Modulation of Calcium Current and Diverse K⁺ Currents in Identified *Hermisenda* Neurons by Small Cardioactive Peptide B

Juan Acosta-Urquidí

Friday Harbor Laboratories, Friday Harbor, Washington 98250, and Department of Biology, Western Washington University, Bellingham, Washington 98225

The molluscan neuropeptides, small cardioactive peptides A and B (SCP_{A,B}), are known to modulate the responses of many molluscan central and peripheral target cells (see review by Lloyd, 1986), but their full range of physiological actions remains unknown. External application of SCP_B (1–10 μ M) modified diverse ionic conductances in a set of giant identifiable neurons in the brain of the marine mollusk *Hermisenda crassicornis*. SCP_B caused a transient depolarization and increased input resistance that enhanced or promoted cell firing. Under voltage-clamp, SCP_B reduced a “background” residual current (I_r), reduced early transient K⁺ current (I_A), reduced a delayed K⁺ current ($I_{K(v)}$), and enhanced I_{Ca} , I_{Ba} , and a Ca²⁺-activated K⁺ current, $I_{K(Ca)}$. In tetraethylammonium chloride (TEA) saline, SCP_B enhanced the amplitude and duration and reduced the threshold of evoked Ca and Ba spikes. Immunocytochemical staining techniques have localized an endogenous SCP_B-like peptide in numerous somata and their neurites in the nervous system of *Hermisenda* (Longley and Longley, 1985; Watson and Willows, 1986). These data are consistent with a role for SCP_B as a neurotransmitter/neurohormone modulator of neuronal excitability in *Hermisenda*. A neurotransmitter role for endogenous SCPs has been proposed for a synaptic pair of cultured neurons in the *Aplysia* buccal ganglion (Lloyd et al., 1986). SCP_B has been implicated in the control of feeding motor output in *Aplysia* (Sossin et al., 1986) and *Tritonia* (Willows and Watson, 1986), and in the presynaptic facilitation of sensory neurons mediating the gill and siphon defensive withdrawal reflex in *Aplysia* (Abrams et al., 1984).

The notion that bioactive peptides, in addition to their roles as neurohormones, can serve as neurotransmitters and modulators is gaining acceptance (Krieger, 1983). Neuropeptides most likely represent the greatest diversity of chemical messengers in the nervous system and peripheral tissues (Scheller et al., 1984). Two small neuropeptides with cardioexcitatory actions, termed small cardioactive peptide A (SCP_A) and B (SCP_B), 11 and 9 amino acids long, respectively, are found in many gastropod

species (see review by Lloyd, 1986). They are derived from a common precursor (Mahon et al., 1985), and have been purified and sequenced (Morris et al., 1982) and are thus available for experimentation. Biochemical and immunocytochemical techniques have localized endogenous SCP_B in the gut and nervous system of *Aplysia* (Lloyd et al., 1985), *Tritonia* (Masinovsky et al., 1985), *Hermisenda* (Longley and Longley, 1985; Watson and Willows, 1986), and other nudibranchs (Watson and Willows, 1986). The SCPs are suspected of playing a role in the initiation and modulation of the feeding motor program, since they are most concentrated in the buccal ganglia, and have been reported to stimulate contractile activity in the gut, enhance the contraction of the radula closer muscle, and increase the output of the burst pattern generator that drives feeding movements in *Aplysia* (see review by Lloyd, 1986). In several different molluscan neurons, the SCPs have been reported to coexist with other peptides (W. H. Watson, personal communication) and conventional transmitters like ACh (Lloyd et al., 1985; Masinovsky et al., 1985). In *Aplysia*, the SCPs act at multiple sites. SCP_A and SCP_B have an excitatory action on identified cultured buccal neurons (Lloyd et al., 1986). SCP_B acts centrally and peripherally to modulate the gill-withdrawal reflex in *Aplysia*, in addition to having cardioexcitatory actions (Cawthorpe et al., 1985; Lukowiak and Colmers, 1987). In siphon sensory neurons that mediate the defensive withdrawal reflex in *Aplysia*, the SCPs mimic the presynaptic action of serotonin by also facilitating transmitter release due to cAMP-dependent closure of the S K⁺ channel (Abrams et al., 1984).

The present study was undertaken to explore the physiological actions of SCP_B on I_{Ca} and diverse K⁺ currents that have been characterized in a set of identified pedal and pleural ganglion neurons in the brain of *Hermisenda*, some of which are also sensitive to serotonin and cAMP (Fig. 1) (Acosta-Urquidí, 1985; Jacklet and Acosta-Urquidí, 1985). This study demonstrates that SCP_B simultaneously suppresses diverse K⁺ currents and enhances I_{Ca} and $I_{K(Ca)}$, with the resultant net effect of enhancing neuronal excitability. A preliminary account of these findings has been reported (Acosta-Urquidí, 1986).

Materials and Methods

Animals were collected locally (Westcott Bay, San Juan Island, WA) and kept in running, ambient (12°C) seawater aquaria at Friday Harbor Labs. The circumesophageal nervous system was dissected free and pretreated with 1 mg/ml protease type VII (Sigma) in seawater for ca. 10 min at 20–21°C in order to digest the connective sheath and permit easy 2-electrode impalements. Most measurements of effective membrane input resistance (R_{in}) and excitability to directly evoked spikes were made using 2 microelectrodes; one for passing current, the other for recording membrane potential changes. Conventional 2-electrode

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Correspondence should be addressed to Dr. Juan Acosta-Urquidí, Biology Department, Western Washington University, Bellingham, WA 98225.

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Figure 1. Schematic map of dorsal surface of *Hermisenda* circumesophageal ganglion, showing relative sizes and locations of the giant identified neurons whose currents are being studied. LP, left pedal ganglion; LPI, left pleural cluster; RPI, right pleural. Shading indicates cells with short-duration spikes (3–8 msec) that undergo very small broadening and little accommodation during repetitive firing. LPI_{tc} and LPI_c or LPI₁ are sometimes pigmented white. Some LPI cluster cells have higher input resistance and wider spikes (10–20 msec) with a shoulder on the repolarizing phase, that undergo pronounced broadening and accommodation during repetitive firing.

voltage-clamp techniques were used. Command pulse rise time was ca. 100 μ sec, settling time 1–2 msec at a gain of 2500. Membrane current was measured using a virtual ground circuit connected to the bath reference Ag/AgCl electrode. Glass electrodes (AM Systems, thin-wall) had resistances of 5–10 M Ω , filled with 3 M KCl or 3 M K acetate. The choice of anion had no effect on the results. Voltage and current records were monitored on a pen writer (Gould 2-Channel, 40 Hz cutoff frequency) and photographed on the CRO screen. Fresh stock solutions of 1 mM SCP_B (Peninsula Labs, Belmont, CA) were made in distilled water and refrigerated before use. Test solutions were added to the bath (ca. 1 ml) and excess fluid was removed by suction. SCP_B was bath-applied in all voltage-clamp experiments. For some current-clamp experiments, SCP_B was “puffed” directly onto the cell being recorded by pressure ejection (Knox-Norton electropneumatic valve; 10–20 psi for 0.3–1 sec) from a micropipette with a broken-off tip loaded with SCP_B (0.1 mM) placed 50–100 μ m from the cell surface.

Saline composition for the study of diverse K⁺ currents was (in mM): 430 tetramethylammonium chloride (TMA; Aldrich), replacing equimolar Na⁺, 10 CaCl₂, 10 KCl, 48 MgCl₂, 10 Trizma base (Sigma), pH adjusted to 7.7. To study I_{Ca}, 100 mM tetraethylammonium chloride (TEA; Aldrich) replaced equimolar TMA to block I_{K(v)}, and 5 mM 4-aminopyridine (4-AP; Aldrich) blocked I_A. In some experiments, 10 mM Ba²⁺ replaced Ca²⁺ in order to study inward current (I_{ba}) free of contaminating I_{K(Ca)}. CdCl₂ (1–5 mM) was effective in blocking I_{Ca} and Ca spikes (Jacklet and Acosta-Urquidí, 1985). All experiments were performed at 16–18°C. As standard protocol for voltage-clamp experiments, current–voltage (*I*–*V*) curves were obtained before (controls) and at various times after SCP_B was added.

Data were taken from a select set of identifiable pedal and pleural ganglion giant cells 80–200 μ m in diameter (LP_{1–3}, LPI_{tc}, LPI_c, LPI_{1–2}, and RPI_{1–2}; see Fig. 1), which were identified on the basis of color, position, relative size and electrophysiological signature. All these cells have a full complement of diverse K⁺ currents and I_{Ca} (cf. Adams et al., 1980), but magnitude and kinetics vary considerably between different cells. This latter feature assists in the recognition of identified neurons (J. Acosta-Urquidí, unpublished observations). The effects of SCP_B on the inward Cl[−] rectifier (cf. Chenoy-Marchais, 1982) exhibited by some cells was not systematically studied. Also, Na⁺ currents were not studied.

Results

SCP_B excitatory action

In seawater, bath application of SCP_B (1–10 μ M) had an excitatory action on the identified left pedal neurons tested. To examine direct effects on a given cell, the technique of “puffed” focal delivery of SCP_B was employed. Within seconds, an excitatory response to the peptide was recorded; it consisted of depolarization and initiation of sustained firing or enhancement of the existing firing rate (Fig. 2A). The SCP_B-induced depolarization (<10 mV) was accompanied by an increased *R*_{in} (25–

50%), which was not secondary to depolarization owing to the presence of anomalous rectification in some of these cells (J. Acosta-Urquidí, unpublished observations). An example of increased *R*_{in} [controlled for changes in membrane potential (*V*_m)] and enhanced excitability after SCP_B was “puffed” onto cell LP₃ is shown in Figure 2B. Repeated “puffed” applications of SCP_B failed to elicit the initial excitatory response, suggesting possible desensitization of the extrasynaptic receptors distributed over the somata.

To further examine the mechanism of direct membrane actions of SCP_B on the set of identified *Hermisenda* neurons under study, voltage-clamp experiments were performed. SCP_B effects were tested on diverse K⁺ currents and I_{Ca}, previously characterized.

Effects on background residual current (*I*_R)

*I*_R is a “background” current present in varying degrees in all the cells studied. Some left pleural cluster cells consistently have very small *I*_R in comparison to other cells, notably LP_{1–3} and RPI_{1–2} (see Fig. 1), which have a sizable *I*_R component. *I*_R is probably carried mostly by K⁺ ions, since its reversal potential (ca. −65 mV) shifts more positive in high external K⁺ solutions (ca. 30 mV for a 3-fold increase in [K_o], in reasonable agreement with the Nernst equation prediction for a K⁺-selective conductance), and the slope conductance also increases (J. Acosta-Urquidí, unpublished observations). In the range −30 to −160 mV, *I*_R is a linear, time-invariant and voltage-insensitive current, since slope conductance is independent of holding potential (*V*_h) in the range −40 to −100 mV. At potentials more positive than −30 mV, *I*_R starts to exhibit moderate voltage-dependence (Fig. 3A, B). *I*_R is resistant to external TEA and is only slightly reduced by 4-AP, Cd, and EGTA injection, but is strongly suppressed by internal Cs⁺ (J. Acosta-Urquidí, unpublished observations). *I*_R in these cells is also consistently suppressed by serotonin and cAMP (Acosta-Urquidí, 1985, 1986; Jacklet and Acosta-Urquidí, 1985).

SCP_B (1–10 μ M) consistently reduced the slope conductance (560 ± 150 nS control versus 370 ± 80 nS after SCP_B; *p* < 0.05, paired samples; *n* = 9 cells) of linear *I*_R measured in the range −30 to −160 mV. The degree of SCP_B reduction of *I*_R was variable between different cells, being most prominent in cells with a sizable *I*_R component. Figure 3 illustrates the re-

Figure 2. Excitatory responses to focal application of SCP_B onto identified pedal neurons recorded in seawater. *A*, SCP_B “puffed” onto cell LP₂ caused depolarization and robust firing for about 3 min. *B*, SCP_B produced a depolarization, increased input resistance (R_{in}), and enhanced excitability in cell LP₃. *a*, Control responses before SCP_B; constant hyperpolarizing pulses (−1.5 nA, 500 msec) monitor R_{in} , and a superimposed depolarizing step (+1.0 nA, 10 sec—arrowheads) elicits a train of spikes interrupted by the hyperpolarizing pulses. Cell LP₃ was silent (V_m , −64 mV) and received excitatory synaptic input (small EPSPs are just detectable). *b*, Depolarization and increased R_{in} seconds after SCP_B was “puffed” onto cell (the increased R_{in} was not secondary to depolarization). Note slower time base. *c*, At 1 min after SCP_B was applied, EPSPs triggered spiking that most likely was due to depolarization and increased R_{in} . V_m was −56 mV. *d*, At 3 min after addition of SCP_B, V_m returned to −63 mV and the cell was silent. R_{in} was slightly enhanced and a +1.0 nA step revealed an increased firing rate (increased excitability). The smaller R_{in} after spike train was transient and associated with postburst hyperpolarization. *e*, At 5 min a volley of EPSP depolarized LP₃ and led to spiking. An imposed hyperpolarization (−1.0 nA) reduced firing rate and revealed further increased R_{in} and EPSPs clearly.

duction of I_R by SCP_B in 2 different cells, LP₁ and LPlc. In LP₁, SCP_B effects on I_R were measured in the ranges −80 to −150 and −30 to −10 mV. For this experiment, steady holding current was reduced approximately 2 nA by SCP_B at V_h −80 mV. Figure 3*B* illustrates the suppression of I_R in LPlc by SCP_B. I_R was measured at −100 and +20 mV, evoked in the presence of TEA plus 4-AP and Cd, which blocked $I_{K(v)}$, I_A , $I_{K(Ca)}$, and I_{Ca} .

No detectable transient shifts in holding current (measured at the usual sensitivity of the recordings, 5–10 nA/cm) were apparent at a V_h of −40 or −80 mV in Na⁺-free saline after SCP_B. If desensitization did occur, bath application of SCP_B may have precluded the recording of rapid transient conductance changes (shifts in holding current). It remains to be determined whether “puffed” SCP_B applications reveal transient conductance changes.

In TEA plus 4-AP saline, SCP_B increased R_{in} an average of $38.3 \pm 5.4\%$; $n = 15$ cells. The SCP_B-induced increase in R_{in} at or near rest is consistent with the reduction of I_R (cf. Jacklet and Acosta-Urquidí, 1985).

Effects on I_A

Two species of A current, distinguished by differences in kinetics and voltage range of activation–inactivation, were encountered in the different cells studied. Cells with short-duration spikes (3–8 msec at half-amplitude) that undergo little broadening and accommodation to sustained depolarization (see Fig. 1), exhibit a “fast” I_A type (I_{Af}), which typically peaks in less than 10 msec, has a τ_{off} of ca. 30–50 msec, activates at ca. −40 mV, and is half-maximally inactivated at ca. −65 mV at 18°C. Some cells in the left pleural cluster, notably LPlc, LPc and LPl₁ (see Fig.

Figure 3. SCP_B reduced a "background" residual current (I_R). *A*, Cell LP_1 in 0 Na, TMA plus Cd saline. *a*, I - V plot of linear component of I_R before (control, open symbols, I_R slope conductance, 318 nS), and after (closed symbols, 200 nS) 5 μ M SCP_B , evoked by hyperpolarizing steps. The reversal potential was ca. -65 mV, V_h = -80 mV. Sample I_R responses at -140 mV are shown. No measurements were made in the linear portion of I_R between -80 to -30 mV in this case. *b*, Same experiment, segment of I - V plot of I_R measured at the end of 800 msec depolarizing command pulses before (control) and after SCP_B . *B*, Cell $LPIc$ in TEA plus 4-AP Ca^{2+} saline with added Cd^{2+} to block I_{Ca} , showing SCP_B (5 μ M) suppression of I_R at negative and positive potentials. In this and all subsequent figures, command pulses are to the indicated potential (absolute value in mV) from the V_h indicated at the foot of each pulse and calibration bar is 2 sec for I_R responses elicited by negative command pulses.

1) have wider spikes (10–20 msec) with a distinct shoulder on the falling phase which exhibit pronounced broadening and accommodation to evoked firing. These cells exhibit a "slow" $I_A(I_{As})$, which typically peaks at ca. 20–30 msec, has τ_{off} of ca. 200–300 msec and an activation-inactivation curve that is shifted more positive (activation at ca. -35 to -20 mV and half-

maximal inactivation at ca. -40 mV). Both I_{Af} and I_{As} are blocked by 5 mM 4-AP. At voltages more positive than -10 mV, I_{Af} usually begins to overlap with $I_{K(v)}$, but the superimposed $I_{K(v)}$ can always be detected as the slower component, which inactivates to paired pulses (refer to Fig. 5). In some pleural cells, I_{As} has kinetics of decay that closely match those

Table 1. Summary of ionic currents modulated by SCP_B in identified *Hermisenda* neurons

	Decreased ^a	Increased ^a
K⁺ currents		
Background residual I_R	32.35 $\pm$ 17, n = 9 ^b	
Early transient		
I_{Af}	67.6 $\pm$ 7.84, n = 5 ^c	
I_{As}	52.5 $\pm$ 8.42, n = 6 ^c	
Delayed rectifier $I_{K(v)}$	43.4 $\pm$ 5.8, n = 6	
Ca ²⁺ -activated $I_{K(Ca)}$		40.6 $\pm$ 13, n = 7 ^d
Ca²⁺ current		
I_{Ca}		87.5 $\pm$ 39.7, n = 6 ^e
I_{Ba}		59.6 $\pm$ 13.9, n = 10 ^e

^a Average percentage change $\pm$ SEM, n = number of cells.

^b Linear slope conductance values.

^c N.S., Student t test.

^d Measured at +30 mV.

^e Measured at 0 mV (N.S., Student t test).

Figure 4. SCP_B suppressed A currents. *A*, “Fast” I_A (I_{Af}) recorded in cell LP₁ from same experiment as in Figure 3*A*. Activation plot of peak I_{Af} amplitude (corrected for linear I_R), evoked from $V_h = -80$ mV, before (Con) and after SCP_B. Inset, Sample responses show SCP_B reduced peak I_{Af} and steady-state amplitude, which is I_R (see text). *B*, “Slow” I_A (I_{As}) recorded in pleural cell LPI₁. Activation curve for peak I_{As} amplitude (I_R corrected) before (Con), and after application of SCP_B (5 μ M). Sample responses at right show SCP_B reduced the I_{As} peak (32% at -10 mV) and steady-state amplitude, which is I_R . Note, also, that SCP_B reduced I_R evoked by negative pulses. Saline for both *A* and *B* was 0 Na, plus TMA with added Cd²⁺ to block I_{Ca} .

of $I_{K(v)}$. However, in these cells, $I_{K(v)}$ is resistant to 4-AP and is blocked by TEA, which does not suppress I_{As} (J. Acosta-Urquidí, unpublished observations).

SCP_B reduced the peak amplitude and steady-state value of both I_{Af} and I_{As} (Fig. 4, *A*, *B*). The steady-state component corresponds to I_R , since it is the residual outward current seen after 4-AP block of I_A (the transient component of outward current). The average percentage reduction in peak I_A (both “fast” and “slow” species pooled) was 59.4 ± 6.03 , $n = 11$ cells. Table 1 lists the average percentage reductions in I_{Af} and I_{As} separately. The suppression of I_{Af} and I_{As} occurred in the presence of Cd²⁺, which blocked I_{Ca} , and hence cannot be attributed to an induction or enhancement of I_{Ca} by SCP_B, which can attenuate peak I_A because of close temporal overlap. Suppression of I_A by SCP_B would tend to promote repetitive firing (cf. Connor, 1980).

Effects on delayed K⁺ current, $I_{K(v)}$

The largest component of outward current in these cells is $I_{K(v)}$. Isolation of $I_{K(v)}$ was accomplished by minimizing contamination by I_A (blocked either with 4-AP or by setting V_h at a level at which I_A is completely inactivated, e.g., -40 mV for cell LP₁), and $I_{K(Ca)}$ (Cd²⁺ was added to block I_{Ca} , monitored by a block of Ca spikes; see Fig. 8*C*). $I_{K(v)}$ is blocked by external TEA (100 mM) and activates more slowly and at more positive potentials (typically ca. -10 mV or more positive) than I_A . $I_{K(v)}$ characteristically displays pronounced voltage- and time-dependent inactivation to identical paired or repetitive pulses. This inactivation can be removed by a conditioning hyperpolarizing (> -20 mV) prepulse (e.g., Fig. 5*A*) or rest (15–30 sec for complete recovery) (cf. Ruben and Thompson, 1984).

SCP_B consistently reduced peak $I_{K(v)}$ ($43.4 \pm 5.8\%$, $n = 6$ cells) and the steady-state component in records of $I_{K(v)}$ in all cases tested (Fig. 5). The reduction of the steady-state component measured at the end of an 800 msec command pulse in records of $I_{K(v)}$ (Fig. 5, *A*, *C*) mainly reflects the suppression of I_R by SCP_B.

Effects on I_{Ca} and Ca²⁺ spikes

In 0-Na TEA saline, overshooting Ca²⁺ spikes, lasting tens of milliseconds, can be evoked in all the cells studied. These Ca²⁺ spikes and their underlying I_{Ca} are blocked by Cd (1–5 mM) (Fig. 8*C*). I_{Ca} amplitude varies considerably across different cells, being largest for the 2 giant cells (LP₁ and RPI₁; see Fig. 1). In all cells I_{Ca} inactivates, but the degree of inactivation (measured as twin-pulse inactivation at 0 mV) varies considerably across different cells. I_{Ca} typically activates at ca. -35 mV, and peaks between 0 and $+10$ mV. Ba²⁺ effectively replaces Ca²⁺ as the charge carrier. Ba²⁺ spikes are of longer duration (up to ca. 1 sec), and I_{Ba} also exhibits twin-pulse inactivation, but the rate of I_{Ba} inactivation is slower than that for I_{Ca} , which is due in part to the absence of a contaminating $I_{K(Ca)}$ (compare Figs. 6*B* and 7*B*).

SCP_B enhanced I_{Ca} and I_{Ba} (measured at 0 mV) by $87.5 \pm 39.7\%$, $n = 6$ cells, and $59.6 \pm 13.9\%$, $n = 10$ cells, respectively (data from cells LP₁, LP_{1c}, LPI₁, and RPI₁). Examples of this effect are shown in Figures 6, *A*, *B*; 7*B*. However, reduction of I_R by SCP_B could lead to an apparent increase in inward current, since Cs⁺ injection, which also suppresses I_R , can produce a slight enhancement of I_{Ba} (J. Acosta-Urquidí, unpublished observations). Despite these considerations, a direct enhancement of I_{Ca} and I_{Ba} by SCP_B is possible, as suggested by the complete I - V plot of I_{Ba} (Fig. 6*B*), and the fact that in some cases SCP_B enhanced I_{Ca} with just detectable suppression of I_R (Fig. 7*B*). Further tests on the I_{Ca} in a Cs⁺-loaded cell to remove I_R contamination are necessary to convincingly demonstrate a direct enhancement of I_{Ca} by SCP_B.

SCP_B affected Ca²⁺ or Ba²⁺ spikes in 2 ways: (1) by augmenting their amplitude ($18.7 \pm 4.4\%$, $n = 9$ cells) and duration (average, $503 \pm 153\%$, $n = 12$ cells); and (2) by reducing spike-initiation threshold (not shown), which resulted in increased excitability. Examples of enhanced spike amplitude and duration are shown in Figure 6*A*, *a*. Enhanced amplitude and increased excitability (reduced threshold not shown) after SCP_B are shown in Figure 7*A* (refer also to Fig. 2*B* for increased excitability after SCP_B). The enhanced amplitude and duration of Ca²⁺ and Ba²⁺ spikes always correlated with enhancement of I_{Ca} and I_{Ba} (Fig. 6*A*, *a*, *b*). The increase in Ca²⁺ and Ba²⁺ spike amplitude by SCP_B was probably due mostly to increased I_{Ca} and I_{Ba} . The sizable in-

Figure 5. SCP_B reduced the delayed rectifier K current, $I_{K(v)}$. **A**, Cell LP₁ in the presence of Cd to remove $I_{K(Ca)}$ contamination. $I_{K(v)}$ responses evoked from V_h -40 mV (at which I_{A_f} is completely inactivated in this cell), exhibit characteristic voltage- and time-dependent inactivation to paired or repetitive pulses, which is removed by conditioning hyperpolarization. In this case, twin-pulse inactivation is evidenced as a decreased amplitude and increased rate of inactivation of the second response. A 2 sec prepulse conditioning hyperpolarization to -80 mV removed inactivation and restored the first response. SCP_B (5 μ M) reduced peak and the steady-state amplitude (which is the I_R component; see text) and also reduced linear I_R (response to -80 mV). **B**, Cell LP₁. Family of $I_{K(v)}$ responses before (Con) and 10 min after SCP_B (35% reduction at +50 mV). **C**, $I_{K(v)}$ responses in pleural cell LP₁ before (Con) and at 13 min after SCP_B, which caused a 90% reduction in peak amplitude and 57% at steady-state, reflecting the suppression of I_R (see text).

crease in duration could be explained by enhanced I_{Ca} and I_{Ba} , in conjunction with suppression of I_R , which appears to contribute to the repolarization of TEA-broadened spikes (cf. Klein et al., 1982). As noted earlier, suppression of I_R could also contribute to enhancement of I_{Ba} , and hence Ba^{2+} spike amplitude, but this may be difficult to quantify.

Effects on $I_{K(Ca)}$

In 0-Na, TEA plus 4-AP and Ca^{2+} saline, depolarization to +10 mV or more positive evokes I_{Ca} and an outward current consisting of I_R plus $I_{K(Ca)}$. $I_{K(Ca)}$ in these cells can be suppressed by EGTA injection (J. Acosta-Urquidí, unpublished observations) or indirectly by Cd blockage of I_{Ca} (Fig. 8C). In some experiments, SCP_B enhanced an outward current that was identified as $I_{K(Ca)}$. The average increase in $I_{K(Ca)}$ was $40.6 \pm 13\%$, $n = 7$ cells. This effect was usually expressed after 10–15 min exposure to SCP_B, suggesting that it was secondary to a buildup of intracellular Ca^{2+} , in addition to the increase in I_{Ca} . In 5/7 cells there was also a clear increase in I_{Ca} detected earlier. Enhancement of $I_{K(Ca)}$ by SCP_B was not always apparent, as it was masked by the simultaneous suppression of I_R by SCP_B, and hence detection of this effect depended on the relative magnitudes of I_R and $I_{K(Ca)}$ and the degree to which these currents were affected by the peptide in a given cell. An example from cell LP₁, showing SCP_B enhancement of $I_{K(Ca)}$, is shown in Figure 8B. In this experiment, SCP_B first produced a 28% increase in I_{Ca} (not shown). The enhanced $I_{K(Ca)}$, which was detected later, caused an apparent decrease in I_{Ca} .

Enhanced $I_{K(Ca)}$ could have resulted from increased Ca influx due to enhancement of I_{Ca} by SCP_B. However, a direct SCP_B effect on $I_{K(Ca)}$ is possible. Further experiments on $I_{K(Ca)}$, elicited directly by Ca^{2+} injection (cf. Gorman and Thomas, 1980), are necessary to test this possibility. It is interesting to note that serotonin, which has effects similar to SCP_B on all of the other K^+ conductances studied in these cells (see Discussion), also enhances $I_{K(Ca)}$ (Acosta-Urquidí, 1985; Jacklet and Acosta-Urquidí, 1985).

In all the aforementioned voltage-clamp experiments, the effects of SCP_B on diverse ionic currents, excepting $I_{K(Ca)}$, were evidenced within 30–60 sec, developed steadily over the next 3–10 min, and usually reached saturation by 15 min. A vigorous washout resulted in only partial reversibility of SCP_B effects. Table 1 summarizes the SCP_B effects on all the currents studied. In conclusion, SCP_B affected multiple ionic conductances, which considerably complicated the analysis of its excitatory membrane effects. The SCP_B-induced conductance changes would favor potentiation of transmitter release (cf. Abrams et al., 1984) and would also promote enhanced neuronal excitability, such as that reported for *Aplysia* sensory neurons (Klein et al., 1986). Such conductance changes in appropriate target cells could sustain or drive a motor pattern generator for stereotyped behaviors, such as feeding. Preliminary voltage-clamp studies of *Hermissenda* buccal ganglion cells, which appear to be homologous to SCP_B-sensitive neurons implicated in feeding behavior in other marine mollusks (Willows and Watson, 1986), revealed the existence of I_A , $I_{K(v)}$, and I_{Ca} (J. Acosta-Urquidí, unpublished observations).

Figure 6. SCP_B enhanced I_{Ba} and Ba²⁺ spike broadening and suppressed I_R . *A*, Cell LPic in 0 Na plus TEA, with 4-AP and Ba²⁺ as the charge carrier (replacing Ca²⁺) to eliminate $I_{K(Ca)}$ contamination (see text). *a*, SCP_B (5 μ M) increased R_{in} (27%) and greatly enhanced duration (14 $\times$) and amplitude (25%) of directly evoked Ba²⁺ spike (V_m , -40 mV, $\pm$ 4 nA, 400 msec pulse). *b*, Same experiment, under voltage-clamp. Paired depolarizing pulses evoked slowly inactivating I_{Ba} . SCP_B reduced linear I_R slightly (arrow) and enhanced I_{Ba} 328%. *c*, SCP_B also reduced outward current at +30 mV, which is I_R (see text). *B*, Cell LP₁ in TEA plus 4-AP Ba²⁺ saline. In this case no outward current was detectable. *I-V* plot of peak I_{Ba} (linear I_R corrected) before (control, open symbols), and after (filled symbols, dotted lines) 5 μ M SCP_B. Inset, I_{Ba} responses to paired pulses were enhanced 77% by SCP_B.

Discussion

Immunocytochemical studies of the *Hermisenda* CNS (Longley and Longley, 1985; Watson and Willows, 1986) reveal clusters of SCP_B-like immunoreactive neurons in the pedal, pleural, cerebral, and buccal ganglia that send axons out of many peripheral nerves. In the brain, some cells have extensive neuritic arborizations that course in the vicinity of the identifiable cells studied. At high resolution, SCP_B-like immunoreactivity in fibers is visible as a fine network of varicosities. The fact that responses to SCP_B were recorded in the select set of cells chosen for this study suggests that they have receptors for SCP_B and thus may

be targets for SCP_B released by neurons within the ganglion. To date, no candidate presynaptic SCP_B-containing neurons have been identified. Interestingly, cell RPl (see Fig. 1) reveals strong SCP_B-like immunoreactivity (Longley and Longley, 1985); it remains for future studies to demonstrate whether it mediates intraganglionic SCP_Bergic transmission.

The actions of SCP_B are very similar to those of serotonin at central and peripheral sites in *Aplysia* (see review by Lloyd, 1986). In *Hermisenda*, serotonin also had effects similar to those of SCP_B on the same ionic conductances in some of the same identified neurons used in this study; for example, serotonin reduced I_R , I_{Af} , I_{As} , and $I_{K(v)}$ and enhanced I_{Ca} , $I_{K(Ca)}$, and

Figure 7. SCP_B enhanced excitability and unmasked I_{Ca} . Cell LP₁ in 0-Na with TEA plus 4-AP Ca²⁺ saline. **A**, Under current-clamp, ± 4 nA pulses monitored R_{in} and evoked only a single Ca²⁺ spike before (Con) application of 5 μ M SCP_B. V_m , -46 mV. R_{in} was increased and ± 4 nA pulse now evoked 2 larger-amplitude Ca²⁺ spikes after SCP_B. V_m was set at -46 mV. Longer-duration control pulses failed to evoke 2 spikes owing to I_{Ca} inactivation (not shown). **B**, Same experiment under voltage-clamp shows SCP_B enhanced I_{Ca} at 0 mV and "unmasked" I_{Ca} at +30 mV. Note that in this case outward current was unaffected and I_R was only slightly reduced by step to -110 mV, suggesting a direct effect of SCP_B of enhancing I_{Ca} (see text).

Ca spike duration (Acosta-Urquidí, 1985, 1986; Jacklet and Acosta-Urquidí, 1985). Serotonin is present in numerous cells and their processes in the *Hermisenda* brain (Land and Crow, 1985; W. Watson, personal communication), and has been implicated as a putative transmitter/modulator in the oculovestibular pathway that mediates the learning-induced conductance changes in the type B photoreceptor, since it mimics these conductance changes (Crow and Bridge, 1985; Farley and Auerbach, 1986). Because some type B photoreceptor dark K conductances (excepting I_{Ca-K} ; cf. Alkon et al., 1984) are similar to those present in the giant cells of this study, and SCP_B has effects similar

to those of serotonin, it is conceivable that SCP_B may play a role in the associative learning model of *Hermisenda*.

In siphon and tail sensory neurons of *Aplysia*, SCP_B increases cAMP levels and mimics the effects of serotonin by also closing the S K⁺ channels via cAMP mediation (Abrams et al., 1984) and inducing an inward current associated with a decreased resting conductance (Occor and Byrne, 1985). The reduction of I_R by SCP_B, serotonin, and cAMP in *Hermisenda* giant cells (Jacklet and Acosta-Urquidí, 1985; Acosta-Urquidí, 1986) resembles the effects of serotonin and cAMP on the macroscopic S K⁺ current in *Aplysia* (Klein et al., 1982; Pollock et al., 1985;

Figure 8. SCP_B enhanced Ca-activated K⁺ current, $I_{K(Ca)}$. Cell LP₁ in 0-Na with TEA and 4-AP, Ca²⁺ saline. **A**, I-V plot of peak I_{Ca} (linear I_R corrected) and steady-state outward current (upper pair of traces) measured at end of the first pulse, before (Control, open symbols) and at 15 min after (closed symbols) 5 μ M SCP_B. SCP_B enhanced outward current and reduced inward current. At an earlier time, a 28% increase in I_{Ca} by SCP_B was recorded (not shown; see text). **B**, Sample recordings from this experiment reveal SCP_B strongly enhanced outward current, which caused an apparent reduction of peak I_{Ca} . Outward current and tail current (arrow) are $I_{K(Ca)}$. Note also slight reduction of linear I_R . **C**, Different experiment on cell LP₁ in same saline demonstrated that outward current was mostly $I_{K(Ca)}$, since Cd blocked Ca spike, blocked I_{Ca} and the component of outward current activated by Ca²⁺ influx, leaving only I_R .

Brezina et al., 1987b) and some *Helix* neurons (Paupardin-Tritsch et al., 1985). Single-channel studies (cf. Siegelbaum et al., 1982) are necessary to demonstrate conclusively whether I_R in *Hermisenda* neurons is the equivalent of the S K⁺ current.

FMRFamide, another related endogenous cardioactive peptide (Price and Greenberg, 1977), has been colocalized with SCP_B by differential immunofluorescence staining in some *Hermisenda* neurons (W. Watson, personal communication). In some *Aplysia* abdominal and pleural ganglion neurons, FMRFamide (independently of cAMP involvement) suppressed I_{Ca} and activated an outward current that resembled the S K⁺ current (Brezina et al., 1987a, b). In some of the same abdominal neurons, FMRFamide induced a Na⁺ current and enhanced I_A (Ruben et al., 1986). In *Aplysia* sensory neurons, FMRFamide opened S K⁺ channels via a cAMP-independent second-messenger system, and reversed the closure of S K⁺ channels induced by serotonin or cAMP (Berladetti et al., 1987). By contrast, in some identified *Helix* neurons FMRFamide suppressed both the S K⁺ current and I_{Ca} (Colombaioni et al., 1985), and in other *Helix* neurons FMRFamide modulated sodium conductance and other diverse K⁺ conductances (Cottrell et al., 1984). These findings demonstrate that molluscan cardioactive peptides modulate many diverse conductances and produce different (even opposite) membrane effects depending on the individual type of neuron tested. The emerging pattern is that in some neurons SCP_B and serotonin appear to have similar membrane actions, whereas FMRFamide and serotonin have opposite effects on some of the same conductances.

The long-lasting and only partially reversible SCP_B effects are consistent with involvement of internal messengers. In *Aplysia* motoneuron R₁₅, treatments that elevate cAMP mimic the physiological response to egg-laying hormone, ELH (Ram, 1983). In *Aplysia* sensory neurons, present indirect evidence is consistent with cAMP mediation of the SCP_B effects (Abrams et al., 1984; Occor and Byrne, 1985). In *Aplysia* central and peripheral tissues, SCP_B increases adenylate cyclase activity (Cawthorpe et al., 1985). In the *Hermisenda* cells studied, I found that treatments that elevated intracellular cAMP mimicked the SCP_B-induced conductance changes. These treatments included application of 8-Br-cAMP (a nonhydrolysable, soluble cAMP analog), forskolin (an activator of adenylate cyclase), and Ro-20-1724 (a phosphodiesterase inhibitor), all of which reduced I_R, I_A, I_{K(v)}, and enhanced I_{Ca} (Acosta-Urquidí, 1985).

The SCP_B-induced modulation of conductances reported in this study would tend to favor a rise in free intracellular Ca²⁺ (Ca²⁺)_i because of the enhanced amplitude and duration of Ca²⁺ spikes and the increased number of spikes during a burst. Additionally, SCP_B might cause release of internal Ca²⁺ or interference with internal Ca²⁺ buffering, which might be detectable as activation of I_{K(Ca)}. In a number of cases, I recorded a secondary enhancement of the steady-state component of I_A in experiments in which SCP_B first reduced I_A peak amplitude and I_R. Such an effect could have resulted from activation of I_{K(Ca)} by SCP_B (see Fig. 8). This interpretation follows from the observation that a depolarization-induced increase in (Ca²⁺)_i, measured with arsenazo III differential absorption spectrophotometry, produces a transient suppression of I_A peak amplitude and a parallel enhancement of the I_A steady-state component owing to the activation of I_{K(Ca)} (Acosta-Urquidí et al., 1983). It was reported that serotonin increased (Ca²⁺)_i in the *Aplysia* sensory neurons in which it also suppressed the S K⁺ current (Boyle et al., 1984). Given the similarity of actions of serotonin and SCP_B,

this possibility should be considered for SCP_B. A rise in (Ca²⁺)_i caused by SCP_B could also lead to activation of calcium calmodulin-dependent kinases, such as type II CaM kinase and/or C-kinase, which could also modulate channel activity (cf. Huganir, 1987) and lead to long-lasting conductance changes. In support of this possibility, I found that injection of exogenous type II CaM kinase purified from rat brain (cf. Kennedy et al., 1983) and phosphorylase kinase (Cohen, 1982) into some of the same giant *Hermisenda* cells sensitive to SCP_B resulted in a reduction of I_R, I_A, and I_{K(v)} and enhancement of I_{Ca}, I_{Na}, and Ca²⁺ spike amplitude and duration (Acosta-Urquidí et al., 1983; Acosta-Urquidí, 1985). In the *Hermisenda* type B photoreceptor, injection of an exogenous phosphorylase kinase and activation of C-kinase suppressed the same K⁺ currents reduced by associative training (Acosta-Urquidí et al., 1984; Farley and Auerbach, 1986). More work is needed to elucidate the involvement of cAMP and/or other internal messengers in the modulation of diverse membrane ionic conductances by SCP_B in central molluscan neurons.

References

- Abrams, T. W., V. R. 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.
- Acosta-Urquidí, J. (1985) Calcium and cyclic AMP-mediated regulation of Ca and K-currents in *Hermisenda* giant neurons. *Soc. Neurosci. Abstr.* 11: 788.
- Acosta-Urquidí, J. (1986) Modulation of Ca and K⁺ currents in giant *Hermisenda* neurons by the neuropeptide SCP_B and serotonin. *Soc. Neurosci. Abstr.* 12: 241.
- Acosta-Urquidí, J., D. L. Alkon, J. A. Connor, and J. T. Neary (1983) Intracellular injection of a Ca²⁺ dependent protein kinase amplifies Ca-mediated inactivation of a transient K⁺ current (I_A) in *Hermisenda* giant neurons. *Soc. Neurosci. Abstr.* 9: 501.
- Acosta-Urquidí, J., D. L. Alkon, and J. T. Neary (1984) Ca²⁺-dependent protein kinase injection in a photoreceptor mimics biophysical effects of associative learning. *Science* 224: 1254-1257.
- Adams, D. J., S. J. Smith, and S. H. Thompson (1980) Ionic currents in molluscan soma. *Annu. Rev. Neurosci.* 3: 141-167.
- Alkon, D. L., J. Farley, M. Sakakibara, and B. Hay (1984) Voltage-dependent calcium and calcium-activated potassium currents of a molluscan photoreceptor. *Biophys. J.* 46: 605-614.
- Berladetti, 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.
- Boyle, M., M. Klein, S. Smith, and E. R. Kandel (1984) 5-HT increases intracellular Ca²⁺ transient in voltage-clamped sensory neurons of *Aplysia californica*. *Proc. Natl. Acad. Sci. USA* 81: 7642-7646.
- Brezina, V., R. Eckert, and C. Erxleben (1987a) Modulation of potassium conductances by an endogenous neuropeptide in neurons of *Aplysia californica*. *J. Physiol. (Lond.)* 382: 267-290.
- Brezina, V., R. Eckert, and C. Erxleben (1987b) Suppression of calcium current by an endogenous neuropeptide in neurons of *Aplysia californica*. *J. Physiol. (Lond.)* 388: 565-595.
- Cawthorpe, D. R. L., J. Rosenberg, W. F. Colmers, and K. Lukowiak (1985) The effects of small cardioactive peptide B on the isolated heart and gill of *Aplysia californica*. *Can. J. Physiol. Pharmacol.* 63: 918-924.
- Chenoy-Marchais, D. (1982) A Cl⁻ conductance activated by hyperpolarization in *Aplysia* neurones. *Nature* 299: 359-361.
- Cohen, P. (1982) The role of protein phosphorylation in neural and hormonal control of cellular activity. *Nature* 296: 613-619.
- Colombaioni, L., D. Paupardin-Tritsch, P. R. Vidal, and H. M. Gerschenfeld (1985) The neuropeptide FMRFamide decreases both the Ca-conductance and a cyclic 3',5'-adenosine monophosphate-dependent K⁺ conductance in identified molluscan neurons. *J. Neurosci.* 5: 2533-2538.
- Connor, J. A. (1980) The fast K⁺ channel and repetitive firing. In *Molluscan Nerve Cells: From Biophysics to Behavior*, J. Koester and

- J. H. Byrne, eds., pp. 125–133, Cold Spring Harbor, New York.
- Cottrell, G. A., N. W. Davies, and K. A. Green (1984) Multiple actions of molluscan cardioexcitatory neuropeptide and related peptides on identified *Helix* neurones. *J. Physiol. (Lond.)* 356: 315–333.
- Crow, T., and M. S. Bridge (1985) Serotonin modulates photoresponses in *Hermisenda* type B photoreceptors. *Neurosci. Lett.* 60: 83–88.
- Farley, J., and S. Auerbach (1986) Protein kinase C activation induces conductance changes in *Hermisenda* photoreceptors like those seen in associative learning. *Nature* 319: 220–223.
- Gorman, A. L. F., and M. V. Thomas (1980) Potassium conductance and internal calcium accumulation in a molluscan neurone. *J. Physiol. (Lond.)* 308: 287–313.
- Huganir, R. C. (1987) Biochemical mechanisms that regulate the properties of ion channels. In *Neuromodulation. The Biochemical Control of Neuronal Excitability*, L. K. Kaczmarek and I. B. Levitan, eds., pp. 64–85, Oxford U. P., New York.
- Jacklet, J. W., and J. Acosta-Urquidí (1985) Serotonin decreases a background current and increases calcium and calcium-activated current in pedal neurons of *Hermisenda*. *Cell. Molec. Neurobiol.* 5: 407–412.
- Kennedy, M. B., T. McGuinness, and P. Greengard (1983) A calcium/calmodulin-dependent protein kinase from mammalian brain that phosphorylates synapsin I. Partial purification and characterization. *J. Neurosci.* 3: 818–831.
- 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.
- Klein, M., B. Hochner, and E. R. Kandel (1986) Facilitatory transmitters and cAMP can modulate accommodation as well as transmitter release in *Aplysia* sensory neurons: Evidence for parallel processing in a single cell. *Proc. Natl. Acad. Sci. USA* 83: 7994–7998.
- Krieger, D. T. (1983) Brain peptides: What, where and why? *Science* 222: 975–985.
- Land, P. W., and T. Crow (1985) Serotonin immunoreactivity in the circumesophageal nervous system of *Hermisenda crassicornis*. *Neurosci. Lett.* 62: 199–205.
- Lloyd, P. E. (1986) The small cardioactive peptides: A class of modulatory neuropeptides in *Aplysia*. *Trends Neurosci.* 9: 428–432.
- Lloyd, P. E., I. Kupfermann, and K. R. Weiss (1984) Evidence for parallel actions of a molluscan neuropeptide (SCP_B) and serotonin in mediating arousal in *Aplysia*. *Proc. Natl. Acad. Sci. USA* 81: 2934–2937.
- Lloyd, P. E., A. C. Mahon, I. Kupfermann, J. L. Cohen, R. H. Scheller, and K. R. Weiss (1985) Biochemical and immunocytological localization of molluscan small cardioactive peptides in the nervous system of *Aplysia californica*. *J. Neurosci.* 5: 1851–1861.
- Lloyd, P. E., S. Schacher, I. Kupfermann, and K. T. Weiss (1986) Release of neuropeptides during intracellular stimulation of single identified *Aplysia* neurons in culture. *Proc. Natl. Acad. Sci. USA* 83: 9794–9798.
- Longley, R., and A. Longley (1985) Immunocytochemical localization of serotonin, FMRFamide, BPP, and SCP_B in nudibranch molluscs. *Soc. Neurosci. Abstr.* 11: 943.
- Lukowiak, K., and W. Colmers (1987) Endogenous peptides work at multiple sites in the nervous system in the control of gill behaviors in *Aplysia*. *Reg. Pep.* 17: 99–109.
- Mahon, A. C., P. E. Lloyd, K. T. Weiss, I. Kupfermann, and R. H. Scheller (1985) The small cardioactive peptides A and B of *Aplysia* are derived from a common precursor molecule. *Proc. Natl. Acad. Sci. USA* 82: 3925–3929.
- Masinovsky, B., P. E. Lloyd, and A. O. D. Willows (1985) Morphology of two pairs of identified peptidergic neurons in the buccal ganglia of the mollusc *Tritonia diomedea*. *J. Neurobiol.* 16: 27–39.
- Morris, H. R., M. Panico, A. Karplus, P. E. Lloyd, and B. Rinicker (1982) Elucidation by FAB-MS of the structure of a new cardioactive peptide from *Aplysia*. *Nature* 300: 643–645.
- Occor, K. A., and J. H. Byrne (1985) Membrane responses and changes in cAMP levels in *Aplysia* sensory neurons produced by serotonin, tryptamine, FMRFamide and small cardioactive peptide B (SCP_B). *Neurosci. Lett.* 55: 113–118.
- Paupardin-Tritsch, D., L. Colombaioni, P. Deterre, and H. Gerschenfeld (1985) Two different mechanisms of calcium spike modulation by dopamine. *J. Neurosci.* 5: 2522–2532.
- Pollock, J. D., L. Bernier, and J. Camardo (1985) Serotonin and cyclic 3',5' monophosphate modulate the potassium current in tail sensory neurons in the pleural ganglion of *Aplysia*. *J. Neurosci.* 5: 1862–1871.
- Price, D. A., and M. J. Greenberg (1977) Structure of a molluscan cardio-excitatory neuropeptide. *Science* 197: 670–671.
- Ram, J. L. (1983) Forskolin activation of an identified peptide-sensitive motoneuron in *Aplysia*. *Br. J. Pharmacol.* 79: 631–633.
- Ruben, P., and S. Thompson (1984) Rapid recovery from K current inactivation on membrane hyperpolarization in molluscan neurons. *J. Gen. Physiol.* 84: 861–875.
- Ruben, P., J. W. Johnson, and S. H. Thompson (1986) Analysis of FMRF-amide effects on *Aplysia* bursting neurons. *J. Neurosci.* 6: 252–259.
- Scheller, R. H., T. Kaldany, T. Kreiner, A. C. Mahon, J. T. Nambu, M. Schaefer, and R. Taussig (1984) Neuropeptides: Mediators of behavior in *Aplysia*. *Science* 225: 1300–1308.
- Siegelbaum, S., J. S. Camardo, and E. R. Kandel (1982) Serotonin and cAMP close single K⁺ channels in *Aplysia* sensory neurons. *Nature* 299: 413–417.
- Sossin, W., M. Kirk, and R. H. Scheller (1986) Peptidergic modulation of the neuronal circuitry controlling feeding in *Aplysia*. *Soc. Neurosci. Abstr.* 12: 241.
- Watson, W. H., and A. O. D. Willows (1986) Homologous peptidergic modulatory neurons in the buccal ganglia of marine molluscs. I. Immunohistochemistry. *Soc. Neurosci. Abstr.* 12: 241.
- Willows, A. O. D., and W. Watson (1986) Homologous peptidergic modulatory neurons in the buccal ganglia of marine molluscs. II. Physiology. *Soc. Neurosci. Abstr.* 12: 241.