{in}, as seen at voltages above −40 mV, and increased the inward rectifier, as seen at potentials below −60 mV.

cluded, although, in the presence of 8-bromo-cAMP, 5-HT consistently (8 of 8 cases) caused an outward shift in holding current associated with an apparent increase in membrane conductance (Fig. 11). However, this apparent increase in conductance was primarily due to a decrease in the previously activated slow inward sodium current between −40 and 0 mV (3 of 4 cases), as shown in Figure 10*C*. This effect persisted in saline, where Cs^+ replaced K^+, and therefore is not due to outward current through the inward-rectifier potassium channel. In normal ASW, pretreatment with 8-bromo-cAMP had no detectable effect on the 5-HT-induced increase of the inward rectifier (4 of 4 cases). Therefore, when I_{in} had previously been enhanced by ELH or 8-bromo-cAMP, 5-HT caused a decrease in this current instead of the increase it produced when applied alone.

When high concentrations of 8-bromo-cAMP were followed by 8-bromo-cGMP, the response to the latter compound was occluded (data not shown), indicating little or no additivity. In addition, the outward shift in holding current following 8-bromo-cAMP (holding potential, −40 mV) in response to 5-HT was unaffected by addition of 8-bromo-cGMP. This outward shift does not appear to be mediated by either cAMP or cGMP, suggesting that another second-messenger system may be involved.

Discussion

By studying the effects of peptides and other transmitters on neurons with known functions, we hope to relate the actions of the transmitters to their specific roles in the control and/or modulation of behavior. ELH appears to mediate certain aspects of the egg-laying behavior, and the inhibition of the consummatory phase of feeding (during egg-laying; Arch and Smock, 1977; Stuart and Strumwasser, 1980) may be mediated by specific actions of ELH on identified feeding neurons found in the buccal or cerebral ganglia (Kupfermann, 1974; Stuart and Strumwasser, 1980).

The role of the serotonergic MCC in the mediation of feeding arousal has been studied extensively by Kupfermann, Weiss, and their colleagues (Weiss et al., 1980; Rosen et al., 1983). The release of 5-HT by the MCC causes an enhanced responsiveness in both B16 and the ARC muscle (Weiss et al., 1978). Therefore, the overall effects of 5-HT and ELH on feeding behavior are clearly quite different, and although both cause a prolonged excitation of B16, the transmitters produce radically different effects on the central pattern generator(s) found in the buccal ganglia (Sossin et al., 1987). In addition, ELH does not appear to act on the ARC muscle directly (M. D. Kirk, unpublished observations), and there may be subtle differences in the effects of ELH and 5-HT on the response of B16 to synaptic input or other neuromodulators.

Peptide and amine effects on voltage-dependent currents

We have used the voltage-clamp technique to identify and characterize the currents in motoneuron B16 that are modulated by ELH and 5-HT. These are the currents responsible for the prolonged excitation of B16 affected by these transmitters. Both ELH and 5-HT increase a voltage-dependent slow inward current carried by Na^+ (I_{in}). Slow inward currents are found in neurons that endogenously produce a bursting pattern of action potentials (Wilson and Wachtel, 1974), although other currents are also likely to be necessary for expression of this property (Kramer and Zucker, 1985; Smith and Thompson, 1987). We

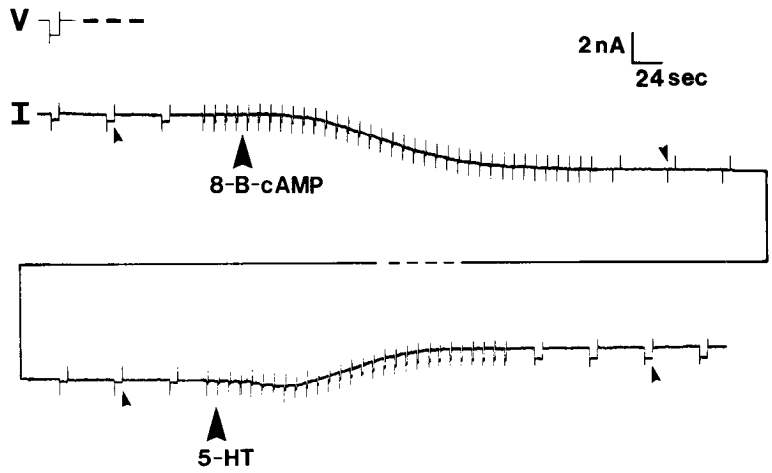


Figure 11. Following the response to 8-bromo-cAMP, 5-HT causes an outward current associated with an apparent increase in conductance. 8-Bromo-cAMP was applied at the large *arrowhead* while the holding current and step current were monitored (holding potential, −40 mV). 8-Bromo-cAMP induced I_{in}, as seen by the inward shift in holding current and the reversal in step current (*small arrowheads*). After approximately 2 min (*dashed line*), 5-HT was applied at the second *large arrowhead* (*bottom trace*). 5-HT caused a much reduced inward shift in holding current, seen immediately following the application, and, with a longer delay, an outward shift in holding current and increase in step-current amplitude (*small arrowheads*).

found that B16 is a conditional burster (Dekin et al., 1985); although the cell is normally silent, it occasionally produces a series of endogenous bursts. This is consistent with the presence of I_{in} and with the observation that B16 (in current-clamp mode) bursts endogenously as the ELH effects subside and when low concentrations of ELH (or atrial gland extracts) are applied (unpublished observations and see fig. 3 of Padgaonkar and Ram, 1983). As stated above, 5-HT also causes a prolonged firing of B16; however, we have not critically tested for effects on firing patterns in B16 at low concentrations of 5-HT. With several currents simultaneously being modulated by 5-HT, we would expect a more complicated influence on spike activity.

In addition to I_{in}, 5-HT also modulated at least 2 other currents in B16. One of these is an inward-rectifier potassium current that is increased in the presence of 5-HT. A similar effect is produced by 5-HT in neuron R15 of *Aplysia* (Benson and Levitan, 1983), although in that cell the effect appears to be mediated by cAMP (Adams and Levitan, 1982). Second, 5-HT decreases an outward current in some preparations at membrane potentials of about −40 mV (near rest) and above. This outward current resembles the potassium-dependent S-current of *Aplysia* sensory neurons, which flow through channels that are open at rest and have a slight voltage dependence at depolarized potentials (Klein et al., 1982; Siegelbaum et al., 1982). We found no convincing evidence to suggest that ELH modulates either of these 2 currents. It should be noted that ELH causes a prolonged excitation of neurons in the lower-left quadrant of the *Aplysia* abdominal ganglion (Mayeri et al., 1985) and that ELH increases both the inward-rectifier potassium current and an inward current active above the resting membrane potential; however, the latter of these currents is dependent upon calcium (Jansen and Mayeri, 1986).

Peptide and amine effects on voltage-dependent ionic currents have been compared in other identified neurons (Deterre et al., 1982; Klein et al., 1982; Siegelbaum et al., 1982; Abrams et al., 1984; Cottrell et al., 1984; Colombaioni et al., 1985; Paupardin-Tritsch et al., 1986a, b). Abrams et al. (1984) found that the small cardioactive peptides A and B (SCP_A and SCP_B) decrease the same potassium current (S-current) decreased by 5-HT in *Aplysia* sensory neurons. In identified neurons of the snail *Helix*, FMRFamide, dopamine, and 5-HT, acting through different receptors, all cause a cAMP-mediated decrease in S-current (Colombaioni et al., 1985). In addition, FMRFamide and dopamine decrease a calcium conductance in *Helix* neurons, but the second messenger involved in that effect has not been identified (Colombaioni et al., 1985). However, in other snail neurons, 5-HT causes an increase in a calcium conductance that is mediated by cGMP (Paupardin-Tritsch et al., 1986a, b). Peptide-induced decreases in calcium conductance have also been observed in vertebrate neurons (Dunlap and Fischbach, 1981).

It is clear that peptides and amines can act through different receptors to produce changes in ionic conductance. When the same neuron is considered, in some cases the currents affected and the second-messenger systems used appear to be the same, while in others they are different. ELH and 5-HT exhibit a unique combination of actions on B16, with both increasing I_{in} and only 5-HT affecting the inward rectifier and an outward current. The functional significance of these unique actions is obscure at this point, although one would expect the integrative properties of B16 to be very different under the 2 conditions.

Second messengers involved in ELH and 5-HT effects

Bath application of 8-bromo-cAMP, 8-bromo-cGMP, and forskolin, and intracellular injection of cAMP, all increase I_{in}, and both 8-bromo-cAMP and 8-bromo-cGMP occlude the inward current response to subsequently applied ELH or 5-HT. Furthermore, the prior application of 8-bromo-cAMP occludes the response to 8-bromo-cGMP. Levitan and Norman (1980) reported that high concentrations of the 8-substituted derivatives of cAMP and cGMP block cAMP and cGMP phosphodiesterases. Therefore, it is possible that the high concentrations of 8-bromo-cGMP used in our experiments caused an indirect elevation of cAMP and increase in I_{in} by inhibiting cAMP phosphodiesterases. We conclude that cAMP is the most likely candidate for the second messenger that mediates the ELH- and 5-HT-induced increase in I_{in}.

Following application of 8-bromo-cAMP or 8-bromo-cGMP, 5-HT causes an increase in the inward-rectifier potassium current and, paradoxically, a decrease in the voltage-dependent slow inward current. The different effects of 5-HT on B16 that we observed are consistent with the multiplicity of receptors for 5-HT on molluscan neurons and the varied responses they mediate (Gerschenfeld and Paupardin-Tritsch, 1974a; Gerschenfeld et al., 1981; Paupardin-Tritsch et al., 1986a). The push–pull effect of 5-HT on I_{in} may result from the binding of 5-HT

to receptors coupled to separate second-messenger pathways that have opposing effects on this current (Wakelam et al., 1986).

Segregation of 5-HT receptors on ink gland motoneurons of *Aplysia* has been documented by Walsh and Byrne (1985). They found that bath application of 5-HT produced a mixture of current responses, and were able to isolate one particular response (a decrease in K^+ conductance) by restricting the application to a localized neuropilar region of the neuron. Therefore, it may be possible to isolate the various responses of B16 to 5-HT by applying the transmitter locally to either the cell body or neuropilar processes. In addition, it will be important to characterize the second messengers mediating the increase in inward rectifier and decreases in I_{in} and outward current induced by 5-HT.

References

Abrams, T. W., V. F. Castellucci, J. S. Camardo, E. R. Kandel, and P. E. Lloyd (1984) Two endogenous neuropeptides modulate the gill and siphon withdrawal reflex in *Aplysia* by presynaptic facilitation involving cAMP-dependent closure of a serotonin-sensitive potassium channel. Proc. Natl. Acad. Sci. USA *81:* 7956–7960.

Adams, W. B., and I. B. Levitan (1982) Intracellular injection of protein kinase inhibitor blocks the serotonin-induced increase in K^+ conductance in *Aplysia* neuron R15. Proc. Natl. Acad. Sci. USA *79:* 3877–3880.

Arch, S., and T. Smock (1977) Egg-laying behavior in *Aplysia californica.* Behav. Biol. *19:* 45–54.

Barish, M. E., and S. H. Thompson (1983) Calcium buffering and slow recovery kinetics of calcium-dependent outward current in molluscan neurones. J. Physiol. (Lond.) *337:* 201–219.

Belardetti, F., E. R. Kandel, and S. A. Siegelbaum (1987) Neuronal inhibition by the peptide FMRFamide involves opening of S K^+ channels. Nature *325:* 153–156.

Benson, J. A., and I. B. Levitan (1983) Serotonin increases an anomalously rectifying K^+ current in the *Aplysia* neuron R15. Proc. Natl. Acad. Sci. USA *80:* 3522–3525.

Brown, A. M., and D. L. Kunze (1974) Ionic activities in identifiable *Aplysia* neurons. Adv. Exp. Med. Biol. *50:* 57–74.

Byrne, J. H. (1985) Neural and molecular mechanisms underlying information storage in *Aplysia*: Implications for learning and memory. Trends Neurosci. *8:* 478–482.

Carew, T. J., and C. L. Sahley (1986) Invertebrate learning and memory: From behavior to molecules. Annu. Rev. Neurosci. *9:* 435–487.

Chiu, A. Y., M. W. Hunkapiller, E. Heller, D. K. Stuart, L. E. Hood, and F. Strumwasser (1979) Purification and primary structure of the neuropeptide egg-laying hormone of *Aplysia californica.* Proc. Natl. Sci. USA *76:* 6656–6660.

Cohen, J. L., K. R. Weiss, and I. Kupfermann (1978) Motor control of buccal muscles in *Aplysia.* J. Neurophysiol. *41:* 157–180.

Colombaioni, L., D. Paupardin-Tritsch, P. P. Vidal, and H. M. Gerschenfeld (1985) The neuropeptide FMRFamide decreases both the Ca^{2+}-conductance and a cAMP-dependent K^+-conductance in identified snail neurons. J. Neurosci. *5:* 2533–2538.

Connor, J. A., and P. Hockberger (1984) A novel sodium current induced by injection of cyclic nucleotides into gastropod neurones. J. Physiol. (Lond.) *354:* 139–162.

Cottrel, G. A., N. W. Davies, and K. A. Green (1984) Multiple actions of a molluscan cardioexcitatory neuropeptide and related peptides on identified *Helix* neurones. J. Physiol. (Lond.) *356:* 315–333.

Dekin, M. S., G. B. Richerson, and P. A. Getting (1985) Thyrotropin-releasing hormone induces rhythmic bursting in neurons of the nucleus tractus solitarius. Science *229:* 67–69.

Deterre, P., D. Paupardin-Tritsch, J. Bockaert, and H. M. Gerschenfeld (1982) cAMP-mediated decrease in K conductance evoked by serotonin and dopamine in the same neuron: A biochemical and physiological single-cell study. Proc. Natl. Acad. Sci. USA *79:* 7934–7938.

Dunlap, K., and G. D. Fischbach (1981) Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. J. Physiol. (Lond.) *317:* 519–535.

Gerschenfeld, H. M., and D. Paupardin-Tritsch (1974a) Ionic mechanisms and receptor properties underlying the responses of molluscan neurones to 5-hydroxytryptamine. J. Physiol. (Lond.) *243:* 427–456.

Gerschenfeld, H. M., and D. Paupardin-Tritsch (1974b) On the transmitter function of 5-hydroxytryptamine at excitatory and inhibitory monosynaptic junctions. J. Physiol. (Lond.) *243:* 457–481.

Gerschenfeld, H. M., P. Deterre, and D. Paupardin-Tritsch (1981) Neuronal responses to serotonin, a second view. In *Serotonin Neurotransmission and Behavior,* B. L. Jacobs and A. Gelperin, eds., pp. 105–130, MIT Press, Cambridge, MA.

Hille, B. (1984) *Ionic Channels of Excitable Membranes,* Sinauer, Sunderland, MA.

Jan, L. Y., and Y. N. Jan (1982) Peptidergic transmission in sympathetic ganglia of the frog. J. Physiol. (Lond.) *327:* 219–246.

Jan, Y. N., and L. Y. Jan (1983) A LHRH-like peptidergic neurotransmitter capable of "action at a distance" in autonomic ganglia. Trends Neurosci. *6:* 320–325.

Jansen, R. F., and E. Mayeri (1986) Voltage clamp analysis of responses to egg-laying hormone and alpha-bag cell peptide shows identity with responses to bag cell discharges in *Aplysia.* Soc. Neurosci. Abstr. *12:* 946.

Kandel, E. R., T. Abrams, L. Bernier, T. J. Carew, R. D. Hawkins, and J. H. Schwartz (1983) Classical conditioning and sensitization share aspects of the same molecular cascade in *Aplysia.* Cold Spring Harbor Symp. Quant. Biol. *48:* 821–830.

Kirk, M. D., and R. H. Scheller (1986a) Egg-laying hormone of *Aplysia* induces a voltage-dependent slow inward current carried by Na in an identified motoneuron. Proc. Natl. Acad. Sci. USA *83:* 3017–3021.

Kirk, M. D., and R. H. Scheller (1986b) Voltage clamp analysis of ELH, 5-HT, and second messenger effects on the identified motoneuron B16 of *Aplysia.* Soc. Neurosci. Abstr. *12:* 947.

Kirk, M. D., S. H. Thompson, and R. H. Scheller (1985) A voltage clamp analysis of egg-laying hormone and serotonin effects on the identified motoneuron B16 of *Aplysia.* Soc. Neurosci. Abstr. *11:* 641.

Klein, M., and E. R. Kandel (1980) Mechanism of calcium current modulation underlying presynaptic facilitation and behavioral sensitization in *Aplysia.* Proc. Natl. Acad. Sci. USA *77:* 6912–6916.

Klein, M., J. Camardo, and E. R. Kandel (1982) Serotonin modulates a specific potassium current in the sensory neurons that show presynaptic facilitation in *Aplysia.* Proc. Natl. Acad. Sci. USA *79:* 5713–5717.

Kramer, R. H., and R. S. Zucker (1985) Calcium-dependent inward current in *Aplysia* bursting pace-maker neurons. J. Physiol. (Lond.) *362:* 107–130.

Krieger, D. T. (1983) Brain peptides: What, where, and why? Science *222:* 975–985.

Kupfermann, I. (1970) Stimulation of egg-laying by extracts of neuroendocrine cells (bag cells) of abdominal ganglion of *Aplysia.* J. Neurophysiol. *33:* 877–881.

Kupfermann, I. (1974) Dissociation of the appetitive and consummatory phases of feeding behavior in *Aplysia*: A lesion study. Behav. Biol. *10:* 89–97.

Levitan, I. B., and J. Norman (1980) Different effects of cAMP and cGMP derivatives on the activity of an identified neuron: Biochemical and electrophysiological analysis. Brain Res. *187:* 415–429.

Lundberg, J. M., and T. Hökfelt (1983) Coexistence of peptides and classical neurotransmitters. Trends Neurosci. *6:* 325–333.

Mackey, S., and T. J. Carew (1983) Locomotion in *Aplysia* triggering by serotonin and modulation by bag cell extract. J. Neurosci. *3:* 1469–1477.

Mayeri, E., B. S. Rothman, P. H. Brownell, W. D. Branton, and L. Padgett (1985) Nonsynaptic characteristics of neurotransmission mediated by egg-laying hormone in the abdominal ganglion of *Aplysia.* J. Neurosci. *5:* 2060–2077.

Padgaonkar, V. A., and J. L. Ram (1983) Atrial gland activation of neuronal input into a buccal mass muscle of *Aplysia.* Comp. Biochem. Physiol. (C) *74:* 387–391.

Paupardin-Tritsch, D., C. Hammond, and H. M. Gerschenfeld (1986a) Serotonin and cyclic GMP both induce an increase of the calcium current in the same identified molluscan neurons. J. Neurosci. *6:* 2715–2723.

Paupardin-Tritsch, D., C. Hammond, H. M. Gerschenfeld, A. C. Nairn, and P. Greengard (1986b) cGMP-dependent protein kinase enhances Ca^{++} current and potentiates the serotonin-induced Ca^{++} current increase in snail neurones. Nature *323:* 812–814.

Pellmar, T. C. (1984) Enhancement of inward current by serotonin in neurons of *Aplysia.* J. Neurobiol. *15:* 13–25.

Pollock, J. D., L. Bernier, and J. S. Camardo (1985) Serotonin and cyclic adenosine 3':5'-monophosphate modulate the potassium cur-

rent in tail sensory neurons in the pleural ganglion of *Aplysia.* J. Neurosci. *5:* 1862–1871.

Ram, J. L. (1983) Neuropeptide activation of an identifiable buccal ganglia motoneuron in *Aplysia.* Brain Res. *288:* 177–186.

Ram, J. L., K. A. Haller, and Z. Levran (1986) Sensitivity of a peptide-activated neuron in *Aplysia* to serotonin and cyclic AMP-relevant agents. Comp. Biochem. Physiol. C *83:* 279–283.

Rosen, S. C., I. Kupfermann, R. S. Golstein, and K. R. Weiss (1983) Lesion of a serotonergic modulatory neuron in *Aplysia* produces a specific defect in feeding behavior. Brain Res. *260:* 151–155.

Siegelbaum, S. A., J. S. Camardo, and E. R. Kandel (1982) Serotonin and cyclic AMP close single K channels in *Aplysia* sensory neurones. Nature *299:* 413–417.

Smith, S., and S. Thompson (1987) Slow membrane currents in bursting pacemaker neurons of *Tritonia.* J. Physiol. *282:* 425–448.

Snyder, S. H. (1980) Brain peptides as neurotransmitters. Science *209:* 976–983.

Snyder, S. H., and R. B. Innis (1979) Peptide neurotransmitters. Annu. Rev. Biochem. *48:* 755–782.

Sossin, W. S., M. D. Kirk, and R. H. Scheller (1987) Peptidergic modulation of neuronal circuitry controlling feeding in *Aplysia.* J. Neurosci. *7:* 671–681.

Stuart, D. K., and F. Strumwasser (1980) Neuronal sites of action of a neurosecretory peptide, egg-laying hormone, in *Aplysia californica.* J. Neurophysiol. *43:* 499–519.

Thompson, S. H. (1977) Three pharmacologically distinct potassium channels in molluscan neurons. J. Physiol. (Lond.) *265:* 465–488.

Wakelam, M. J. O., G. J. Murphy, V. J. Hruby, and M. D. Houslay (1986) Activation of two signal-transduction systems in hepatocytes by glucagon. Nature *323:* 68–71.

Walsh, J. P., and J. H. Byrne (1985) Analysis of decreased conductance serotonergic response in *Aplysia* ink motor neurons. J. Neurophysiol. *53:* 590–602.

Weiss, K. R., J. L. Cohen, and I. Kupfermann (1978) Modulatory control of buccal musculature by a serotonergic neuron (metacerebral cell) in *Aplysia.* J. Neurophysiol. *41:* 181–203.

Weiss, K. R., U. T. Koch, J. Koester, D. E. Mandelbaum, and I. Kupfermann (1980) Neural and molecular mechanisms of food-induced arousal in *Aplysia californica.* In *Advanced Physiological Science, vol. 23: Neurobiology of Invertebrates,* J. Salanki, ed., pp. 305–344, Pergamon, Budapest.

Wilson, W. A., and H. Wachtel (1974) Negative resistance characteristics essential for maintenance of slow oscillations in bursting neurons. Science *186:* 932–934.