Dual Effects of Proctolin on the Rhythmic Burst Activity of the Cardiac Ganglion

ROBERT E. SULLIVAN and MARK W. MILLER*

Békésy Laboratory of Neurobiology, University of Hawaii, 1993 East-West Road, Honolulu, Hawaii 96822

Received July 28, 1983; revised November 15, 1983

SUMMARY

The neuropeptide proctolin has distinguishable excitatory effects upon premotor cells and motorneurons of Homarus cardiac ganglion. Proctolin’s excitation of the small, premotor, posterior cells is rapid in onset (5–10 s) and readily reversible (< 3 min). Prolonged bursts in small cells often produce a “doublet” ganglionic burst mode via interactions with large motorneuron burst-generating driver potentials. In contrast to small cell response, proctolin’s direct excitatory effects upon motorneuron are slow in onset (60–90 s to peak) and long-lasting (10–20 min). The latter include: (a) a concentration-dependent (10^{-9}–10^{-7}M) depolarization of the somatic membrane potential; (b) increases in burst frequency and (c) enhancement of the rate of depolarization of the interburst pacemaker potential. Experiments on isolated large cells indicate: (a) the slow depolarization is produced by a decrease in the resting $G_K$ and (b) proctolin can produce or enhance motorneuron autorhythmicity. A two-tiered non-hierarchical network model is proposed. The differential pharmacodynamics exhibited by the two cell types accounts for the sequential modes of ganglionic burst activity produced by proctolin.

INTRODUCTION

The nine-cell crustacean cardiac ganglion is a simple neural network which exhibits spontaneous rhythmic bursting activity (see Hagiwara, 1961 and Hartline, 1979, for reviews). It consists of five large (80–100 $\mu$m) motorneurons which transmit ganglionic output to the heart musculature and four small (30–40$\mu$m) premotor cells which excite the motorneurons via chemical and electrotonic synapses.

Ganglionic pattern generation results from the electrotonic and chemical synaptic interactions and endogenous burst- and rhythm-generating mechanisms. Slow (200–300 ms), TTX-resistant, $Ca^{++}$-dependent, regenerative depolarizing responses termed “driver potentials” (DPs) have been demonstrated in the motorneurons of crab and lobster ganglia (Tazaki and Cooke, 1979a–c, 1983a,b). It was postulated that the driver potential serves as a source of current for impulse initiation and acts to produce a patterned spike train in response to single nonpatterned inputs. Ganglionic rhythmicity is attributed to the presence of slow ramp or pacemaker potentials (Watanabe, Obara, and Akiyama, 1969; Connor, 1969; Tazaki and Cooke, 1979a; Miller

* Current address: Neurobiology Unit, Institute of Life Sciences, Hebrew University, Jerusalem, Israel.
and Sullivan, 1981). However, the mechanism and localization of the pace-maker activity in the cardiac ganglion are not well understood.

Recently the crustacean pericardial neurohormone, proctolin (Arg-Tyr-Leu-Pro-Thr), has been shown to have direct and specific effects on cardiac motorneurons (Sullivan, 1979; Miller and Sullivan, 1981; Cooke and Sullivan, 1982). Using pulsed applications of proctolin to restricted areas of intact cardiac ganglia, we demonstrated long-lasting, excitatory effects of proctolin on motorneurons. Furthermore, higher concentration pulses produced rhythmic driver potentials in TTX-treated or ligatured cells. Additional effects of proctolin on the small premotor neurons have been noted in a previous abstract (Sullivan, Tazaki and Miller, 1981).

We report here: (1) the responses of intact cardiac ganglia to proctolin, in which differential effects on the two ganglionic cell types are evident; (2) the dose-response relationship of motorneuron responses to bath applied proctolin; and (3) the results of experiments with isolated motorneurons designed to determine the ionic basis of the proctolin response.

**METHODS**

Maine lobster, *Homarus americanus*, (0.5 kg, males) were obtained from local vendors and held in a recirculated seawater tank at 11°C. The recording chamber and recording methodologies were identical to those described previously (Miller and Sullivan, 1981), except where noted.

The physiological saline had the following composition: NaCl, 460mM; KCl, 16mM; MgCl₂, 8mM; CaCl₂, 26mM; and 4 mM HEPES buffer at pH 7.4. Altered saline compositions were as follows: For cadmium experiments, 0.4 – 2.6mM CdCl₂ was added to the normal saline. In 0.1 (K⁺) saline (KCl = 1.6mM), NaCl was increased to 475mM. Sodium free saline was prepared by substituting 462mM Tris-HCl (Tris [hydroxymethyl] aminomethanehydrochloride) for NaCl; the pH was adjusted to 7.4 with Tris base. Tetraethylammonium chloride (TEA; Aldrich), 4-aminopyridine (4-AP; Sigma), tetrodotoxin (TTX; Sigma), SITS (4-acetomido-4'-isothiocyanostilbene-2,2' disulfonic acid; Pierce Chem. Co.), quinine (Sigma), and cesium chloride (ICN; K and K Labs) were added to the saline in the concentrations noted.

Proctolin was obtained from Vega Biochemicals (Tucson, AZ) or Peninsula Labs (San Carlos, CA). Atropine, picrotoxin, octopamine, and ergonovine were obtained from Sigma. Bradykinin potentiating factor 5a (BPP₅ₐ) was obtained from Chemical Dynamics Corporation. Cinanserin, metergoline, and chlorisondamine were gifts from Squibb and Sons, Inc., Farmitalia, and CIBA respectively.

**Drug application**

Two methods of proctolin administration were employed. A 'pulsed' application was made by introducing a small aliquot (50–100μL) of proctolin-containing saline with an Eppendorf pipette to a point 'upstream' from the preparation while maintaining a continuous perfusion. Experiments with 10X K⁺ pulses provided an estimate of concentration profiles in the perfusion chamber, and normal saline pulses controlled for movement artifacts. The pulsed application was designed to mimic the release of proctolin from pericardial organs. Pulsed application resulted in a very rapid increase and subsequent decrease in the concentration of proctolin near specific receptor areas under constant perfusion conditions. As such rapid changes could not be achieved by bath application methods, these experiments provided the best empirical measurements of proctolin's time course of action on motor and premotor neurons. 'Bath' application was achieved by means of a two-way switching valve. Steady state concentrations (as estimated by observation of dye) were obtained within 2–3 min. Bath application was primarily used to examine concentration dependence and drug interactions.

A two-pooled Sylgard (Dow Corning) chamber was utilized to achieve a measure of topographical selectivity in drug administration. The ganglion was placed into a small groove connecting two 0.4 mL U-shaped pools [see Fig. 1(A) for gap placements]; the groove was filled with a paraffin/vaseline mixture and each side of the chamber was perfused independently. Rapid perfusion rates (0.5–1.5 mL/min) were used in experiments designed to examine the time course of drug effects. In the majority of the two-pool experiments (n = 23) the vaseline gap
Fig. 1. Anatomy of *Homarus* cardiac ganglion. (A) Diagram of the ganglion drawn approximately to scale (modified from Cooke, 1962). The positions of the two principle gap placements (G1 and G2) and ligatures (L1 and L2) are indicated. Ganglia are approximately 1 cm. in length. (B) Schematic representation of functional anatomy of the nine neurons comprising the ganglion (adapted from Cooke and Hartline, 1975). Intracellular recordings in this study were made from the five large motorneurons (1–5) located in the anterior trunk. The pharmacologically sensitive region of each cell is denoted by the cross-hatched segment (Cooke and Hartline, 1975). These regions include the proximal inactive axon segment (dashed line) between the soma and the spike trigger zone (X).

was placed across the main trunk in the region of the soma of cell four [G1 in Fig. 1(A)]. With this arrangement, the anterior pool contains the somata of large cells 1–3, the pharmacologically sensitive (Cooke and Hartline, 1975) regions of these large cells as well as those of 4 and 5, terminals from extrinsic fibers, small cell axons and their terminals. The posterior pool contains the somata and chemically sensitive regions of the small cells (6–9), and the soma of cell 5.

Alternatively, the vaseline gap was placed at a point slightly posterior to the posterolateral nerves \((n = 9)\). With this position \([G2 in Fig. 1(A)]\), the posterior pool contains only the somata and pharmacologically sensitive zones of small cells 8 and 9. The anterior chamber holds all of the large cells and the sensitive regions of small cells 6 and 7.

An extracellular monitor of total ganglionic impulse activity across the vaseline gap was obtained by placing a platinum wire electrode in each pool. The motor output of the ganglion was sometimes recorded from cut ends of branch nerves with saline-filled suction electrodes. Extracellular signals were amplified \((\times1000)\) by a Grass P15 AC preamplifier.

Isolation of the most anterior large cells (No. 1 or No. 2) from their normal ganglionic inputs with fine silk ligatures has been utilized to study driver potentials in *Homarus* motorneurons (Cooke and Tazaki, 1979; Miller and Sullivan, 1981; Tazaki and Cooke, 1983b). If the ligature is placed within 1.2 mm of the soma \([L1 in Fig. 1(A)]\), the anterior cell retains its driver potential capability. Placement of the ligature more distal to the cell soma (i.e., close to the
anterior bifurcation) preserves both the driver potential and spike initiating zone of the isolated cell [L2 in Fig. 1(A)]. In this study we found that overly tight ligatures may adversely affect the physiological properties and pharmacological responsiveness of isolated cells. Isolation of cells by transection with scissors consistently produced viable preparations.

Intracellular recordings of large cell membrane potentials were made by impaling somata with micropipettes (10–20MΩ) filled with 2M KCl. For experiments requiring current passage, a second electrode equipped with a constant-current injection “bridge” circuit (WPI M707) was introduced independently. A third pipette in another soma was occasionally used to examine electrotonic coupling. Currents were measured by means of a virtual ground (WPI 180).

RESULTS

Application of proctolin to whole ganglia

A single pulse of proctolin (100 μL of 10⁻⁶M, applied to the ganglion with constant continuing normal perfusion) results in a fixed sequence of altered motor output in the spontaneously bursting ganglion. Intracellular recording from large cell somata reveals: (1) increases in burst duration; (2) biphasic changes in burst frequency; and (3) a depolarization which is slow in onset and long lasting [Figs. 2, 3(a,b)]. Within 5–10 s of proctolin administration, bursts become progressively prolonged. This prolongation becomes maximal between 15 and 30 s, at which time ganglia typically exhibit a “doublet” mode of bursting [Fig. 2(A), panel 2]. If each such event is considered as a single burst, the burst duration reaches a maximum during this period of doubletting [Fig. 2(D)]. Coincidentally, the burst frequency is observed to

![Fig. 2. Proctolin induces sequential changes in burst parameters of the isolated ganglion. (A) Intracellular recording from motorneuron 1 (V_m = -59 mV) demonstrates the time-dependent proctolin effects. Samples of burst activity (1–5) were taken at the times indicated on the continuous plot of instantaneous burst frequency [shown in (B)]. A total of 14 min is shown. Time 1 is control. Within 10 s of application of a pulse of proctolin (1.5 × 10⁻⁶M; 100 μL), burst frequency began to decline; (B) reaching a minimum of 65 per min after approximately 25 s. Concurrently, the ganglion exhibited a period of double bursting (Time 2). Considering each doublet as a single burst, there was a coincident peak in burst duration (D). (C) Maximum V_m of cell 1: the membrane potential underwent a slow depolarization which began approximately 15 s after application and became maximal after 1.5 min. Maximum frequency in this preparation was obtained approximately 7 min after application of proctolin (Time 4). The ganglionic burst frequency exceeded the control rate for the ensuing 7 min returning to the control frequency concurrently with motorneuron repolarization (Time 5). (D) Burst duration: a peak during the period of double bursting corresponded with the time of minimal burst rate.]
undergo a progressive decrease [Fig. 2(B)]. The burst rate becomes minimal concurrently with the peak in burst duration; i.e., the period of doubletting. Burst rate then begins to increase again and for a period lasting up to 20 min is greater than control rates.

The membrane potential ($V_m$) of the large cell, as measured from the maximum postburst hyperpolarization, undergoes a slow depolarization in response to a proctolin pulse [Fig. 2(C)]. Onset of this response occurs 20–30 s after administration and peaks between 60 and 90 s. Thereafter, it decays slowly, requiring 10 to 20 min to return to the original $V_m$ (see Miller and Sullivan, 1981). Throughout the duration of this depolarization, the rate of ganglionic bursting exceeds the control frequency. The burst duration, as measured at the base of the intracellurally recorded burst, usually remains slightly prolonged throughout the recovery from the effect [Fig. 2(D)] and a marked 'shouldering' may be observed on the form of the burst potential (compare A4 with A1). All effects eventually reverse [Fig. 2(A), panel 5].

Intra-axonal recording from an unidentified motorneuron permits an examination of drug effects upon ganglionic motor output. Construction of a

![Fig. 3. Effects of proctolin on ganglionic output. (A) Dot pattern constructed from intra-axonal recording from unidentified motorneuron. Each dot represents an action potential and each horizontal row corresponds to a burst. A slow vertical sawtooth caused successive bursts to appear directly below one another so that vertical displacement of each row provides a measure of burst frequency. The onset of a ganglionic response to a proctolin pulse ($1 \times 10^{-5} M; 100 \mu L$; arrow) is shown. Double bursting occurred within seconds of proctolin administration. Additional effects included: (1) an increase in the number and frequency of impulses comprising the first part of the burst; and (2) a slight decrease in burst frequency. Note the comparatively small number (1–4) of impulses comprising the second phase of each double burst. (B) Alterations in axonal spike trains at different times following pulsed ($1 \times 10^{-5} M; 100 \mu L$) proctolin application. Top: zero reference for intracellular recording. Middle: extracellular recording of ganglionic motor output recorded from the right anterolateral branch. Only motorneuron activity is monitored with this arrangement (see Hartline, 1979). Bottom: intra-axonal recording from unidentified axon on left anterolateral branch. Control: three superimposed bursts. Each intracellular trace consisted of five or six grouped impulses and a single impulse occurring approximately 150 ms later. Fifty seconds after proctolin application (50 s) the burst exhibited a doublet form, with four impulses comprising its second part. Note also the doublet burst as it appears in the composite extracellular trace. Four minutes after peptide application (4 min) the burst consisted of a single train of 14 impulses which lasted approximately 250 ms. Sweeps were triggered by the first impulse.](image-url)
“dot” pattern (see Cooke and Hartline, 1975) for the analysis of spike trains during the onset of a proctolin response revealed: (1) the rapid development of a double burst mode; (2) a slight decrease in burst frequency; and (3) gradual increases in the number and intraburst frequency of impulses comprising the first part of the double bursts (Fig. 3). At most, the second part of the double burst consisted of four impulses, well below the number comprising the initial part. We should note that this two-phase nature of cardiac ganglion motorneuron spike patterning was initially studied by Maynard (1961). The possible role of the relative refractory period for motorneuron driver potential generation in the production of “doublet” burst mode motor output is considered in the discussion.

Another intra-axonal recording demonstrated marked alterations in motor output sampled at different times following a pulsed proctolin application [Fig. 3(B)]. After 50 s, the impulse train exhibited a doublet form with four spikes grouped into the second segment. Later in the response (4 min) the spike pattern consisted of an uninterrupted prolonged train of impulses with an initial high frequency which progressively decayed. Experiments described below demonstrate that the initial short-term effects are caused by excitatory actions of proctolin on the small posterior cells and that the delayed and long-lasting effects are due to direct actions on the large motorneurons.

**Split chamber experiments**

The two general types of split chamber arrangements described in materials and Methods are designed to provide a means of selective drug administration to pharmacologically sensitive areas of large and/or small cells. With the vaseline gap placed in the region of cell body 4 [G1 in Fig. 1(A); see Methods], anterior proctolin application affects primarily the large motorneurons 1–4 and results in several long-lasting effects. Recording from any one of the anterior large cells reveals: (1) an increase in burst frequency; (2) increases in burst duration; and (3) a prolonged depolarization. All of these effects are slow in onset (60–90 sec to peak) and require 10–20 min of wash with normal saline for complete reversal (Fig. 4; see also Miller and Sullivan, 1981). Introduction of a pulse of 10X K⁺ saline in a manner identical to the proctolin administration produced a depolarization of the anterior motorneuron which reversed within one min [Fig. 4(A)]. This depolarization provides an estimate of the perfusion system flow parameters and indicates that the delayed and prolonged time course of the proctolin response reflects actual properties of the receptor-effector system. Note that the high K⁺ induced depolarization of the anterior region did not alter ganglionic burst frequency. This observation suggests that the effect of proctolin on ganglionic burst frequency is not simply the result of motorneuron depolarization.

In addition to its depolarizing properties, proctolin also appears to markedly alter the form of the motorneuron membrane potential trajectory during the interburst interval [Fig. 4(B)]. In untreated *Homarus* ganglia, bursts are typically followed by a small (2–3 mV) after-hyperpolarization which diminishes within 1 s and is followed by a ‘plateau’ period during which the membrane potential is stable (see also Connor, 1969). In the presence of proctolin the interburst interval assumes a steeper (up to 5 mV/s) trajectory (Fig. 4(B), 50 s panel). The burst potential continues to be initiated by a marked inflection, which is presumed to be of synaptic origin.
Fig. 4. Effects of a proctolin pulse applied to the anterior region isolated with a vaseline gap at G1 site. (A) Intracellular recording from cell 3 (V_m = -54 mV) and cell 5 (V_m = -56 mV). Vaseline gap was placed directly over the region of soma 4. Introduction of a pulse of proctolin (1 x 10^-6 M; 50 μL) to the anterior chamber produced a depolarization which was slow in onset and long lasting. Ganglionic burst rate underwent an increase which was maximal approximately 80 s after application. A prepulse of 10 x K+ saline (50 μL) applied in an identical manner produced a depolarization which reversed within 1 min. The baseline membrane potential of cell 5 was unaffected by the two treatments, indicating the efficacy of the vaseline gap. (B) Same preparation as (A). Records taken from anterior cell 3 at various times after application of the proctolin pulse to the anterior chamber. At 50 s the ganglion was close to its maximal frequency and a marked increase in the trajectory of the interburst pacemaker potential may be noted. The effect required 14 min of wash for complete reversal. (For further discussion of anterior applications see Miller and Sullivan, 1981.)

Vaseline gap placement G2 is expected to provide a “pure” small cell response, with posterior drug application, since only the pharmacologically sensitive regions of cells 8 and 9 are present in the posterior pool. With this
posterior gap placement, a pulse of proctolin (100 μL; $1 \times 10^{-6} M$) to the posterior chamber alters ganglionic bursting in a reversible manner which is rapid in onset and decay [Fig. 5(A) and Fig. 6, posterior application]. Effects include: (1) increase in burst duration; (2) increase in impulses per burst; (3) decrease in the interburst interval; and (4) decrease in burst frequency in ganglia with normal control rates (>30/min). The effects begin within 5–10 s after application, reach a maximum between 15 and 30 s, and are reversed within 3 min of washout with normal saline [Fig. 5(A)]. Comparable changes in ganglionic bursting are obtained with a 10X K⁺ saline pulse applied to the posterior cells (not shown), suggesting that proctolin may simply be depolarizing the small cells.

Bath application of proctolin restricted to the small cell region never produced a change in the baseline $V_m$ of large anterior cells. With increasing concentrations, a dose-dependent prolongation in the duration of small cell bursting was observed. At low concentrations, the prolongation of the burst consisted entirely of EPSPs reflecting small cell activity as seen in the

![Fig. 5. Effect of application of proctolin to small posterior cells. Vaseline gap at G2 site located slightly posterior to posterolateral nerves, placing the pharmacologically sensitive zones of cells 8 and 9 in the posterior pool. (A) Recording from anterior motorneuron 3; $V_m = -53$ mV. Pulse of proctolin (3 $\times$ $10^{-6} M$; 50 μL) to posterior pool had excitatory effects within 5 s which were maximal after approximately 15 s, at which time the ganglion exhibited a double bursting mode. All effects were reversed within two min. There was no change in the basal membrane potential of cell 3. (B) Different preparation with same gap placement as (A). Bath application of proctolin at low concentrations to the posterior cells produced prolonged bursting observed as PSPs in the monitored anterior cell 3 ($V_m = -56$ mV). With a higher proctolin concentration, the small cell excitation was sufficient to elicit a second abbreviated motorneuron burst.](image-url)
Fig. 6. Differential timecourse for anterior versus posterior effects. Gap placement (G2) slightly posterior to posterolateral nerves. Posterior proctolin application (1 × 10^{-6}M; 50 μL) to the pharmacologically sensitive areas of small cells 8 and 9 produced an effect which was rapid in onset and reversed within three min. Anterior effects were also rapid in onset (due to small cells in the anterior pool), but required 15 min for complete reversal (large cell effects). Identical flow rates (approx. 1 mL/min) used for the two administrations.

motorneuron recording [Fig. 5(B), middle panel]. Extracellular recordings show long trains (>600 ms) of small cell spikes (not shown) suggesting that proctolin produces plateau potentials in small cells. At a higher concentration (6 × 10^{-6}M), the small cell input was sufficient to produce a second burst in the large cells [Fig. 5(B), right panel]. The form of the membrane potential record suggests the presence of driver potentials underlying the two large cell bursts. The interaction between small cell excitation and motorneuron driver potentials probably accounts for the “double bursts” noted in whole ganglion responses to proctolin [Fig. 2(A); see Discussion].

Anterior drug application with gap position G2 would be expected to produce mixed effects due to the presence of all of the large cells and the pharmacologically sensitive zones of some small cells in the anterior pool. As seen in Figure 6, anterior application of proctolin produced effects which were rapid in onset, presumably due to actions on small cells in the anterior pool (cells 6 and 7), and long-lasting, due to large cell effects (Fig. 6, anterior application).

As noted in our previous article (Miller and Sullivan, 1981), it seems improbable that differences in the geometry of the small and large cell region can account for the different time courses of action, i.e., small cell versus large cell. Since anterior application of proctolin produces immediate small cell effects (Fig. 6), one must assume that no gross diffusional barriers prevent proctolin’s access to the anterior region and large cell receptor areas. Note also the rapid time course of 10x K^+ saline [Fig. 4(A), Cell 3; Miller and Sullivan, 1981, Fig. 1] effect after anterior application. Similar time constants (τ_s < 60 s) have been obtained for the efflux of radiolabeled choline
from the extracellular space of this ganglion (Sullivan, unpublished). Moreover, the half time for proctolin's release from receptor would be in the order of $10^{-2}$ to $10^{-1}$ s given the ED$_{50}$ = $K_d$. Thus the prolonged time course of the large cell effect must be due to secondary intracellular processes. We interpret the different time courses as prima-facie evidence supporting our conclusion that proctolin has separate and distinct actions on large and small cells.

**Effects of proctolin on isolated large cells**

Two additional procedures were utilized in order to demonstrate direct effects of proctolin on the large anterior cells: (1) application of tetrodotoxin (TTX, $3-6 \times 10^{-7}M$) was used to eliminate spike mediated interactions between small and large cells (see Tazaki, 1971; Miller and Sullivan, 1981); (2) transection or ligature of the two most anterior somata.

Isolated somata are generally inactive, exhibiting stable resting potentials between $-40$ and $-50$ mV with a nonpatterned low-level (1-3 mV) background activity. This activity probably consists of: (1) decremented impulses from the damaged motorneuron axons; (2) PSPs from damaged small cell axons and extrinsic input fibers; and (3) spontaneous endogenous subthreshold oscillations (e.g., Fig. 12). All were eliminated by TTX (Fig. 7). Application of proctolin produces a depolarization which is slow in onset, long lasting, and concentration-dependent. With the ligature or transection placed at a point distal to the cell soma (L2 in Fig. 1), the proctolin-treated cell usually exhibits a period of rhythmic bursting lasting for several minutes (Fig. 7, top panels). The somatic waveform of these bursts consists of large (15–20 mV; 200–300 ms) driver potentials upon which an indistinguishable number

![Fig. 7. Effects of proctolin on a motorneuron isolated by transection. Recording from soma of cell 1 ($V_m$; $-46$ mV) with transection at the L2 position; see Figure 1. One minute bath application of proctolin ($1.5 \times 10^{-5}M$) produced a long-lasting depolarization which was delayed and slow in onset (top left panel). Repetitive burst activity (shown at faster paper speed in top right panel) occurred during the initial six min of the response. In the presence of TTX ($3 \times 10^{-7}M$) the low level background activity is eliminated, as evidenced by the reduction in thickness of the baseline (middle left panel). $V_m$ was reduced 2 mV. Application of proctolin produced a depolarization and rhythmic driver potential activity (middle right panel) which damped out after two–three min. Addition of cadmium (400$\mu$M) eliminated the DPs leaving only a 12 mV slow depolarizing response to proctolin (bottom panel). Note the sodium dependent component of the “driver potential.”](image-url)
Fig. 8. Concentration-dependence of proctolin-induced depolarization of isolated motorneurons. Points represent mean (± S.E.M.) responses of several cells (n = 5–7) from four ganglia isolated by ligature at the L1 position. Line represents a least squares fit of the data points between 12–92% saturation. The ED50 is 2.1 × 10⁻⁸M; estimate based on average maximum depolarization between 3 × 10⁻⁷M and 1.5 × 10⁻⁷M (n = 18). Resting Vm ranged from 40–48 mV (m = 45 mV). Proctolin was bath applied for three min. Insert: Hill plot from a typical experiment. Abscissa: log of proctolin concentration; ordinate: log (mV depolarization/maximal mV depolarization minus mV depolarization). Slope of the least squares fit through the data points is 1.0 (r² = 0.99).

of attenuated action potentials is superimposed.* A rapidly rising ramp or pacemaker potential precedes each burst. These results further support the hypothesis that proctolin has direct actions on the motorneurons (Miller and Sullivan, 1981).

In the presence of tetrodotoxin (3 × 10⁻⁷M), application of proctolin produces a depolarization and repetitive rhythmic driver potentials in the isolated motorneuron (Fig. 7, middle panels). Driver potential frequency (100/min) is nearly the same as bursting in the absence of TTX. However, the train of DPs terminates earlier than the bursts (Fig. 7, top panels), generally damping out with a series of subthreshold oscillations. Note that the magnitude of the drug induced DP in TTX is less than that in normal saline suggesting that TTX sensitive currents contribute to the bursts. Again, a pacemaker potential leads into each DP often with a clear demarcating inflection.

Application of proctolin to an isolated motorneuron in the presence of TTX and Cd⁺⁺ results in a long-lasting depolarization without the rhythmic Ca⁺⁺-dependent activity observed with TTX only (Fig. 7, bottom panel). (Cadmium is also a partial blocker of the early outward current; see Tazaki

---

* It is worth noting that extracellular recordings from bursting, isolated cells reveal abnormal intra-burst spike patterning (unpublished observations). Thus the “driver potential” (Ca⁺⁺ and Na⁺ dependent) functions as a frequency dependent Boolean switch and affords a permissive time for integrated drive to effect the highly characteristic intraburst spike pattern. See Figures 7, 11C, and 13.
and Cooke, 1983c; Salkoff, 1983). The comparative ease of measuring peak proctolin-induced depolarizations in the absence of active currents made TTX-Cd⁺⁺ saline the preferred medium for examining current–voltage relationships (Fig. 9) and possible blocking agents (Fig. 10).

The concentration-dependence of the proctolin-induced depolarization was examined in motorneurons isolated by ligature (Fig. 8). Threshold for measurable responses was consistently found to be approximately $1 \times 10^{-9} M$. With bath application of proctolin, the $ED_{50}$ was $2.1 \times 10^{-9} M$; this value is notably different from that obtained by the pulse method ($ED_{50} = 10^{-7} - 10^{-6} M$; Miller and Sullivan, 1981). Replotting a typical dose response curve as the Logit transform (insert, Fig. 8) produced a Hill coefficient of 1.0 ($r^2 = 0.99$). While this may indicate independent and noninteracting proctolin-receptor kinetics, the data are clearly subject to multiple interpretations. It is, however, interesting to note the phenomenological similarities between these data and the proctolin responses obtained with *Periplaneta* hindguts ($ED_{50} = 2 \times 10^{-9} M$; Sullivan and Newcomb, 1982) and cardiac muscle ($ED_{50} = 2 \times 10^{-9} M$; Benson et al., 1981).

Current-voltage relationships of motorneurons in TTX or TTX + Cd⁺⁺ revealed an increase in the apparent input resistance (19.6 ± 9.6%, $n = 5$) in the presence of proctolin. One particularly vital preparation exhibited an input resistance of 3.2 MΩ, permitting an investigation of the I–V relationship at very hyperpolarized membrane potentials [Fig. 9(A)]. In this

![Graph: Fig. 9. Motorneuron depolarization involves a decrease of resting potassium conductance.](image)

(A) Current-peak voltage relationships in the presence and absence of bath-applied proctolin (1.5 × 10⁻⁶ M) under current clamp conditions. TTX (3 × 10⁻⁷ M) and Cd⁺⁺ (4 × 10⁻⁴ M) present to block active responses. Transected cell 2 ($V_m$: −48 mV). Proctolin produced a 10 mV depolarization and a 37.5% increase in input resistance (4.4 MΩ / 3.2 MΩ). The intersection of the least squares fit ($r^2 > 0.999$) indicates a reversal potential of 79 mV for the proctolin response close to $E_k$. Abscissa: peak mV deflection (at 160 ms); ordinate: nanoamps.
cell, proctolin produced a 26% increase in the peak input resistance and the two I–V curves intersected at -79 mV, a value which would be consistent with a K⁺ equilibrium potential where |[K⁺]|/[K⁻] = 23. Additional experiments revealed similar intercepts (m = 78.2 ± 7.7 mV, n = 4) as estimated from the least square linear fits. Recordings from cells of high input impedance (3–5 MΩ) often exhibited a slowly activating (τᵱ = 200 ms), anomalously rectifying conductance; data not shown. This current was most apparent in the hyperpolarizing region more negative than E_K; thus we used peak conductance values obtained at 160 ms to produce the I–V plots in Figure 9(A).

While the linearity of the current–voltage relationships indicated that the resistance increases noted in the presence of proctolin were drug-induced rather than a secondary potential-dependent phenomenon, a manual voltage clamp was utilized to further verify this observation [Fig. 9(b)]. Passage of hyperpolarizing current maintained the membrane potential at the pre-drug resting value while constant current test pulses were passed throughout the response. In these experiments the apparent membrane input resistance, as evidenced by the magnitudes of the voltage deflections produced by the pulses, increased by 22–50% (m = 32.7 ± 15.1%; n = 3) at the peak of the proctolin response.

The response of motorneurons to proctolin was examined in media containing altered K⁺ concentrations. Lowering the K⁺ concentration in TTX-containing saline to 1.6mM (0.1 × normal) produced a 5–10 mV hyperpolarization.
Bath application of proctolin to 0.1x K+ treated cells produced slow depolarizations which were 25–44% (m = 33.0 ± 9.8%; n = 3) greater than control responses (not shown). Additional experiments in which the hyperpolarization caused by the 0.1x K+ saline was nullified by passing a steady depolarizing current throughout the proctolin test dose gave similar results; the average increase in proctolin response was 51.3 ± 16.0%; n = 3. In the experiment shown in Figure 9(C), bath application of proctolin (3 min) produced a 19 mV depolarization in 0.1x K+ medium, compared to 12 mV under control conditions. An increase in the number of spontaneous DPs and an enhancement of the magnitude of the DP after-hyperpolarization were also observed in the 0.1x K+ saline.

Further pharmacological experiments with transected motorneurons were designed to reveal the ionic basis of the slow, proctolin-induced depolarization. Bath applied TEA (20–50mM) produced a 5–10 mV depolarization and an increase in input resistance (Fig. 10(A), middle panel). Application of proctolin in the presence of TEA resulted in depolarizations which slightly exceeded control responses. Similarly, bath applied 4-aminopyridine (4-AP) had no effect on Vm or the magnitude of proctolin-induced depolarization [Fig. 10(A), bottom panel]. These experiments indicate that proctolin does not depolarize the motorneuron by decreasing either the delayed rectifier current, or an early outward potassium current. The description and rather complex pharmacology of these crustacean neuronal currents have been previously noted (Mirolli, 1981; Tazaki and Cooke, 1983; see also Salkoff, 1983).

The involvement of additional potassium currents such as the anomalous rectified (Ih) or the calcium-activated potassium currents (Ica) in the proctolin-induced depolarization were similarly ruled out. Hanani and Shaw (1977)

![Fig. 10. Tests for ionic basis of the proctolin-induced depolarization of motorneurons. All experiments from transected large cells (1 or 2) in the presence of TTX (3 x 10^-3M) and cadmium (4 x 10^-4M). A two min bath applications of proctolin (1.5 x 10^-5M) was used for all tests tests. (A) Proctolin response is not affected by blocking the delayed rectifier (Ik) or the early outward potassium currents: TEA (3 x 10^-2M) produced a 5 mV reduction of Vm and slightly enhanced the proctolin responses; 4-AP (1 x 10^-2M) had no effect on Vm or the proctolin response. (B) The proctolin response is not affected by agents that block other known potassium currents: Cesium (2 x 10^-3M) which blocks Ik and quinine (5 x 10^-3M) which blocks Ica, had no effect on Vm (−46 mV) or the response to proctolin. (C) The chloride channel blocking agent SITS (4 x 10^-2M) had no effect on membrane potential, input resistance, or the proctolin response. O-Na+ Tris-substituted saline produced a 5 mV reduction of Vm (−49 to −44 mV) and a 47% (2.5MO/1.7MO) increase in apparent input resistance. Note that the proctolin response was reduced by approximately 40% (4.5 mV/7.5 mV) (see Results and Discussion for further explanations).]
have demonstrated that quinine is an effective blocker of $I_{K_{Ca}}$ in crustacean neurons whereas cesium has been shown to block the former in mouse neurons (Westbrook and Mayer, 1982) and Aplysia (Benson and Levitan, 1983). Bath application of $2 \times 10^{-3}M$ cesium and $5 \times 10^{-4}M$ quinine had no effect on $V_m$ or the proctolin response [Fig. 10(B)]. These observations, coupled with the apparent absence of $I_m$ in these somata [see Fig. 9(A) and Discussion] indicate that proctolin does not act by decreasing any of the previously characterized potassium conductances ($G_K$).

Possible contributions of ions other than potassium were also tested. The anion blocking agent SITS had no effect on $V_m$ or the proctolin-induced depolarization [Fig. 10(C), middle panel] suggesting that a chloride conductance is not involved. Finally, eliminating sodium from the medium with Tris-substituted saline produced an initial biphasic response resulting in a net (2–3 mV) depolarization and marked increase in the apparent input resistance (ca. 2X). Proctolin responses in O-Na⁺, Tris-substituted media were reduced by $44.7 \pm 5.0(3)\%$.

We can propose two possible interpretations of the above observations. First, if a small concurrent increase in sodium conductance accompanied the larger decrease in potassium conductance, the proctolin response should be reduced in zero sodium saline, i.e., the increased sodium conductance would be negated. This would be especially true if the resting sodium conductance is negligible. If the large cell membrane potential obeyed the relationship dictated by the Goldman (1943) equation with $P_{Na}$ contributing significantly to $E_{rest}$, we would expect zero sodium saline to cause a hyperpolarization. As noted above, it produces a net depolarization. Therefore, it is possible that $P_{Na}$ is insignificant at $E_{rest}$, but more likely that the absence of sodium ions (or the presence of Tris cation) is responsible for additional specific ion permeability changes which underlie the small depolarization and increased input resistance. Although a further complication is the presence of an electrogenic sodium pump (Livengood and Kusano, 1972), we should note that ouabain (20μM) has no effect on the proctolin response (our unpublished observations).

The second interpretation assumes that $P_{Na}$ at rest is significant with secondary “Tris” saline effects” giving rise to the curious observations just noted. In this case the diminished response to proctolin directly supports the reduced $G_K$ hypothesis, i.e., resting $G_{Na}$ is responsible for the depolarization produced by a proctolin-induced reduction in resting $G_K$. Therefore, a reduced sodium equilibrium potential would result in a diminished proctolin response. Moreover, it is probable that the zero sodium salines were actually more effective in blocking the proctolin response than our measurements indicate considering that the Tris-substituted salines increased the membrane resistance by a factor of approximately two. Both interpretations do require a reduced $G_K$ in response to proctolin and we feel this has also been demonstrated by the position of the I-V intercepts [Fig. 9(A)] and effects of reduced potassium [Fig. 9(C)].

As noted in our preceding brief communication (Miller and Sullivan, 1981), the cardiac ganglion proctolin receptors show a high degree of specificity when proctolin responses are compared to those of structurally similar peptide analogues. It is also clear that proctolin is not partially agonizing any of the known pharmacon receptors of the cardiac ganglion because the proctolin response is unaffected by the following drugs: atropine (1mM), picrotoxin
Fig. 11. Proctolin-induced bursting in ligatured neurons. (A) Intracellular recording from anterior cell 2 offset 20 mV ($V_m = -44$ mV) and cell 3 ($V_m = -47$ mV). Ligature (LIG) was placed close to the anterior bifurcation. Although a small amount of coupling could be seen to persist through the ligature [(A); control panel], proctolin-induced ($1 \times 10^{-7}M$; bath application) repetitive bursting in cell 2 (3 min panel) bore no relationship to ganglionic bursting (monitored in cell 3). Note presence of impulse(s) in isolated cell due to location of the ligature (compare with Fig. 7). Effects reversed in ten min wash with normal saline (10 min panel). (B) Chart records of ligatured cell response to bath application of proctolin reveal oscillatory prepotentials in the ligatured cell. Same preparation as (A); shows typical time course of response for motorneuron isolated by ligature. Note that during onset of response (30 s panel) oscillatory prepotentials gradually developed into uninterrupted repetitive burst activity (1 min and 3 min panels). During course of wash with normal saline, subthreshold oscillations were again present (7 min wash panel). (C) Plot of burst frequency versus duration (measured at base of burst) for ligatured cell 2 during response shown in (B). Mean ± S.D.; n for each point in parentheses.

(1 $\times 10^{-4}M$), cinanserin ($2 \times 10^{-4}M$), octopamine ($5 \times 10^{-5}M$), metergoline ($5 \times 10^{-4}M$), ergonovine ($3 \times 10^{-6}M$), and chlorisondamine ($5 \times 10^{-4}M$); these data are not shown.

Experiments with isolated large cells reveal that certain burst characteristics which have been noted with intact ganglia may be attributable to endogenous properties of the motorneuron. Placing a ligature close to the anterior bifurcation typically renders ligatured anterior motorneuron (1 or 2) silent (nonbursting) while cell 3 exhibits normal burst activity [Fig. 11(A)]. Application of proctolin produced repetitive burst activity of the isolated anterior cell at frequencies which, at their maximum, approached those of the remainder
of the ganglion (3 min panel). During the onset of the proctolin response, subthreshold oscillatory prepotentials often appeared in the ligatured cell [Fig. 11(B), 30 s panel]. The frequency of bursting in the ligatured cell increased as it depolarized (1 min panel) and at its maximum (3 min panel) the subthreshold oscillations were no longer present. As the burst rate of the ligatured cell increased during onset [Fig. 11(B)], there was a progressive decrease in the duration of each burst. Likewise, during washout, the burst duration increased as the frequency decreased (11A,C). Such an inverse relationship has been pointed out previously for total ganglionic burst activity (Maynard, 1955; Mayeri, 1973; Cooke and Hartline, 1975). Its presence in proctolin-activated large cells isolated by ligature demonstrates that this phenomenon is an intrinsic property of the motorneuron. A similar inverse relationship between the magnitude of the slow potential underlying bursting and its recurrence frequency has been described in motorneurons (Panulirus japonicus) isolated by transection (Tazaki, 1973).

Fig. 12. Proctolin effects on isolated motorneuron exhibiting spontaneous oscillatory prepotentials and bursting. Intracellular recording from cell 1 ($V_m = -45$ mV) with ganglion transected near anterior bifurcation. (A) Cell exhibited spontaneous subthreshold oscillatory activity of progressively increasing magnitude which was reset following the occurrence of a driver potential with superimposed impulses (a burst). (B) Injection of depolarizing current through recording electrode produced repetitive burst activity. Hyperpolarization eliminated oscillatory activity. (C) Application of proctolin ($1 \times 10^{-6} M$; $100 \mu L$ aliquot) produced a slow depolarization and a progressive increase in burst frequency and decrease in the amount of interburst prepotential activity which resulted in an uninterrupted train of bursts (not shown). A primary action of proctolin in reducing leak $G_K$ may lead to secondary effects on both oscillatory prepotentials and driver potentials in motorneurons.
Fig. 13. Current and alternative models for rhythmic pattern generation in the isolated cardiac ganglion of Homarus americanus. Left: Endogenously oscillating premotor small cells (\(\sim\sim\)) impose their rhythmic activity upon large motorneurons via chemical and electrical synapses. Large cells possess intrinsic burst forming mechanisms, i.e., "driver potentials" (\(\sim\)), but have no endogenous rhythmicity. Right: In the presence of neurohormones, proctolin or the trypsin-sensitive frequency peptide (Sullivan, 1979), or excitatory neuro-transmitters, all five motorneurons become endogenous oscillators (\(\sim\sim\sim\)) while the small cells may produce "plateau potentials" (\(\sim\)). Under these conditions, large cell rhythmicity may control ganglionic burst frequency. (See text for further discussion.) Intraburst impulse patterning is an integrated function of small cell synaptic drive, large cell "driver potentials" and the repetitive firing characteristics of motorneuron spike initiating zones.

Occasionally, spontaneous "driver potentials" in transected cells were observed. Such "driver potentials" were preceded by a series of oscillatory prepotentials of progressively increasing magnitude [Fig. 12(A)]. The progressive growth of the prepotentials appears to be reset by the occurrence of a driver potential, but this may simply reflect the high conductance state of the DP afterhyperpolarization (Tazaki and Cooke, 1979b). Passage of hyperpolarizing current [Fig. 12(B)] eliminated the prepotentials indicating that they represent an endogenous voltage-dependent mechanism in the isolated motorneuron. As in ligatured preparations, application of proctolin caused a progressive decrease in the number of prepotentials preceding successive DPs [Fig. 12(C)]. It is possible that the proctolin-induced increase in input resistance serves to enhance the endogenous oscillatory prepotentials. This, in turn, could bring about an uninterrupted train of driver potentials.

DISCUSSION

The above data provide physiological evidence for two sites of proctolin action on the lobster cardiac ganglion. While the fast proctolin response elicited from small cells is clearly demonstrable it is difficult to study because
of our inability to visualize and impale the premotor neurons. The proctolin induced slow depolarization of isolated motorneurons was studied in detail. The present study which utilized bath application to achieve steady state concentrations of ligand revealed an $ED_{50}$ of $2.1 \times 10^{-5} M$ for the proctolin response; these data strongly support our previous hypothesis that cardiac ganglion proctolin receptors are occupied by hormonal proctolin. Based on current clamp studies and ionic manipulations we have concluded that proctolin reduces an outward potassium conductance to produce the slow depolarizing response. Moreover, we have used proctolin, a natural hormonal modulator, for perturbation analysis of cardiac ganglia activity. We are now able to briefly comment on the roles of endogenous cellular properties and synaptic interactions in rhythmic pattern generation and the dynamics of neuronal function in coordinated network activity.

Hormonal modulation of neural systems

These experiments address the question of how a single pharmacon (in this case a peptide neurohormone) could produce a fixed sequence of altered motor outputs via differential actions on network elements. Although the effects of proctolin on the two cell types are both excitatory, their differential time courses and characteristic alterations of ganglionic activity make it possible to delineate their separate contributions to observed network behavior.

Exposure of the ganglion to a pulse of proctolin initially produces an excitation of small cells. This results in an immediate prolongation of ganglionic burst duration, which usually includes a period of double bursting. It should be reemphasized that the small cell effect decays with a time constant of approximately 1 min (Fig. 6: posterior application). This is nearly one order of magnitude faster than the decay of proctolin effects on the motorneurons. This indicates that the pharmacodynamic phase (Ariens et al., 1979) of proctolin action on small cells is different from that on motorneurons. The prolongation of the small cell burst, as evidenced by postsynaptic and extracellular recording, may simply be the result of a rapid depolarization produced by a classical receptor-gated ion channel. However, we might also speculate that the prolonged bursts of impulses are the direct result of proctolin-induced plateau potentials (i.e., prolonged driver potentials) in the small cells. The small premotor neurons of *Portunus* and *Squilla* cardiac ganglia are reported to respond similarly to dopamine and accelerator stimulation, respectively (Miller et al., 1981; Watanabe, Obara, and Akiyama, 1969).

In ganglia with high or moderate basal rates of bursting (>30 bursts/min), premotor excitation typically produces a decrease in burst frequency [Figs. 2(A), 5(A)]. However, simultaneous decreases in interburst intervals [Figs. 2(A), 3(A); 6] result in a larger proportion of each cycle being occupied by bursting. Increases in ganglionic burst frequency have been noted in preparations with low basal rates (<20/min).

Previous investigators have observed double bursts in untreated ganglia (Maynard, 1955; Hartline, 1967). Maynard noted that small cell impulse activity continued through such bursts and also pointed out that there were fewer large cell impulses in the second half of the burst. Our proctolin-induced double bursts have a similar form. We suggest that if the duration of the small cell burst exceeds a certain critical level, it may outlast the
refractory period of the motorneuron driver potential and trigger a second large cell burst. Relative refractory periods of 500–700 ms have been measured for driver potential generation in isolated Homarus motorneurons (Tazaki and Cooke, 1983b; our unpublished observations). The 200–500 ms interval between the two halves of observed double bursts indicates that the second half is produced during the relative refractory period, which may account for its diminished number of impulses. Similar double bursts associated with epileptiform events have been recorded from penicillin-treated hippocampal slices and were attributed to excitatory synaptic interactions between neurons possessing intrinsic burst capabilities (Traub and Wong, 1982).

In marked contrast to the small cell response, effects of proctolin on the large motorneurons are slow in onset (60–90 s to peak) and persist for 10–20 min. Throughout this period, ganglionic burst frequency is elevated over control rates, interburst pacemaker potentials assume steeper trajectories, and somatic burst potentials often exhibit broadened or more pronounced “shoulders” (Figs. 4 and 6; see also Miller and Sullivan, 1981). The long-lasting motorneuron depolarization appears to be produced by a decrease in resting $G_K$ as indicated by the intercept of the I-V plots and an enhancement by low potassium saline [Fig. 9(A)–(C)]. The physiological result of the reduced conductance is to promote spontaneous bursting in the motorneurons.

**Oscillatory prepotentials**

In motorneurons, oscillatory prepotentials are often observed in association with autorhythmicity [Figs. 11(B) and 12(D)]. It appears as though one effect of proctolin may be to elicit or enhance such oscillatory prepotentials and that they may in turn serve to trigger repetitive driver potentials. The relationship, if any, between these prepotentials and pacemaker potentials is not clear. Oscillatory prepotentials have been reported in molluscan neurons (Arvanitaki and Chalazonitis, 1968) and canine Purkinje fibers (Cranefield, 1975). More recently, this type of potential oscillation was noted in rat sympathetic neurons exposed to muscarine (Brown, Constanti, and Marsh, 1980). These potentials may participate in the production of repetitive spike activity in these various cells. However, their apparent ability to trigger driver potentials in cardiac ganglion motorneurons suggests an additional role, i.e., the generation of repetitive burst activity.

**Comparative physiology of $G_K$ inhibition**

The responses of cardiac motorneurons to proctolin and muscarinic cholinomimetics (Sullivan and Miller, 1982a) is surprisingly similar to those reported for luteinizing hormone-releasing hormone and muscarinic agonists on vertebrate sympathetic ganglion cells (Jan, Jan, and Kuffler, 1979; Adams and Brown, 1980; Brown, Constanti, and Marsh, 1980). In the latter case both agents are known to suppress a voltage-sensitive $K^+$ conductance, $m$-current (Adams, Brown, and Constanti, 1982). The linearity of our I-V plots obtained with pulses in the 0.5–1.0 s range (Fig. 9; Miller and Sullivan, 1981, and other unpublished studies) suggest that $m$-current is not present in cardiac motorneurons. However, the possibility of additional active currents such as the anomolous rectifier masking the $m$-current should not be overlooked. Preliminary voltage clamp analyses revealed a clear effect on “leak” conductance but little or no effects on the voltage- and time-dependent con-
ductances which underlie motorneuron driver potentials (Tazaki and Sullivan, unpublished observation). This is in agreement with the observed insensitivity of the proctolin response to pharmacological agents which block the active currents (Figs. 7 and 10). Thus we suggest that proctolin inhibits a resting potassium conductance ($G_{K\cdot p}$) and would not be surprised if the current was in fact a potassium leak conductance (low voltage sensitivity) possibly similar to a current recently described by Klein, Camardo, and Kandel (1982) in Aplysia sensory neurons. The conclusion that an arthropod neurohormone, proctolin, also acts by decreasing a resting potassium conductance suggests that long-lasting synaptic or hormonal inhibition of extant potassium conductances may be a common “up modulatory” cellular mechanism for increasing spontaneity (Sullivan and Miller, 1982).

At present the transduction processes which underlie the slow inhibition of resting potassium conductances are unknown (see Adams et al., 1982). Excepting the possibility of novel kinetic schemes it is likely that slow inhibition of $G_{K\cdot p}$ is mediated by intracellular processes whose relaxation kinetics exhibit time constants on the order of minutes. In our limited studies we have found that the proctolin-induced slow depolarization of motor neurons is substantially potentiated by only trifluoperazine ($8 \times 10^{-5} M$) and the calcium ionophore A23187 (Sullivan and Miller, 1982b). While these are suggestive, the isolated motorneuron preparation should prove useful in future studies of this nature.

Network architecture and rhythmic pattern generation: an alternative view

Two general theoretical mechanisms have been proposed to account for the neural generation of invertebrate rhythmic motor activity: (1) imposition of rhythmic activity upon motorneurons by a single endogenously oscillating premotor interneuron or by a group of coupled oscillators; (2) rhythm generation as an emergent property of cellular networks, the constituents of which do not possess independent oscillatory capabilities (see Kennedy and Davis, 1977). Studies on the crustacean cardiac ganglion, one of the simplest known burst generating networks, have led to diverse hypotheses regarding rhythmic motor pattern generation. In his “modified closed chain” model of the ganglion, Maynard (1955) considered that each unit in isolation would tend toward a uniform low spontaneous firing frequency and that rhythmic bursting arises from reciprocal synaptic interactions which result in alternating periods of high excitation and postexcitatory depression. A second two-layered model, proposing that one or more small cells act as endogenous bursting oscillators which impose the burst pattern upon the motorneurons, gained support from the extracellular stimulation and transection experiments of Mayeri (1973) who concluded that the “large cells, individually or as a group, do not have an independent capability for burst generation.” Meanwhile, detailed transection and pharmacological experiments led several investigators to stress the endogenous nature of motorneuron rhythm and burst generation (Matsui, 1955; Watanabe, 1958; Connor, 1969; Matsui, Arinobu, and Naohiro, 1972; Tazaki, 1973; Miller and Sullivan, 1981). The demonstration of TTX-resistant, $Ca^{++}$-dependent driver potentials in motorneurons (Tazaki, 1971; Tazaki and Cooke, 1979b) led to the current view that rhythmic motor pattern generation results from a combination of integrated small cell inputs with the regenerative driver potential of large cells (Tazaki and Cooke,
Ganglionic rhythmicity is attributed to ramp or pacemaker potentials which have been recorded in small cells (Tameyasu, 1976; Tazaki and Cooke, 1979a). This model is depicted in Figure 13 (left side).

Our experiments with large cells whose synaptic interactions have been reduced by: (1) ion channel blocking agents, TTX and Cd\(^{++}\); (2) ligature; and (3) transection, lead us to propose that in the presence of neurohormones and certain excitatory transmitters motorneuron autorhythmicity may be expressed and/or enhanced (Fig. 13, right side). At such times, we suggest that a large cell pacemaker potential would govern ganglionic rhythmicity, either directly or via electrotonic feedback to small cells. According to this model, it is the current flow during the interburst interval, regardless of its cell type of origin, which controls the slope \(\frac{dv}{dt}\) of the “pacemaker potential” and thus determines the point in time at which the lowest threshold network element is activated. While this element is often a small cell axon (see Maynard, 1961; Mayeri, 1973; Hartline, 1979), its \textit{a priori} designation as the ganglionic “pacemaker” seems inappropriate. While the pacemaker potential, and therefore control over ganglionic rhythmicity appears to occur within the small cells in isolated preparations (Fig. 13, left side), we suggest that large cell rhythm-generating mechanisms may contribute to the \textit{in vivo} burst rate where the ganglion is exposed to its normal complement of stretch-sensitive sensory, excitatory, synaptic, and hormonal inputs.

The existence of distributed burst-generating and rhythm-producing mechanisms in the cardiac ganglion raises several points of interest. If we attribute burst formation and rhythm generation to the \textit{cellular} properties of network elements (Figs. 7, 11, 12), we must ask: what is the fundamental purpose of network architecture (Fig. 13, left)? We propose that it is designed as a two-tiered, multireceptor target for modulatory inputs. Its function is to orchestrate the fine control of burst patterning (Fig. 3) and alternative burst modes (Fig. 2). For a given network the actual number of alternative burst modes is a complex permutation of specific receptor display at each tier level, the number of nonequivalent tiers and receptor occupation. Possible adaptive features of a multi-tiered system include a means by which temporal sequences of altered network output can result from differential cellular sensitivities to a single chemical messenger. The latter may be a simple solution for the production of fixed action patterns in component networks.

REFERENCES


