Inositol Trisphosphate and Activators of Protein Kinase C Modulate Membrane Currents in Tail Motor Neurons of *Aplysia*

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**SUMMARY AND CONCLUSIONS**

1. We have investigated how activation of the inositol lipid second messenger pathway may contribute to modulation of membrane currents in tail motor neurons of *Aplysia.* Specifically, we examined the effects of injected inositol 1,4,5-trisphosphate (IP$_3$) and analogues of diacylglycerol (DAG), both of which are products of the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP$_2$).

2. Injection of IP$_3$ produced an outward current associated with an apparent increase in membrane conductance. Ion substitution experiments, the sensitivity of the response to low concentrations of TEA and its attenuation by intracellular injections of EGTA suggest that the current produced by injection of IP$_3$ is a calcium-activated K$^+$ current ($I_{K,Ca}$).

3. The response to IP$_3$ was mimicked by intracellular injection of Ca$^{2+}$. Injection of Ca$^{2+}$ also produced an outward current that was associated with an apparent increase in input conductance of the membrane. The same manipulations that affected the response to IP$_3$ also affected the response to injections of Ca$^{2+}$.

4. Injections of activators of protein kinase C (PKC) produced a relatively slow inward current. The inward current has not been fully analyzed, but it does not appear to be due to the actions of any single conventional ion channel.

5. Activators of PKC attenuated responses to subsequent injections of IP$_3$, indicating that one component of PIP$_2$ hydrolysis can attenuate the other.

6. The results suggest that hydrolysis of inositol phospholipids is a mechanism for regulation of membrane properties in tail motor neurons of *Aplysia.*

**INTRODUCTION**

The tail withdrawal reflex is one of several defensive behaviors displayed by *Aplysia californica* that exhibit simple forms of learning. The sensory neurons mediating the reflexes are at least one locus for the plasticity underlying these behavioral modifications. In the sensory neurons which comprise the afferent pathway of reflex responses activated by stimulation of the tail, cAMP appears to be a critical second messenger (2, 3, 27–29, 31, 43–45).

Much interest has recently been expressed in another second messenger system, the inositol lipid pathway. Hydrolysis of phosphatidylinositol bisphosphate (PIP$_2$) results in the generation of two active second messengers, inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG) (4, 5). IP$_3$ appears to act by releasing Ca$^{2+}$, another messenger, from intracellular stores. DAG has been shown to activate protein kinase C (PKC; Ref. 26). Several lines of evidence indicate that both branches of this pathway are utilized in the nervous system of *Aplysia.* For example, this enzyme system is present in its nervous tissue (9, 34). In addition, components of this system or their analogues affect membrane properties of individual neurons. IP$_3$ produces a hyperpolarization after injection into bag cell neurons (14), left upper quadrant (LUQ) bursting neurons (38), and right upper quadrant (RUQ) neurons (37). Application of phorbol esters or injection of PKC has been shown to increase a calcium conductance in bag cells (10). Finally, phorbol esters modulate the connections between sensory neurons and motor neurons in the abdominal ganglion, which mediate the gill and siphon withdrawal reflex (18).

We were interested in examining the possibility that the inositol lipid pathway contributes to modulation of neurons mediating the tail withdrawal reflex. As a first step, we studied the effects of these messengers when injected directly into somata of tail motor neurons located in the pedal ganglion. Motor neurons were voltage clamped at fixed holding potentials. Intracellular injection was used to avoid any nonspecific actions of these agents through the activation of other neurons in the pedal ganglion. Injection of IP$_3$ produced an outward current that was similar to that produced by injection of Ca$^{2+}$, indicating that IP$_3$ was activating the Ca$^{2+}$-activated potassium current ($I_{K,Ca}$). In contrast, injection of 1-oleoyl-2-acetylgllycerol (OAG), an active analogue of DAG, produced an inward current that was relatively voltage-independent. Interestingly, OAG reduced the outward current produced by a subsequent injection of IP$_3$. These results indicate that hydrolysis of inositol lipids in tail motor neurons generates two second messengers that appear to act in a coordinated way to alter the membrane potential.

A preliminary report of these results has been presented (36).

**MATERIALS AND METHODS**

*Aplysia californica* (150–350 g) were obtained from several sources, including Marine Specimens Unlimited (Pacific Pali- sades, CA), Alacrity Marine Biological Specimens (Redondo Beach, CA), Marinus Biomarine (Westchester, CA), and Sea Life Supply (Sand City, CA). The animals were maintained in artificial sea water (Instant Ocean) at 15°C. Prior to dissection, animals were anesthetized by injection of a volume of isotonic MgCl$_2$ equal to approximately one-half of their body volume. Isolated
pleural-pedal ganglia were pinned to a plexiglas chamber, and the connective tissue sheath of the pedal ganglion was removed surgically. The ganglia were superfused with buffered saline containing the following (in mM): 10, KCl; 460, NaCl; 55, MgCl₂; 10, CaCl₂; and 10, HEPES at pH 7.6) at room temperature (19–20°C). When necessary, the concentration of K⁺ in the saline was altered without osmotic compensation by adding KCl.

Tail motor neurons were identified by location, electrophysiological properties, and antidromic stimulation of the posterior pedal nerve (45). The cells were impaled with one single-barreled microelectrode and one triple-barreled microelectrode. Cells were not used if their resting potentials after impalement with both electrodes was less than -35 mV. One barrel of the triple-barreled electrode, filled with 4 M K⁺-acetate, was used to record membrane potential; the other two barrels were used to inject substances into the neuron with pressure pulses controlled by a Picospritzer (General Valve Co.). The single barreled microelectrode was used as the current passing electrode for two-electrode voltage clamp.

Several different substances were injected. Solutions of (in mM): 1.4, IP₃ (Amershon); 10, CaCl₂ (Fisher); 50, ethylene glycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA; Sigma), and 10, myo-inositol (Sigma) were used. In addition, 30 μM OAG (Nakarai Chem.) and 200 μM phorbol 12,13-dibutyrate (PDBu; Sigma) were injected. OAG and PDBu were first dissolved in dimethylsulfoxide (DMSO) and then diluted to a final concentration of 0.05% DMSO. Control injections of DMSO alone were without effect (see Fig. 8). Solutions were made in 1% procion red (Inolex Co.) for visualization of successful injections. Volumes of solution <2 pl were injected (37). The amount of injected solution varied from one experiment to another because of the difficulty in controlling tip diameter. Consequently, the duration and magnitude of the parameters for pressure injection provided in the text are useful for comparing injection volumes within an experiment but are not useful for quantitatively comparing injection volumes among different experiments.

In some experiments, the extracellular saline was changed locally by pressure ejection from a blunt-tipped glass micropipette. An electrode was placed close to the motor neuron and solutions (in mM) of 2, KCl saline (low-K⁺); 30, KCl saline (high-K⁺); or 5, tetraethylammonium chloride (TEA Eastern Kodak) were superfused locally over the cell with low pressure pulses (<5 psi).

**RESULTS**

**IP₃-induced outward current**

In unclamped cells, injection of IP₃ into the somata of motor neurons produced a marked hyperpolarization (Fig. 1A1). When the same neuron was voltage clamped at a fixed holding potential, reinjection of IP₃ resulted in an outward current with approximately the same time course (Fig. 1A2). In general, the IP₃-induced outward current reached a maximum amplitude (5–15 nA) within 1 s and lasted ~10–30 s. Repetitive injection of IP₃ with brief duration pulses at short intervals (2.5 s) led to an increased amplitude of the IP₃-induced outward currents, since the individual currents summated (Fig. 1B1). On the other hand, repetitive injections of IP₃ with longer duration pulses at intervals of 5 s led to saturation of the outward current (Fig. 1B2). With these large injections, there was
FIG. 3. Voltage dependence of the IP$_3$-induced outward currents. A: IP$_3$ was injected by constant pressure pulses (50 ms, 20 psi) at different holding potentials, as indicated to the left of the traces. Pulses were separated by at least 2 min. Successive injections at the same holding potential gave similar responses (not shown). B: the relationship between membrane potential and peak amplitude of the outward current is illustrated. The reversal potential was approximately -72 mV.

FIG. 4. Effect of low-[K$^+$] seawater on the Ca$^{2+}$-activated and the IP$_3$-induced outward current. A: CaCl$_2$ and IP$_3$ were successively injected through different barrels of the same triple-barreled electrode by constant pressure pulses (15 ms, 15 psi and 15 ms, 20 psi, respectively). B: when low-[K$^+$] seawater was applied locally by pressure ejection from a blunt-tipped glass microelectrode, an outward current was produced. Ca$^{2+}$ and IP$_3$ were again injected during this period, and the amplitudes of the outward current responses were increased. Holding potential was -35 mV.

FIG. 5. Voltage dependence of the Ca$^{2+}$-activated outward current. A: CaCl$_2$ was injected by constant pressure pulses (40 ms, 40 psi) at different holding potentials, as indicated to the left of the traces. Pulses were separated by at least 2 min. Successive injections at the same holding potential gave similar responses (not shown). B: the relationship between membrane potential and the amplitude of the peak outward current is illustrated. The reversal potential was approximately -64 mV.
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A: ASW

B: +TEA

C: ASW

FIG. 6. Effects of focal application of 5 mM TEA on the IP$_3$-induced and the Ca$^{2+}$-activated outward currents. A: IP$_3$ and CaCl$_2$ were injected by a constant pressure pulse (35 ms, 30 psi and 30 ms, 30 psi, respectively). B: 5 mM TEA was applied locally by pressure ejection from a blunt-tipped glass microelectrode. IP$_3$ and CaCl$_2$ were then injected. Note the similar effects of 5 mM TEA; both the IP$_3$-induced and the Ca$^{2+}$-activated outward currents were greatly reduced. C: after the TEA was washed off, the outward currents recovered. Holding potential was −35 mV.

Some indication of desensitization in the final pulse of that series. The amplitude of the outward current produced by individual injections of IP$_3$ increased in a dose-dependent manner (Fig. 2A). When the duration of the pressure pulse was increased, more IP$_3$ entered the cell, resulting in a greater peak outward current and a shorter time to peak.

Changes in membrane input conductance produced by IP$_3$ were examined by applying a series of constant-voltage pulses. Injection of IP$_3$ was typically associated with an apparent increase in the membrane input conductance (Fig. 2B). In this example, the membrane current produced in response to the constant-voltage pulses increased by 800% at the peak of the IP$_3$-induced outward current.

The outward current response to injected IP$_3$ was observed in 62 of 65 motor neurons studied. In contrast, injections of myo-inositol (n = 3, Fig. 2C) or procion red alone (n = 3, data not shown) had no apparent effects on the membrane holding current.

**Ionic mechanism of the IP$_3$-induced outward current**

Because the outward current produced by IP$_3$ was associated with an increased input conductance, it is likely that the current is carried predominantly by K$^+$. As a first step in testing this hypothesis, we examined the current-voltage relationship of the response to IP$_3$.

**EFFECT OF ALTERED [K$^+$]$_o$**

If the outward current is carried by K$^+$, then its amplitude should be sensitive to the concentration of K$^+$ in the extracellular medium ([K$^+$]$_o$). Decreasing [K$^+$]$_o$ from 10 to 2 mM produced an increase in the IP$_3$-induced outward current (Fig. 4). On average, reducing the concentration of K$^+$ to one-fifth normal increased the response to IP$_3$ by 32 ± 4.6% (mean ± SEM, n = 2). In one experiment, increasing the concentration of K$^+$ to three times normal decreased the response to IP$_3$ by 24% (data not shown). Although we did not systematically examine the response over an extensive range of concentrations of extracellular K$^+$, both the reversal potential of the response and its sensitivity to the changes in the concentration of K$^+$ that were examined suggest that the response to IP$_3$ is due, at least in part, to an increase in membrane conductance to K$^+$.

**Role of Ca$^{2+}$ in mediating the response to IP$_3$**

In many cell types, IP$_3$ acts by releasing Ca$^{2+}$ from intracellular stores (4, 5). Thus the outward current produced by IP$_3$ may be due to I$_{K,CA}$. We therefore compared the outward current induced by injection of IP$_3$ with that produced by injection of Ca$^{2+}$. We would expect the response to injections of IP$_3$ and Ca$^{2+}$ to be affected in the same way by a variety of manipulations that are known to affect I$_{K,CA}$.

**INJECTION OF Ca$^{2+}$ MIMICS THE RESPONSE TO INJECTIONS OF IP$_3$**

As with IP$_3$, intracellular injection of Ca$^{2+}$ at fixed holding potentials produced a dose-dependent outward current associated with an apparent increase in input con-
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\[ A_{21 \pm 7.8\%}, n = 2, \text{data not shown}. \] These results are consistent with the expected changes in Nernst potential as a result of altered concentration in extracellular K⁺, and suggest that the outward current elicited by calcium is carried by K⁺.

**EFFECTS OF TEA.** In neurons of *Aplysia*, low concentrations of TEA (5 mM) are sufficient to block a Ca²⁺-activated K⁺ channel, whereas other currents, such as I_{K,L} and I_{K,v}, are relatively unaffected (2, 8, 17, 19, 31, 39, 43). If IP₃ is in fact activating I_{K,Ca}, then it might be sensitive to low concentrations of TEA. Indeed, the IP₃-induced outward current was reversibly abolished by focal application of 5 mM TEA (n = 6). The Ca²⁺-activated outward current was similarly sensitive to low concentrations of TEA (n = 5). In one instance, the effect of TEA on both IP₃- and Ca²⁺-induced currents was observed in the same experiment (Fig. 6). These results suggest that pressure microinjection of IP₃ into identified motor neurons can induce an outward current associated with an increase in K⁺ conductance. Moreover, based on the ionic mechanism and pharmacological properties, this current is identical to the current produced by injection of Ca²⁺ into the same neuron.

**EFFECTS OF INTRACELLULAR INJECTION OF EGTA.** The experiments described above are consistent with the hypothesis that IP₃ exerts its actions by releasing Ca²⁺ from intracellular stores, activating a membrane K⁺ conductance. This hypothesis is supported by the observation that injection of 50 mM EGTA from the triple-barreled electrode attenuated the response to subsequent injections of IP₃ (n = 5; Fig. 7). Presumably, the Ca²⁺ released from intracellular stores was chelated by the injected EGTA. With time, however, the outward current recovered (Fig. 7C). This reversal may be due to the diffusion of EGTA from the soma. Similar results were obtained when EGTA and IP₃ were injected from different microelectrodes located in different regions of the soma (n = 3).

**Effects of activators of PKC on membrane currents**

The other compound produced by hydrolysis of inositol lipids is DAG. There is some evidence that the DAG/PKC pathway can interact with the IP₃/Ca²⁺ pathway in either a positive or negative manner (5, 6). Since the effects of DAG can be mimicked by OAG and PDBu, we examined the

![FIG. 8. Effects of OAG and PDBu on holding current. A: intracellular injection of 0.05% dimethylsulfoxide (DMSO) did not affect holding current, but injection of OAG into the same neuron produced an inward current. DMSO and OAG were injected by a constant pressure pulse (100 ms, 40 psi and 100 ms, 20 psi, respectively). B: intracellular injection of PDBu (B1) and OAG (B2) into the same neuron. PDBu and OAG were injected by constant pressure pulses (70 ms, 20 psi and 40 ms, 20 psi, respectively). Constant hyperpolarizing command pulses (2 mV) of 0.5-s duration were delivered every 5 s to monitor the input conductance of the membrane. Note that both PDBu and OAG induced an inward current, but there was little if any change in membrane conductance. Holding potential for (A) and (B) was -50 mV.

![FIG. 9. Voltage dependence of the response to OAG. A: OAG was injected by constant pressure pulses (10 ms, 15 psi) at different holding potentials, as indicated to the left of the trace. Pulses were separated by intervals of at least 2 min. B: the relationship between the membrane potential and the peak inward current is illustrated. The current did not reverse within this range of holding potentials. Moreover, the voltage dependence of the inward current was not linear. In this experiment, constant hyperpolarizing command pulses (1 mV) of 500-ms duration were delivered every 6 s to monitor the input conductance of the membrane.]
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FIG. 10. Effect of repeated injections of PDBu on the IP3-induced outward current. IP3 and PDBu were injected by constant pressure pulses (15 ms, 20 psi and 65 ms, 20 psi, respectively). PDBu was injected as a train of 5 pulses. Holding potential was -60 mV. PDBu produced an inward shift in the holding current and responses to subsequent injections of IP3 were attenuated.

FIG. 11. Effects of a single injection (A) and repeated injections (B) of PDBu on the Ca2+-activated outward current recorded from a tail motor neuron. A1: CaCl2 and PDBu were injected by constant pressure pulses (30 ms, 20 psi and 30 ms, 20 psi, respectively). PDBu was injected as a train of 5 pulses. A single pulse of PDBu was sufficient to reduce the response to Ca2+ 42 s after the injection of PDBu. B: response was reduced to a greater extent by injecting more PDBu. Holding potential was -30 mV.

Effects of these agents on membrane currents in tail motor neurons.

OAG was injected into somata of motor neurons held at a constant holding potential. A prolonged slow inward current was produced (Fig. 8A). In general, the OAG-induced inward current reached a maximum amplitude of ~1 nA within 5–10 s and lasted 60–120 s (n = 5). The duration of this current is consistent with that observed in embryonic dorsal root ganglia (32), although longer responses have been observed (12, 16). In contrast, injection of 0.05% DMSO did not produce any observable shift in holding current (n = 4; Fig. 8A).

To examine whether the effects of OAG are specific, we also injected PDBu, another known activator of PKC. Injection of PDBu into the same tail motor neuron as that receiving OAG had the same effect. It produced a slow inward current with the same time course as the OAG-induced slow inward current (Fig. 8B). In this experiment, we also monitored the input conductance of the membrane during the injection. Neither of these drugs appeared to have a significant effect on the membrane input conductance (Fig. 8B). To further characterize the responses produced by OAG and PDBu, we examined the current-voltage relationship in six cells. In general, the response to injection of OAG tended to increase as the cell was hyperpolarized (Fig. 9), but our results were not consistent. Moreover, compared with that of IP3 or Ca2+, the current-voltage relationship was not steep (compare the ordinate scale of Fig. 9 with those of Figs. 3 and 5). This is consistent with the lack of effect on membrane input conductance. Similar results were obtained with PDBu (data not shown). These results suggest that OAG and PDBu do not act via the opening or closing of a single conventional ion channel.

Modulatory effects of OAG and PDBu on both the IP3-induced outward current and IK,Ca

IP3 and DAG are both products of the hydrolysis of PIP2. Since both IP3 and compounds that mimic the effects of DAG affect membrane currents of tail motor neurons, we were interested in examining the possibility that there is an interaction between the IP3/Ca2+ pathway and the DAG/PKC pathway. We therefore injected PDBu or OAG and IP3 or Ca2+ into the same neuron through different barrels of the same triple-barreled electrode.

EFFECTS OF OAG AND PDBU ON THE RESPONSE TO IP3.

A single injection of PDBu that did not affect holding current was sufficient to reduce the response to IP3 by ~15% (n = 3, data not shown). Repeated injections of PDBu produced an inward current (Fig. 10), and the subsequent outward current produced in response to IP3 was diminished by 32% (n = 5). The attenuation of the responses to IP3 by prior injection of PDBu was relatively transient, and persisted for ~2 min. Similar results were obtained after injection of OAG (n = 4, data not shown). Interestingly, injections of OAG that did not by themselves produce an inward current were also capable of attenuating subsequent responses to IP3 (n = 4, data not shown). Thus there does not appear to be a direct correspondence between the ability of OAG or PDBu to produce the inward current and its ability to modulate the IP3 response (i.e., they are mechanistically independent).

EFFECTS OF OAG AND PDBU ON IK,Ca. The mechanism by which PKC affects the response to IP3 has yet to be determined. As a first step towards answering this question, we examined the effects of OAG and PDBu on IK,Ca produced by the opening or closing of a single conventional ion channel.
intracellular stores. A single injection of PDBu reduced the amplitude of \( I_{K, Ca} \) by 13% (Fig. 11A) and by 19% after five pulses (Fig. 11B). The effects of PDBu, like OAG, were relatively transient. Similar results were obtained in 18 other experiments. These studies suggest that PKC reduces the ability of \( Ca^{2+} \) to open \( K^+ \) channels rather than affecting the ability of IP3 to release \( Ca^{2+} \) from intracellular stores.

**DISCUSSION**

**Effects of PIP2 hydrolysis on tail motor neurons**

The mechanism by which we propose that IP3 and DAG affect membrane currents in tail motor neurons of *Aplysia* is summarized in Fig. 12. A modulatory neurotransmitter or hormone (which we have not yet identified) binds to a receptor on the plasma membrane of the tail motor neuron, activating phospholipase C (Fig. 12, a), which in turn hydrolyzes PIP2 in the membrane. Both products of this hydrolysis, IP3 and DAG, appear capable of modulating membrane currents. Our results are consistent with those from a number of other systems in which it has been shown more directly that IP3 releases \( Ca^{2+} \) from nonmitochondrial stores such as endoplasmic reticulum (ER; Fig. 12, b). For example, we have shown that the effects of injected IP3 are virtually identical to those of injected \( Ca^{2+} \). Moreover, the outward currents produced by both reversal at the same membrane potential, are sensitive to alterations in the concentration of extracellular \( K^+ \), are sensitive to low concentrations of extracellular TEA, and are blocked by injection of EGTA. These same characteristics suggest very strongly that the outward currents are due to activation of \( I_{K, Ca} \) (Fig. 12, c).

Another possibility is that IP3 activates a \( Cl^- \) channel (30). Although we did not test this possibility directly, we believe that it is unlikely for several reasons. For example, the equilibrium potential for \( Cl^- \) in *Aplysia* is approximately -56 mV (33), whereas the equilibrium potential for both the IP3- and \( Ca^{2+} \)-activated currents is approximately -67 mV, closer to the equilibrium potential for \( K^+ \) (42). Moreover, there is no evidence that \( Cl^- \) currents in *Aplysia* are sensitive to TEA.

Our results also suggest that DAG affects membrane properties in the tail motor neurons of *Aplysia*. Injection of OAG or PDBu produced a slow inward current, which we have not characterized in detail. Nevertheless, we presume this effect is mediated by activation of PKC (Fig. 12, d) and may result in phosphorylation of one or more membrane proteins (Fig. 12, e). Moreover, the two pathways appear to interact (Fig. 12, f). OAG and PDBu, drugs which mimic the activation of PKC by DAG, reduce the amplitude of outward currents produced by injection of IP3, or \( Ca^{2+} \). On the other hand, the increased levels of intracellular calcium produced by IP3 may also increase the activity of PKC, but we have no results to support this suggestion.

**IP3 acts by a similar mechanism in other neurons**

Although the physiological effect of IP3 may be dependent on the particular neuron injected, it appears to have a common action through release of intracellular \( Ca^{2+} \). As in the tail motor neurons, injection of IP3 into RUQ neurons produces a hyperpolarization that appears to be due to activation of \( I_{K, Ca} \) (37). In these cells, the properties of the currents induced by IP3 are slightly different, however. In particular, it was difficult to reverse the IP3-induced current in the RUQ neurons (37), but not in the tail motor neurons. This could be due to several factors. First, the RUQ neurons are much larger than the motor neurons, so it is more difficult to control the membrane potential in these cells. Second, the distribution of \( Ca^{2+} \)-activated \( K^+ \) channels, the channels affected by IP3, may differ in the two types of cells. Similarly, injection of IP3 into bag cells results in a hyperpolarization that seems to be due to activation of \( I_{K, Ca} \) (14). On the other hand, injection of IP3 into the bursting LUQ neurons produces a hyperpolarization that is produced by a different mechanism (38); elevated \( Ca^{2+} \) levels result in inactivation of a steady-state \( Ca^{2+} \) current. Thus IP3 appears to act via a common mechanism even though the released \( Ca^{2+} \) may have diverse actions. Similar results have been obtained in neural tissues from other animals (7, 13, 35, 41).

**Activators of PKC have multiple actions**

Our results also suggest that in the tail motor neurons DAG affects membrane currents. Pressure microinjection of OAG and PDBu (activators of PKC) produced a modest inward current. Possible mechanisms underlying the inward current include inhibition of a steady-state \( I_{K, Ca} \), the same current affected by IP3, and activation of a \( Ca^{2+} \) current. These possibilities are unlikely, however, since the current was not associated with a change in membrane conductance and was only slightly dependent on membrane potential. Therefore, these agents do not appear to act via the opening or closing of a single conventional ion channel, unlike IP3.

We have not fully investigated the mechanism of action in tail motor neurons, but effects of PKC have been ob-
served more directly in other neurons of *Aplysia*. For example, PKC acts in bag cells by recruitment of a previously covert class of Ca\(^{2+}\) channels (10, 40). Although it cannot fully explain our results, this mechanism may contribute to the inward current produced by OAG and PDBu in the tail motor neurons.

PKC, or its activators, has effects in other neuronal tissues. For example, activation of protein kinase C reduces \(I_{K_{Ca}}\) (1, 23) and a voltage-dependent chloride conductance (22) in rat hippocampal neurons. In dissociated mouse dorsal root ganglion and cerebral cortical neurons, multiple \(K^+\) conductances may be reduced (15).

**Interactions between the two intracellular pathways**

One of our more interesting results indicates a complex interaction between the two pathways initiated by hydrolysis of PIP\(_2\). Since IP\(_3\) raises the intracellular concentration of Ca\(^{2+}\), one might expect PKC to be stimulated. We have not investigated this possibility directly, however. Nevertheless, activators of PKC reduced the subsequent response to injected IP\(_3\) or Ca\(^{2+}\). These modulatory effects were achieved with concentrations of activators consistent with those used in other systems (11, 20, 26).

Similar inhibitory interactions have been observed elsewhere (21, 25). Our results indicate that activators of PKC are equally effective at reducing the outward current produced by IP\(_3\) and by Ca\(^{2+}\). At present we do not know the site of this inhibition, however. These results are consistent with the modulatory role of the DAG/PKC pathway proposed by Berridge (5).

The interactions we have observed in this series of experiments suggest that other second messengers may interact with these pathways as well. For example, in pleural nerve cells, CAMP and agents that increase the levels of CAMP, such as serotonin, reduce the conductances activated by intracellular injection of Ca\(^{2+}\) (19, 43). Thus in these cells, \(I_{K_{Ca}}\) may a target for both the cyclic nucleotide- and inositol phospholipid-mediated intracellular pathways.

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