Inositol 1,4,5-Trisphosphate Alters Bursting Pacemaker Activity in Aplysia Neurons: Voltage-Clamp Analysis of Effects on Calcium Currents

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SUMMARY AND CONCLUSIONS

1. The left upper-quadrant bursting neurons (cells L2, L3, L4, and L6) of the abdominal ganglion of Aplysia display a regular burst-firing pattern that is controlled by cyclic changes of intracellular Ca\(^{2+}\) that occur during the bursting rhythm. The characteristic bursting pattern of these neurons occurs within a range of membrane potentials (−35 to −50 mV) called the pacemaker range.

2. Intracellular pressure injection of inositol-1,4,5-trisphosphate (IP\(_3\)) altered the bursting rhythm of the left upper-quadrant bursting (LUQB) cells for up to 15 min. Injection of IP\(_3\) induced a brief depolarization that was followed by a long-lasting (2–15 min) hyperpolarization. The hyperpolarizing phase of the response was accompanied by prolonged interburst intervals.

3. When cells were voltage-clamped at potentials within the pacemaker range, injection of IP\(_3\) generally induced a biphasic response that had a total duration of 2–15 min. Injection of IP\(_3\) induced a brief depolarization that was followed by a long-lasting (2–15 min) hyperpolarization. The hyperpolarizing phase of the response was accompanied by prolonged interburst intervals.

4. At membrane potentials more negative than −40 mV, \(I_{in}\) was associated with a small and relatively voltage-independent increase in membrane conductance. \(I_{in}\) was not blocked by bath application of tetrodotoxin (TTX) or Co\(^{2+}\). Although \(I_{in}\) was activated by injection of IP\(_3\), we were unable to block it by iontophoretic injection of ethylene glycol-bis (β-aminoethyl ether)-N,N',N\(^\prime\),N\(^\prime\)-tetraacetic acid (EGTA) sufficient to block the Ca\(^{2+}\)-activated inward tail current (\(I_{Ca}^t\)). The ionic mechanism that produces \(I_{in}\) has not been analyzed.

5. In normal bathing solution, \(I_{out}\) was present at membrane potentials more positive than \(\sim 50\) mV. \(I_{out}\) was not blocked by 50 mM tetraethylammonium (TEA), which is known to block Ca\(^{2+}\)-activated K\(^+\) currents (\(I_{Ca}^K\)) in these cells. However, it was blocked by 30 mM Co\(^{2+}\), which blocks \(I_{Ca}^K\). These results indicate that a steady-state \(I_{Ca}^K\) is necessary for the generation of \(I_{out}\) following injection of IP\(_3\), suggesting that \(I_{out}\) is due to inactivation of \(I_{Ca}^K\) and not to activation of a K\(^+\) conductance.

6. Intracellular iontophoresis of EGTA abolished \(I_{out}\) indicating that elevation of intracellular Ca\(^{2+}\) is necessary.

7. To test directly whether injection of IP\(_3\) leads to inactivation of \(I_{Ca}^K\), the effect of IP\(_3\) on the voltage-dependent \(I_{Ca}^K\) was studied with small depolarizing pulses to potentials near −40 mV, in cells bathed in a solution that blocked outward currents. Under such conditions the voltage-dependent \(I_{Ca}^K\) was partially inactivated by intracellular pressure injection of IP\(_3\). The effect of IP\(_3\) on \(I_{Ca}^K\) was blocked by intracellular iontophoresis of EGTA.

8. The results indicate that the outward component of the steady-state response to IP\(_3\) is due to inactivation of the steady-state Ca\(^{2+}\) current. This inactivation produces a prolonged interburst hyperpolarization by reducing the amount of inward current contributing to the depolarization that precedes.
the burst. The sensitivity of the response to intracellular iontophoresis of EGTA indicates that inactivation of \( I_{\text{Ca}} \) may occur through \( \text{Ca}^{2+} \)-dependent inactivation, stimulated by \( \text{IP}_3 \)-induced release of intracellular \( \text{Ca}^{2+} \). The finding that \( I_{\text{out}} \) is dependent on elevations of intracellular \( \text{Ca}^{2+} \) is consistent with previously described cellular actions of \( \text{IP}_3 \) in nonneuronal cells (e.g., Ref. 8).

**INTRODUCTION**

The phosphatidylinositol cycle is believed to be an important signaling pathway in a variety of cell types (8, 47). Receptor-mediated hydrolysis of phosphatidylinositol bisphosphate (PIP\(_2\)) yields two active products, inositol-1,4,5-trisphosphate (IP\(_3\)) and diacylglycerol (DG). IP\(_3\) has been reported to stimulate release of \( \text{Ca}^{2+} \) from intracellular stores (55), while DG promotes protein phosphorylation through activation of protein kinase C (PKC; 58). The importance of this messenger system in nerve cells has been inferred from studies that have found high concentrations of PKC in the brain (37, 46) and from the variety of neurotransmitters that appear to stimulate PIP\(_2\) breakdown (for review see Refs. 34, 47). Previous studies have confirmed the presence of elements of the phosphatidylinositol system in nervous tissue of *Aplysia* (15). While a variety of studies in the nervous system have focused on the effects of activation of PKC by phorbol esters or synthetic DG (e.g., 6, 16, 21, 43, 56), the physiological effects of IP\(_3\) are only beginning to be examined (13, 22, 33, 51, 52). To examine further the effects of the phosphatidylinositol cycle on neuronal function, we have directly injected IP\(_3\) into individual identified bursting pacemaker neurons of *Aplysia*.

The first slow tail current that is observed following a depolarizing voltage-clamp pulse is an outward current that is activated by intracellular \( \text{Ca}^{2+} \) and appears to be carried by \( \text{K}^+ \) ions (40). It has been shown to be blocked by 50 mM tetraethylammonium (TEA), which also blocks the \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) current (\( I_{\text{K,CA}} \)) in these cells (31, 32, 40). \( I_{\text{K,CA}} \) appears to participate in repolarization of the action potential and generates a hyperpolarizing afterpotential following the spike.

The second slow tail current that is observed is an inward current that lasts up to 1 s. It has been proposed that this current...
(\(I_B;40\)) is carried by channels that are nonselective for cations (\(\text{Na}^+, \text{Ca}^{2+}, \text{K}^+\)) and that are also activated by intracellular \(\text{Ca}^{2+}\) (1, 39, 57). A pharmacological agent that blocks this current has not been identified. \(I_B\) generates a depolarizing afterpotential after each action potential, which often triggers another spike and appears to contribute to the clustering of action potentials into bursts (2, 39, 40).

The third slow tail current is an outward current that lasts up to 20 s. It has been proposed that this current is carried by \(\text{K}^+\) ions and is thus a \(\text{Ca}^{2+}\)-activated \(\text{K}^+\) current (27, 36, 61). However, this slow tail current is not blocked by TEA, whereas \(I_{\text{K,ca}}\) is blocked by TEA (31, 32). A likely alternative explanation is that the slow outward tail current is due to \(\text{Ca}^{2+}\)-dependent inactivation of the steady-state \(I_{\text{ca}}\) (2, 40). Inactivation of \(I_{\text{ca}}\) reduces the amount of steady-state inward current and thus causes a hyperpolarization. Buffering or extrusion of intracellular \(\text{Ca}^{2+}\) allows \(I_{\text{ca}}\) to be activated, thereby producing a depolarizing drive that brings the cell to threshold. The slow outward tail current will be referred to as \(I_H\) following the notation of Adams (1).

We have found that intracellular injection of IP3 has effects on the bursting rhythm of the LUQB cells that last as long as 15 min. The mechanism of action of IP3 was studied by voltage-clamp analysis of the membrane currents that are involved in generation of the bursting rhythm. At least two membrane conductances were affected by injection of IP3 into LUQB cells. One of these was \(I_{\text{ca}}\), which was inactivated by IP3, apparently through \(\text{Ca}^{2+}\)-dependent inactivation stimulated by IP3-induced release of intracellular \(\text{Ca}^{2+}\). In addition, IP3 activated an inward membrane current of an unknown character. This report primarily focuses on how IP3 affects \(I_{\text{ca}}\) and produces a gradual slowing of the bursting rhythm in the LUQB cells. Some of these results have been presented in preliminary form (13, 52).

METHODS

General methodology

*Aplysia californica* (150–300 g) were obtained from Marinus (Long Beach, CA), Marine Specimens (Pacific Palisades, CA) and Alacrity Marine Biological (Redondo Beach, CA) and maintained in isolated containers in artificial seawater (ASW) at 15°C. Animals were anesthetized by an injection of a volume of isotonic MgCl2 equal to approximately one-half their volume. The abdominal ganglion was then removed and pinned to the floor of a Sylgard- (Dow Corning) lined chamber. The cells in the left hemiganglion were exposed by surgical removal of the connective tissue sheath that covers the ganglion. In most experiments, the LUQB cells were axotomized by a cut across the left hemiganglion about 600 μm from the soma (see also below). This procedure greatly improved the spatial control of the membrane potential and reduced synaptic input and the generation of spikes from uncontrolled regions of the axon. Experiments were done at room temperature (20–22°C). Due to the strong temperature dependence of \(I_{\text{ca}}\) in these neurons (17, 64), all solutions were equilibrated to room temperature before addition to the bath.

The preparation was bathed in an aerated ASW solution that contained in mM: 495 NaCl, 10 KCl, 10 CaCl2, 55 MgCl2 and was buffered to pH 7.6 with 10 mM tris (hydroxymethyl) aminomethane (Tris). In some experiments, 30–60 μM tetrodotoxin (11X) was added to decrease synaptic input onto LUQB cells and to block the voltage-dependent sodium current (\(I_{\text{Na}}\)). Solutions that contained 30 mM CoCl2 or 15 mM NiCl2 were made by addition of the desired compound to ASW, without adjustment of osmolarity. Solutions containing 50–200 mM TEA were made by addition of a stock solution of 1 M TEA to ASW. TEA stock was prepared by extraction in ether to remove triethylamines (66).

Intracellular recording and voltage-clamp procedures were done with two glass microelectrodes that were filled with 3 M potassium acetate and had resistances of 2–5 MΩ. Standard two-electrode voltage-clamp procedures were utilized. Clamp current was measured by a virtual ground circuit connected to the bath by an Ag-AgCl pellet. Iontophoresis of ethylene glycol-bis(β-aminethylether)-N,N,N',N'-tetraacetic acid (EGTA) was done by passing negative current pulses (200–300 nA) through a third intracellular electrode that contained 200 mM EGTA. This was performed while the cell was voltage-clamped in order to maintain a net membrane current of zero during iontophoresis.

The pacemaker rhythm of the LUQB cells consists of approximately two to seven action potentials per burst, with bursts separated by 5–40 s. A typical example is illustrated in Fig. 1A. Only cells that demonstrated a healthy bursting rhythm after all micropipettes were in place were used in this study. This is an important consideration because the currents that were studied were sensitive to intracellular \(\text{Ca}^{2+}\), which might be elevated in damaged cells (3). Indeed, in preliminary studies,
FIG. 1. Alteration of spontaneous bursting rhythm by injection of inositol-1,4,5-trisphosphate (IP₃). A: injection of IP₃ into a left upper-quadrant bursting (LUQB) cell (arrow). Initial transient hyperpolarization can sometimes be seen in control injections. B: individual burst cycles from A at an expanded time base. Numbers correspond to those in A. IP₃ induced a gradual hyperpolarization and decrease in bursting frequency that follows an earlier depolarization. C: injection of D-myo-inositol into LUQB cells yielded no consistent response.

Cells that did not display a healthy bursting rhythm commonly failed to respond to injection of IP₃. Furthermore, the Ca²⁺-dependent tail currents that follow a brief depolarizing voltage-clamp pulse (see Fig. 4A2), appeared to have different characteristics in cells that were not capable of bursting (unpublished observations). Experiments were begun no sooner than 20 min after all electrodes were in place.

Pressure injection of IP₃ and Ca²⁺

Single-barreled glass micropipettes (without internal glass filaments) were used for pressure injection. The pipettes were beveled and selected for tip size and smoothness. A typical pipette could pass nitrogen gas through the tip into water when a pressure of 50–70 psi was applied. Pipettes were then filled through the tip to where the meniscus could be clearly seen under a dissecting microscope. Visually guided insertion of the injection pipette into the cell was confirmed by observing a brief depolarization on impalement. Injection pipettes were advanced toward the center of the cell to reduce the possibility of damaging the membrane during injection. Injection was accomplished by application of pressure pulses (20–40 psi for 100–300 ms) to the back of the pipette and was monitored by observing movement of the meniscus within the pipette.

Solutions inside the injection pipettes were at a concentration of 10 mM in distilled water. The distilled water that was used for making injection solutions was passed through a Chelex-100 (Bio-Rad Laboratories, Richmond, CA) column to remove as much Ca²⁺ as possible. Approximate determinations of the final intracellular concentration of IP₃ following injection of a 10 mM solution were obtained by injecting a known radioactive sample into a small drop and counting the number of radioactive decays detected in the.
drop. By this method, typical injection volumes were estimated to be one-tenth to one-fifth of 1% of the cell volume, yielding intracellular concentrations of IP3 of 10–20 μM. This concentration appeared to yield maximal responses as larger responses could not be obtained with larger injections. Three batches of IP3 were used. Two of these were lithium salts generously provided by Dr. J. Eichberg. The third was a K⁺ salt purchased from Amersham, Arlington Heights, IL. Control injections consisted of 10 mM D-myo-inositol (Sigma Chemical, St. Louis, MO) in distilled water.

Calcium chloride (10 mM) was injected into LUQB cells by the same technique used for injection of IP3. Since the IP3 solution was used at a concentration of 10 mM, a Ca²⁺ solution of 10 mM served as a control because it is unlikely that the sample contained more Ca²⁺ than IP3. Furthermore, the concentration of Ca²⁺ in the bath is 10 mM. Since a small amount of the bathing solution could be drawn into the micropipette before impalement of the cell, injection of 10 mM Ca²⁺ (as well as D-myo-inositol) served as a control for this possibility.

Isolation of Ca²⁺ currents

Several experiments were performed to examine whether injection of IP3 affected Iₖa. These experiments were carried out by delivering 4–8 mV depolarizing pulses from a holding potential of −38 to −45 mV. At these membrane potentials, few voltage-dependent membrane currents are active that could confound measurements of Iₖa (19, 29, 32). Nevertheless, to minimize contamination of Iₖa by other currents, the ganglion was bathed in seawater containing the following compounds. TEA (200 mM) was added by equimolar substitution with Na, to reduce the delayed potassium current (Iₖ, delayed) and Iₖ,(21, 31, 32, 60). This concentration of TEA also significantly reduces S current (7, 53). TEA seawater was made from a stock solution of 1 M TEA that was extracted in ether (see above). 5 mM 4-aminopyridine (4-AP) was added to block the transient K⁺ current (Iₖ, transient) (12, 60) and Iₖ,V (32). TTX (30–60 μM) was used to block voltage-dependent Na⁺ current. In addition, the current-passing electrode contained 5 M CsCl to further reduce K⁺ currents (17). The remaining outward current is nearly voltage-independent at the membrane potentials used (14; unpublished observations). These experiments were conducted on cells that had been axotomized within about 200 μm of the soma in order to improve spatial control of the membrane potential. Axon lengths were estimated by measuring the distance from the edge of the cell to the point where the ganglion had been cut during axotomy.

RESULTS

IP₃ slows the bursting rhythm

A representative example of a cell injected with 10 mM IP₃ (final intracellular concentration approximately 10–20 μM), is illustrated in Fig. 1A. Individual burst cycles are displayed with an expanded time base in Fig. 1B. In this example, the injection was immediately followed by a hyperpolarizing transient. A similar transient can sometimes be observed following control injections. The portion of the response that is uniquely produced by IP₃ is an initial depolarization (lasting up to 2 min in some cells) followed by a long hyperpolarization that lasts 2–15 min (n = 10). In addition to the changes in membrane potential, IP₃ also caused an increase in the duration of the interburst intervals (Fig. 1B). This effect may be secondary to hyperpolarization of the cell, since a similar increase in interburst interval occurs when hyperpolarizing current is passed into the cell (4). Nonetheless, the remainder of the experiments in this study indicate that at least one of the membrane currents involved in burst generation is regulated by IP₃.

Pressure injection of the control substance 10 mM D-myo-inositol into current-clamped LUQB neurons, yielded no consistent changes in the membrane potential or firing frequency (Fig. 1C, n = 11).

Modulation of holding current and membrane conductance

To analyze further the mechanisms underlying the effects of IP₃ on the bursting rhythm, we examined changes in membrane currents in voltage-clamped cells. A typical response to injection of IP₃ is shown in Fig. 2A. The response lasted between 2 and 15 min in various cells and consisted of an initial inward shift in holding current (Iₖₗ) that lasted up to 2 min in some cells. Iₖₗ was followed by a long-lasting outward shift in holding current (Iₖₒₗ) (n = 49 of 66). In 17 of 66 cells, monophasic outward currents were observed (e.g., Fig. 7A).

Control injections of 10 mM D-myo-inositol yielded no consistent response (Fig. 2B; n = 27). In a separate set of experiments, cells were concurrently impaled with two injection electrodes, one containing IP₃ and one containing D-myo-inositol. All cells re-
IP₃ activates a biphasic series of membrane currents in LUQB cells held at a constant holding potential within the pacemaker range (−35 to −50 mV). A: LUQB cell injected with IP₃ while voltage clamped at a constant holding potential of −37 mV. The biphasic response lasts up to 15 min in some cells and corresponds with the change in resting potential seen in unclamped cells (e.g., Fig. 1). B: another cell injected with D-myo-inositol while voltage clamped at a holding potential of −42 mV. Only occasional injection artifacts that lasted 1–2 s were seen in these cells.

The conductance changes associated with the IP₃ response were studied at two different holding potentials, one potential within the pacemaker region (−35 to −50 mV) and one potential more negative than this region. Complete steady-state current-voltage relationships could not be obtained because the response began to desensitize after about four injections of IP₃. Steady-state input conductance was measured by giving small hyperpolarizing voltage pulses from the holding potential. When cells were voltage clamped within the pacemaker range, IP₃ induced a biphasic response with complex conductance changes (Fig. 3A). Individual voltage pulses from the same cell are displayed at an expanded time base in Fig. 3B.

Before injection, small hyperpolarizing pulses yielded outward shifts in holding current (Fig. 3A, arrow I and Fig. 3B, trace I). This “negative resistance” characteristic has been attributed to the voltage dependence of \( I_{\text{Ca}} \), which is present at relatively hyperpolarized potentials in these neurons (19, 27, 40). Although slow activation and inactivation of \( I_{\text{Ca}} \) (≈200 ms rise time) has been reported during small pulses (19), the much slower kinetics observed here (2–3 s rise time) appear to be due to poor space clamp of the axon or axon stump (unpublished observations).

After injection of IP₃, the inward component of the response was associated with an apparent increase in input conductance (see also below). During both the inward and the outward phase of the response, the hyperpolarizing conductance pulses produced less outward current than prior to the injection of IP₃ (Fig. 3B, traces 2 and 3). However, due to the voltage dependence of \( I_{\text{Ca}} \), the conductance changes observed do not reflect true changes in steady-state conductance (40).

At holding potentials more negative than the pacemaker region (i.e., −60 to −70 mV), hyperpolarizing pulses yielded inward current deflections, as expected for an ohmic conductance. This indicates that the steady-state \( I_{\text{Ca}} \) is small at these potentials. Injection of IP₃ into LUQB cells held at potentials more negative than −60 mV yielded mono-
FIG. 3. IP$_3$ induces complex changes in the membrane conductance that are dependent on the resting potential. Small hyperpolarizing pulses were elicited in voltage-clamped LUQB cells in order to monitor changes in conductance induced by IP$_3$. A: response to injection of IP$_3$ in a cell voltage clamped at $-37$ mV. Upward capacitive transients were clipped by the pen recorder during the peak of the outward current. B: conductance pulses from A at an expanded time base. Numbers correspond to those with arrows in A. Biphasic response induced by IP$_3$ was associated with a reduction of the "negative conductance" seen in the pacemaker region (see text). C: IP$_3$ response in another cell voltage clamped at $-60$ mV. A monophasic response was obtained at this potential that was associated with a small increase in membrane conductance. Axons in A and C were ~2 mm long.

Phasic inward currents associated with a small increase in input conductance (Fig. 3C; $n = 7$).

In the cells studied at potentials more negative than the pacemaker region, $I_{out}$ was greatly reduced or absent (Fig. 3C), whereas $I_{in}$ was qualitatively similar in amplitude to that seen at more depolarized potentials. The responses obtained at hyperpolarized potentials revealed that the duration of $I_{in}$ was longer than could be observed within the pacemaker range. This indicates that $I_{out}$ and $I_{in}$ may overlap considerably in time. There are several possible explanations for the voltage sensitivity of $I_{out}$; these were examined in several experiments (see below).

**Outward response to IP$_3$ is dependent on elevation of intracellular Ca$^{2+}$**

Since IP$_3$ has been shown to release Ca$^{2+}$ from intracellular stores in other cell types (55), intracellular Ca$^{2+}$ is a likely candidate to be involved in the response to IP$_3$ seen in LUQB cells. For this reason we attempted to block the response to IP$_3$ by iontophoretic injection of EGTA. We also examined the Ca$^{2+}$-dependent slow tail currents elicited by brief depolarizing pulses in order to test the
efficacy of EGTA in chelating intracellular Ca\(^{2+}\). This also allowed for comparison of the properties of the slow tail currents with the properties of the shifts in holding current produced by IP\(_3\). These experiments were conducted on cells that had been axotomized. Although the response to IP\(_3\) was still present in these axotomized cells if they were capable of bursting, the response was often smaller and decayed faster than the response in cells that had not been axotomized. It is unclear whether this was due to damage or to localization of the channels or sources of Ca\(^{2+}\) that mediate the response.

Before EGTA was injected, an IP\(_3\) response was obtained (Fig. 4A1). In the same cell, a 30-ms depolarizing pulse to -10 mV yielded a triphasic series of slow tail currents (Fig. 4A2) that were described in the INTRODUCTION. EGTA was injected into LUQB cells by iontophoresis while the cells were voltage-clamped at potentials more negative than -50 mV. The cells were held hyperpolarized by the voltage clamp in order to reduce Ca\(^{2+}\) influx and to maintain a constant net membrane current during iontophoresis (see METHODS). During iontophoresis, EGTA induced a gradual inward shift in holding current of 10-15 nA (not shown). There are two possible interpretations for this effect of EGTA on the holding current. First, EGTA chelates intracellular Ca\(^{2+}\) causing reduction of a steady-state Z\(_{ca}\). Second, EGTA chelates intracellular Ca\(^{2+}\) and reduces the amount of steady-state inactivation of \(I_{Ca}\), thus producing an inward current (see DISCUSSION). Figure 4B1 demonstrates that, following injection of EGTA into the same cell, the outward component (\(I_{out}\)) of the IP\(_3\) response is abolished and a rather persistent \(I_{in}\) is revealed (\(n = 5\)). Similarly, \(I_{H}\), as well as the inward tail current (\(I_{B}\)), were dramatically reduced (Fig. 4B2). These results indicate that the slow outward tail current elicited by a depolarizing pulse (\(I_{H}\)) and the slow outward current produced by IP\(_3\) (\(I_{out}\)) may be related and that both require an elevation of intracellular Ca\(^{2+}\). The relationship between \(I_{B}\) and \(I_{in}\), however, is less clear. Whereas \(I_{B}\) was blocked by EGTA (Fig. 4B2), \(I_{in}\) was not (Fig. 4B1). Although we cannot be certain that the chelation of intracellular Ca\(^{2+}\) is complete following intracellular iontophoresis of EGTA, it seems unlikely that \(I_{B}\) would be blocked while \(I_{in}\) was not if the two currents were carried through the same Ca\(^{2+}\)-activated channels. Although other interpretations must be considered, it is possible that \(I_{in}\) is produced by a mechanism that is distinct from the mechanism that produces \(I_{B}\).

Response to Ca\(^{2+}\) injection does not mimic time course of response to IP\(_3\)

The following experiments were performed to test the possibility that the IP\(_3\) response was due to contamination of the injection pipette contents with Ca\(^{2+}\). In one cell, an IP\(_3\) injection was followed by an injection of an approximately equal volume of 10 mM CaCl\(_2\). Figure 5A1 demonstrates that IP\(_3\) yielded a biphasic response when injected near the center of the cell. After return of the response to baseline, injection of the same volume of CaCl\(_2\) near the center of the cell yielded no response (Fig. 5A2).

In another cell, injection of a small volume of CaCl\(_2\) (typical of an injection of IP\(_3\)) near the membrane yielded a quickly decaying outward current (Fig. 5B1). Injection of the same volume into the center of the cell yielded a small brief biphasic response (Fig. 5B2; see also Ref. 24). We also attempted to simulate a response to IP\(_3\) by a prolonged injection of CaCl\(_2\). Figure 5B3 illustrates the response of the same cell to a 1 min injection of CaCl\(_2\). The volume injected was ~200 times the volume of one IP\(_3\) injection (~20% of the cell volume). The response was a gradual outward shift in holding current. After the injection, the response recovered within ~5 min. Thus it was possible to obtain a response from injection of Ca\(^{2+}\) that superficially appeared to be similar to the response to injection of IP\(_3\). However, a very large amount of Ca\(^{2+}\) had to be injected. The data of Fig. 5B3 does not necessarily indicate that IP\(_3\) causes a very large release of Ca\(^{2+}\) from intracellular stores. Rather, it is likely that IP\(_3\) induces release of Ca\(^{2+}\) from stores that are close to the membrane and thus near the membrane channels that are affected. This presumably allows a relatively small increase in intracellular free Ca\(^{2+}\) to affect the properties of membrane channels before the Ca\(^{2+}\) is buffered or extruded from the cell. In addition, it is likely that the actions of IP\(_3\) (with regards to \(I_{out}\)) are the same as the actions of...
Ca\(^{2+}\) (see below); however, the time course of
the two responses are different. This might
be expected if IP\(_3\) is not inactivated rapidly.
The striking differences between the volume
of Ca\(^{2+}\) and the volume of IP\(_3\) that had to be
injected to obtain a response, indicate that
the response to injection of IP\(_3\) is not due to
contamination by Ca\(^{2+}\) in the injection pi-
Fig. 5. Pressure injection of calcium chloride does not mimic the time course of the response to injection of IP$_3$. A1: response to injection of 10 mM IP$_3$ into an LQUB cell. A2: response to injection of 10 mM CaCl$_2$ (approximately same volume) in the same cell. Both injection electrodes had been advanced into the cell away from the membrane. Whereas IP$_3$ yielded a slow response, no response was detected from the injection of CaCl$_2$. Holding potential, -43 mV. B1: in another cell, injection of 10 mM CaCl$_2$ near the membrane yielded a quickly decaying outward current. B2: injection after the electrode had been advanced further into the cell yielded a small biphasic (inward-outward) response that decayed within ~30 s. B3: prolonged injection of CaCl$_2$ (1 min) yielded a slowly decaying outward current. Holding potential, -38 mV.

Outward current requires a steady-state Ca$^{2+}$ current but not a Ca$^{2+}$-dependent K$^+$ current

Since the results with EGTA indicated that $I_{out}$ is produced by a Ca$^{2+}$-dependent mechanism, we next performed several experiments to determine whether $I_{out}$ is due to activation of $I_{K,CA}$ or to Ca$^{2+}$-dependent inactivation of the steady-state $I_{Ca}$. To investigate the possible role of $I_{K,CA}$, we examined the response to IP$_3$ while the cells were bathed in a solution containing 50 mM TEA. This concentration of TEA is well above the concentration known to block $I_{K,CA}$ in these cells (31, 32, 40). Figure 6A1 illustrates the response to IP$_3$ in a cell bathed in ASW containing 50 mM TEA. Figure 6A2 illustrates the response in the same cell after washing in ASW. It is clear that the slow outward response to IP$_3$ is not due to an increase in conductance through Ca$^{2+}$-activated K$^+$ channels.

In all of the cells studied in 50 mM TEA, $I_{out}$ was larger in TEA compared with $I_{out}$ observed in ASW (e.g., Fig. 6A). This does not appear to be due to a reduction of re-
response amplitude during multiple injections, because the same effect was observed when responses in ASW were obtained before responses in TEA (not shown). Although the reasons for this effect are unclear, it is possible that TEA enhances the spatial control of the membrane potential in the axon, due to a decrease in total membrane conductance. This might increase the amount of $I_{\text{out}}$ recorded by the voltage-clamp circuit and cause an apparent masking of $I_{\text{in}}$. This would be particularly true if the density of channels contributing to $I_{\text{out}}$ was greater in the axon than in the soma. Alternatively, TEA may partially block $I_{\text{in}}$, thus making the net shift in membrane current appear greater in the outward direction.

To further assess the roles of inactivation of $I_{\text{Ca}}$ and activation of $I_{\text{K,Ca}}$ in producing $I_{\text{out}}$, we examined the effects of divalent cations that block $Ca^{2+}$ channels. If activation of $I_{\text{K,Ca}}$ by $Ca^{2+}$ released from intracellular
stores is involved in producing $I_{\text{out}}$, it should still be activated when $\text{Co}^{2+}$ is added to the bathing medium (5, 10). However, if inactivation of $I_{\text{Ca}}$ is involved, $I_{\text{out}}$ should be blocked by the presence of $\text{Co}^{2+}$ in the extracellular medium.

Addition of 30 mM $\text{Co}^{2+}$ to the bathing medium caused an outward shift in holding current when cells were voltage-clamped within the pacemaker range (not shown). These results indicate that in ASW, there is a steady-state $I_{\text{Ca}}$ that contributes to the holding current. Furthermore, if a steady-state $I_{\text{K, Ca}}$ contributes to the holding current, it must be small in comparison to $I_{\text{Ca}}$. Figure 6B1 illustrates the response to an injection of IP$_3$ when the cell was bathed in ASW, followed by a response in ASW containing 30 mM $\text{Co}^{2+}$ (Fig. 6B2). $I_{\text{out}}$ was blocked by $\text{Co}^{2+}$-containing medium ($n = 14$), suggesting that $I_{\text{out}}$ requires the presence of a steady-state $I_{\text{Ca}}$. Bathing medium containing $\text{Co}^{2+}$ did not block $I_{\text{in}}$ (Fig. 6B2), indicating that $I_{\text{in}}$ is not due purely to activation of $I_{\text{Ca}}$.

In some experiments (17 of 66 cells), the response to IP$_3$ in ASW appeared to be a monophasic outward current (Fig. 7A). In two of these experiments, 30 mM $\text{Co}^{2+}$ was added to the solution following a response in ASW. In the presence of $\text{Co}^{2+}$, injection of IP$_3$ produced an inward current (Fig. 7B). This inward current appears to be identical to $I_{\text{in}}$. Thus the variability in the responses to IP$_3$ appears to be due to variability in the relative contribution of $I_{\text{in}}$ and $I_{\text{out}}$ to the total response. This experiment also indicates that there is considerable temporal overlap in the two components.

Taken together, the experiments with TEA and $\text{Co}^{2+}$ indicate that inactivation of a steady-state $I_{\text{Ca}}$ may be the mechanism for production of $I_{\text{out}}$. However, these experiments are not entirely conclusive, since both $\text{Co}^{2+}$ and TEA may have nonspecific pharmacological effects. To further examine the role of inactivation of $I_{\text{Ca}}$ in producing $I_{\text{out}}$, the following experiment was designed to test directly whether IP$_3$ can induce inactivation of $I_{\text{Ca}}$ and whether this action requires an elevation of intracellular Ca$^{2+}$.

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**Fig. 7.** Components of the response to IP$_3$ overlap in time. A: apparent monophasic $I_{\text{out}}$ response obtained after injection of IP$_3$ into an IJQB cell bathed in ASW. B: addition of 30 mM $\text{Co}^{2+}$ to the bathing medium abolished $I_{\text{out}}$ and revealed an inward current that was not blocked by $\text{Co}^{2+}$. Holding potential, $-45$ mV. Axon was $\sim600$ $\mu$m long.
IP$_3$ reduces a voltage-dependent Ca$^{2+}$ conductance

Eckert and Lux (19) have described a voltage-dependent $I_{Ca}$ in bursting neurons of *Helix* that is activated by small depolarizing pulses within the pacemaker range and inactivates very slowly. This current appears to be identical to the current that is responsible for the steady-state $I_{Ca}$ present in the LUQB cells (40). We have examined how $I_{Ca}$ is altered by injection of IP$_3$ in order to gain insight into the ionic mechanism of the action of IP$_3$ in LUQB cells.

Small (4–8 mV) depolarizing voltage-clamp pulses to potentials near $-40$ mV were used to activate $I_{Ca}$. Such pulses would be expected to activate few voltage-dependent outward currents. However, to minimize the contribution of any outward currents that might be present, experiments were carried out while cells were bathed in seawater containing compounds that block outward currents (see METHODS). To maximize spatial control of the membrane potential, cells were axotomized within about 200 µm of the soma. Under these conditions, it appeared that virtually no voltage-dependent outward currents were present at the potentials used (14, 17), as indicated by the lack of any apparent "inactivation" of the inward current during a small depolarizing pulse (see Fig. 8A2).

To examine changes in $I_{Ca}$ as well as changes in steady-state conductance, pairs of equal hyperpolarizing and depolarizing pulses were elicited every 60 s. Pulses were 1

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**FIG. 8.** IP$_3$ reduces the voltage-dependent calcium current ($I_{Ca}$) through a calcium-dependent process. LUQB cell in a bathing solution that contained 200 mM TEA, 5 mM 4-aminopyridine (4-AP), and TTX. A: responses to 8 mV (1 s) hyperpolarizing (A1) and depolarizing (A2) pulses before and 5 s after injection of IP$_3$. IP$_3$ reduces $I_{Ca}$ obtained during a depolarizing pulse (A2) and induces an apparent increase in membrane conductance (A1). Note that the increase in conductance is not large enough to account for the decrease in the voltage-dependent inward current seen during the depolarizing pulse. A3: amplitude of the inward current induced during the depolarizing pulse is plotted as a function of time, before and after injection of IP$_3$. B: after injection of EGTA into the cell by iontophoresis, a second injection of IP$_3$ produced no change in membrane conductance (B1) and no change in $I_{Ca}$ induced by the depolarizing pulse (B2). C: addition of 15 mM Ni$^{2+}$ to the bathing medium blocked $I_{Ca}$. Ni$^{2+}$ also blocked the effect of IP$_3$ on the current elicited by the depolarizing pulse in cells that had not been injected with EGTA (not shown). Holding potential, $-40$ mV. Axon was $\sim 200$ µm long. In this and all other experiments, only cells with normal bursting rhythms after impalement were used (see METHODS).
s in duration with the depolarizing pulse following the hyperpolarizing pulse by 2 s. Two consecutive pairs yielded identical currents for the hyperpolarizing and depolarizing pulse. The depolarizing pulse yielded a net inward current, apparently due to activation of $I_{Ca}$ (Fig. 8.42). Five seconds prior to a third pair of pulses, IP$_3$ was injected into the cell. IP$_3$ reduced the net inward current during the depolarizing pulse (Fig. 8.42; $n = 12$). IP$_3$ also induced a slight increase in steady-state membrane conductance that could be due to two mechanisms (see below), but this change in steady-state conductance cannot account for the entire change in current elicited by the depolarizing pulse. Thus it appears that $I_{Ca}$ is reduced by injection of IP$_3$. This effect of IP$_3$ on $I_{Ca}$ generally lasted ~6-10 min (Fig. 8A3), at which time $I_{Ca}$ returned to preinjection amplitude.

After return of the response to baseline, EGTA was injected by iontophoresis for 20 min while the cell was voltage-clamped at a potential more negative than $-50$ mV. When the same paradigm was followed after EGTA injection, IP$_3$ failed to alter the input conductance (Fig. 8B1) and failed to reduce $I_{Ca}$ (Fig. 8B2) during the third pair of pulses ($n = 5$). Thus the IP$_3$-induced inactivation of $I_{Ca}$ appears to require an elevation of intracellular Ca$^{2+}$. The lack of response to IP$_3$ in Fig. 8B is probably not due to desensitization of the response because in cells that were not injected with EGTA, the action of IP$_3$ could be observed following at least three consecutive injections of IP$_3$ (not shown; $n = 5$).

Addition of 15 mM Ni$^{2+}$ (Fig. 8C) or 30 mM Co$^{2+}$ to the bathing medium induced an outward shift in holding current and blocked $I_{Ca}$. Under these conditions, the response to a depolarizing pulse was not altered by injection of IP$_3$ (not shown). This also demonstrates that no voltage-dependent outward currents were present that could contaminate measurements of $I_{Ca}$.

The increase in steady-state membrane conductance measured during hyperpolarizing pulses that was caused by injection of IP$_3$ could have two explanations. One is the increase in membrane conductance caused by $I_{in}$. The other relates to the fact that at the holding potential used for this experiment, part of the steady-state $I_{Ca}$ is active. Thus, as a result of injection of IP$_3$ and subsequent release of Ca$^{2+}$, part of the steady-state $I_{Ca}$ would be expected to be inactivated. Inactivation of the steady-state $I_{Ca}$ would make less $I_{Ca}$ available for voltage-dependent deactivation, thus causing an apparent increase in membrane conductance as measured by the hyperpolarizing pulse (see Fig. 13 of Ref. 40).

This second interpretation is supported by the finding that EGTA blocks the response to IP$_3$ measured during the hyperpolarizing pulse.

Taken together, these results indicate that IP$_3$ causes inactivation of $I_{Ca}$. In addition, this action requires elevation of intracellular Ca$^{2+}$, most likely due to IP$_3$-induced release of intracellular stores of Ca$^{2+}$. These findings support the results obtained using pharmacological techniques (see Fig. 6).

**DISCUSSION**

**Early inward component of response to IP$_3$**

The early response to IP$_3$, $I_{in}$, is not blocked by Co$^{2+}$ (Fig. 6B2) or TTX and does not appear to be strongly voltage dependent at membrane potentials more negative than $-40$ mV. Although the timing of $I_{in}$ relative to $I_{out}$ is reminiscent of the timing of $I_{B}$ relative to $I_{H}$, it appears that $I_{in}$ is distinct from $I_{B}$. This conclusion is based solely on the observation that $I_{B}$ was blocked by EGTA, whereas $I_{in}$ was not. It is possible that $I_{in}$ is activated by IP$_3$ in a manner that is independent of intracellular Ca$^{2+}$ (see Ref. 63). For example, in Xenopus oocytes IP$_3$ is converted to inositol tetrakisphosphate (IP$_4$) (35); IP$_4$ could possibly mediate part of the response to injection of IP$_3$ in LUQB cells. Further work is required to determine the ionic nature of $I_{in}$.

**Slow outward component of response to IP$_3$**

Recent studies have reported that intracellular injection of IP$_3$ into *Aplysia* neurons (22, 50, 51) and a neuroblastoma cell line (33), activates a Ca$^{2+}$-dependent K$^+$ conductance. In this report, we have described two other membrane conductances that are affected by injection of IP$_3$ into bursting neurons of *Aplysia*. Although one of these is an outward current, it does not appear to be carried by K$^+$ ions, and thus is distinct from $I_{K,ca}$. In LUQB cells, $I_{K,ca}$ is blocked by 50 mM TEA (31, 32, 40), whereas $I_{out}$ in response to IP$_3$ was not blocked by TEA (Fig. 8C).
6A). In addition, \( I_{K,\text{Ca}} \) can still be activated by intracellular \( Ca^{2+} \) when the cell is bathed in \( Co^{2+}\)-containing medium (5, 10); \( I_{\text{out}} \), however, was blocked (Fig. 6B2). These experiments indicate that a \( K^+ \) conductance is not predominantly involved in generating \( I_{\text{out}} \) and suggest that inactivation of \( I_{\text{Ca}} \) is responsible for producing \( I_{\text{out}} \). Indeed, the experiments of Fig. 8 provide further evidence that IP3 can cause inactivation of \( I_{\text{Ca}} \) that is present at relatively hyperpolarized potentials in these cells. At a constant holding potential, inactivation of \( I_{\text{Ca}} \) would lead to an outward shift in holding current.

Although \( I_{\text{out}} \) appears to be due to inactivation of \( I_{\text{Ca}} \), we have not excluded the possibility that IP3 does activate \( I_{K,\text{Ca}} \) in LUQB cells. Since \( I_{K,\text{Ca}} \) decays quickly when observed as a tail current, it may require a higher level of intracellular \( Ca^{2+} \) than the other \( Ca^{2+}\)-activated currents (40). Thus it is possible that \( I_{\text{in}} \) and \( I_{\text{out}} \) may mask a small contribution from \( I_{K,\text{Ca}} \). Indeed, in some experiments where \( Co^{2+} \) was present in the bathing medium, a brief net outward current (2-10 s) could be observed (e.g., Fig. 7B). Although this could be an artifact of injection, it is possible that it represents a slow activation of \( I_{K,\text{Ca}} \) and may also account for the apparent slow onset of \( I_{\text{in}} \). In addition, the time course of this early outward current is similar to the time course of \( I_{K,\text{Ca}} \) activated by IP3 in other neurons (e.g., Ref. 50). However, the slow outward current produced by IP3 (\( I_{\text{out}} \)) is of much longer duration and has been distinguished from \( I_{K,\text{Ca}} \) by pharmacological techniques.

The mechanism that produces the slow \( I_{\text{out}} \) appears to be identical to that responsible for the slow outward tail current, \( I_{\text{H}} \) (2, 40). Although several reports have concluded that all or part of \( I_{\text{H}} \) in \textit{Aplysia} bursting neurons is carried by \( K^+ \) ions (36, 61), these conclusions were based on experiments that required an elevation of extracellular \( K^+ \), which may give misleading results (40; unpublished observations). We observed no net outward currents that lasted longer than 10 s in any of the 14 cells that were injected with IP3 while in a \( Co^{2+}\)-containing medium. Although we cannot completely rule out the possibility that activation of a \( K^+ \) current contributes to \( I_{\text{out}} \), the magnitude of such a current would appear to be small when compared with the effects of IP3-induced inactivation of \( I_{\text{Ca}} \).

The changes in holding current induced by various pharmacological treatments lend further support to the assertion that \( I_{\text{out}} \) is due to inactivation of \( I_{\text{Ca}} \). Intracellular injection of EGTA induces a net inward shift in holding current. If we assume this is caused by chelation of intracellular \( Ca^{2+} \), it could reflect a decrease in \( I_{K,\text{Ca}} \) or an increase in \( I_{\text{Ca}} \), due to recovery from \( Ca^{2+}\)-dependent inactivation of \( I_{\text{Ca}} \). In support of the latter interpretation, \( I_{\text{Ca}} \) was enhanced following injection of EGTA (Fig. 8; compare A2 with B2). This suggests that in a resting cell voltage clamped within the pacemaker range, some steady-state \( Ca^{2+}\)-dependent inactivation of \( I_{\text{Ca}} \) is present. Thus even a slight elevation of intracellular \( Ca^{2+} \) (induced by IP3) could cause further inactivation of \( I_{\text{Ca}} \). In further support of this interpretation, addition of \( Co^{2+} \) to the bathing medium induced a net outward shift in holding current. \( Co^{2+} \) would be expected to block any steady-state \( I_{\text{Ca}} \), and any steady-state \( I_{K,\text{Ca}} \) that was due to \( Ca^{2+} \) entry. However, since \( Co^{2+} \) induces a net outward shift in holding current, it appears that in ASW the contribution of \( I_{\text{Ca}} \) to the holding current is considerably greater than the contribution of \( I_{K,\text{Ca}} \). Together, these results suggest that a larger elevation of intracellular \( Ca^{2+} \) is required to activate \( I_{K,\text{Ca}} \) than is required to inactivate \( I_{\text{Ca}} \). Thus elevation of intracellular \( Ca^{2+} \) following injection of IP3 would be expected to cause greater inactivation of \( I_{\text{Ca}} \) than activation of \( I_{K,\text{Ca}} \).

**Temporal aspects of the action of IP3 in LUQB cells**

The most striking aspect of the response to injection of IP3 into LUQB cells is its duration. In some cells the response lasted as long as 15 min. This long duration could arise from two possible mechanisms. The first is that once \( Ca^{2+} \) is released by IP3, it takes a long time before it is buffered or extruded from the cytoplasm. The second possibility is that IP3 is degraded to an inactive product relatively slowly.

The time courses of \( I_{\text{in}} \) and \( I_{\text{out}} \) that produced the characteristic biphasic response were variable between different preparations. It was also apparent that cutting the axon of the cell affected the time course of the response. Thus uneven spatial localization of channels and the location of the injection of IP3 with respect to the channels that mediate
the response could alter the time course of the response as measured in the soma. Uneven spatial localization of channels may also affect the degree to which the two components are activated (see also Ref. 38). Figure 7 illustrates that some cells appeared to have a monophasic $I_{\text{out}}$. However, when $I_{\text{out}}$ was blocked by $\text{Co}^{2+}$, $I_{\text{in}}$ could be detected. Thus $I_{\text{in}}$ is not absent in these cells; it is merely obscured by $I_{\text{out}}$. Figure 8 also suggests that $I_{\text{in}}$ and $I_{\text{out}}$ overlap in time. Reduction of $I_{\text{Ca}}$ is evident quite early in the response to IP3, indicating that the underlying mechanism that is responsible for $I_{\text{out}}$ may be present even while the net shift in membrane current would be expected to be inward during a response in ASW. This also suggests that the slow time to peak of $I_{\text{out}}$ may be due as much to the decay of $I_{\text{in}}$ as to the activation of $I_{\text{out}}$.

A model for regulation of bursting pacemaker activity

Gorman and Thomas (28) demonstrated that intracellular $\text{Ca}^{2+}$ participates in generating the pacemaker rhythm of the bursting neuron R15 of Aplysia. They also demonstrated that when a burst is made more intense by passing depolarizing current during the burst, the subsequent interburst hyperpolarization is larger and more prolonged. Since the interburst hyperpolarization appears to be produced at least in part, by $\text{Ca}^{2+}$-induced inactivation of $I_{\text{Ca}}$ (2, 40), it is likely that enhanced entry of $\text{Ca}^{2+}$ contributes to the prolonged hyperpolarization following a more intense burst. Similarly, a neurotransmitter or intracellular messenger that causes an elevation of intracellular $\text{Ca}^{2+}$ might also prolong the interburst interval.

We have demonstrated that the intracellular messenger IP3 can produce such a response in the LUQB cells (Fig. 1). Furthermore, the mechanism by which this occurs appears to require an elevation of intracellular $\text{Ca}^{2+}$ (Figs. 4 and 8). Thus hydrolysis of PIP2 and production of IP3 is a putative intracellular mechanism by which a neurotransmitter or hormone could control bursting activity.

The effects of neurotransmitter-stimulated IP3 synthesis on membrane currents are illustrated schematically in Fig. 9. Activation of a receptor at the cell membrane would cause release of IP3 into the cell and activation of PKC by DG. The increased levels of IP3 would lead to release of $\text{Ca}^{2+}$ from intracellular stores and an elevation of intracellular $\text{Ca}^{2+}$. Increased levels of intracellular $\text{Ca}^{2+}$ would then contribute to inactivation of $I_{\text{Ca}}$. Since $I_{\text{Ca}}$ produces a depolarizing

![Diagram](https://via.placeholder.com/150)

**FIG. 9.** Model of the regulation of bursting pacemaker activity by IP3 (see text for details). DG, diacylglycerol; PKC, protein kinase C; ER, endoplasmic reticulum; $I_{\text{in}}$, inward current; IP3, inositol-1,4,5-trisphosphate.
drive that brings the cell to threshold (1, 19), the inactivation of this current would weaken the depolarizing drive, thereby prolonging the interburst interval. It is unclear whether the effects of IP3 on \( I_{\text{in}} \) are mediated by a direct action of IP3 or require additional intracellular messengers.

The role of PKC in control of membrane currents in the LUQB cells will require further study. Although a number of previous studies have indicated that PKC can modulate the \( \text{Ca}^{2+} \) current of neurons in Aplysia and other preparations (16, 30, 48, 56), we have been unable to detect any effects of phorbol esters (activators of PKC) on the \( \text{Ca}^{2+} \) current of LUQB cells (unpublished observations).

\( \text{Ca}^{2+} \)-dependent inactivation of \( I_{\text{Ca}} \) has been reported in Paramecium (9), Aplysia neurons (18, 20), Helix neurons (11, 49, 62), and recently in frog heart atrial muscle (45) and cardiac ventricular myocytes (41). In these studies, inactivation of \( I_{\text{Ca}} \) was seen as a mechanism to provide negative feedback control of \( I_{\text{Ca}} \) during a depolarization, whereby \( \text{Ca}^{2+} \) entering through \( \text{Ca}^{2+} \) channels inactivates \( I_{\text{Ca}} \). In this report, we have described a putative mechanism by which neurotransmitters or hormones could utilize \( \text{Ca}^{2+} \)-dependent inactivation to alter the electrical properties of neurons. The unique aspects of this type of mechanism are that the \( \text{Ca}^{2+} \) comes from intracellular stores that are released by elevations of intracellular IP3, and the effect alters the electrical characteristics of the cell for a much longer time.

Wilson and Wachtel (65) reported that the steady-state \( I_{\text{Ca}} \) in LUQB cells is susceptible to synaptic inactivation. They showed that the cholinergic cell L10 produces a long-lasting inhibitory postsynaptic potential in the LUQB cells that is associated with inactivation of the steady-state \( I_{\text{Ca}} \). Although we have not studied this synapse in detail, it is intriguing that acetylcholine (ACh) produces a response that shares at least one common locus with the response produced by intracellular IP3. In a variety of other systems, muscarinic cholinergic receptors produce an intracellular response by activating phosphatidylinositol hydrolysis (see Ref. 34). It may be possible to test biochemically whether ACh induces production of IP3 in LUQB cells by isolating the left rostral quadrant of the abdominal ganglion and testing whether ACh stimulates phosphatidylinositol turnover. In addition to ACh, the LUQB cells are known to be inhibited by peptides released during discharge of the neuroendocrine bag cells during egg laying (44, 54). This inhibition also has many characteristics in common with the response to injection of IP3. Further study is required in order to identify a neurotransmitter that activates the phosphatidylinositol cycle in the LUQB cells.

ACKNOWLEDGMENTS

We thank D. Baxter and R. Kramer for their comments on an earlier draft of the paper.

This work was supported by National Institute of Mental Health Award K02 MH00649 and National Institute of Neurological and Communicative Disorders and Stroke Grant NS-19895.

Received 9 October 1987; accepted in final form 24 February 1988.

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