model is dependent on prostaglandin generation.

Prostaglandins are known to be potent inhibitors of acid secretion (*9*), and have been shown to protect gastric mucosa from ulcer and erosion formation (*10*). In the complex model developed by Soll (*11*) with isolated canine parietal cells, acid secretion is regulated by mutually potentiating gastrin, histamine, and cholinergic receptors. Prostaglandins appear to act by inhibiting histamine-stimulated cyclic AMP production through inhibition of the enzyme adenylate cyclase (*6*). While the actions of cholinergic agonists alone are not mediated directly by cyclic AMP, they are always acting in concert with histamine in vivo. The potentiating interactions between the two secretagogues is blocked by prostaglandins (*6*). Our observations that prostaglandin synthesis and release from the stomach is enhanced by somatostatin (in the presence of carbamylcholine) and that somatostatin's ability to inhibit bethanechol-stimulated acid secretion is blocked by indomethacin suggest that somatostatin action on gastric secretion is mediated by prostaglandins. Although we did not measure gastric cyclic AMP in our experiments, Gespach *et al.* (*5*) have reported that cyclic AMP production in rat gastric glands is markedly inhibited in a noncompetitive fashion by somatostatin. This is consistent with our hypothesis regarding the actions of somatostatin. Since prostaglandins, in turn, stimulate somatostatin secretion (*12*) it is possible that their release further amplifies the acid inhibitory action of somatostatin. The implication of our results regarding a potential prostaglandin-mediated cytoprotective action of somatostatin requires further investigation.

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Protein Kinase Injection Reduces Voltage-Dependent Potassium Currents

Abstract. *Intracellular iontophoretic injection of the catalytic subunit of cyclic adenosine monophosphate–dependent protein kinase increased input resistance and decreased a delayed voltage-dependent K^+ current of the type B photoreceptor in the nudibranch* Hermissenda crassicornis *to a greater extent than an early, rapidly inactivating K^+ current (I_A). This injection also enhanced the long-lasting depolarization of type B cells after a light step. These findings suggest the involvement of cyclic adenosine monophosphate–dependent phosphorylation in the differential regulation of photoreceptor K^+ currents particularly during illumination. On the other hand, conditioning-induced changes in I_A may also be regulated by a different type of phosphorylation (for example, Ca^{2+}-dependent).*

Numerous studies have suggested a role for protein phosphorylation in many types of neuronal processes, including synaptic transmission, regulation of membrane permeability, phototransduction, and learning (*1*). Recently a change in the level of phosphate incorporation into a specific phosphoprotein band (*2*) has been detected in the eyes of the nudibranch *Hermissenda crassicornis* after training with paired light and rotation (*3, 4*) but not after control procedures. Type B photoreceptors (of which there are three in each eye) also showed biophysical changes both before and after isolation from the central nervous system (*5, 6*) during acquisition and retention of the learned behavior specifically produced by this associative training (*4*). An early voltage-dependent K^+ current (I_A) was reduced and inactivated more rapidly in associatively trained than in control type B cells (*6*). Approximately 60 percent of the resting input resistance of the type B soma membrane is due to this I_A conductance (*7, 8*). The reduction of I_A produced by training, therefore, can account for an increased input resistance and, at least partially, an enhanced long-lasting depolarization (LLD) to a light response also found for conditioned cells (*5, 9*). Since type B cell impulses affect interneuron and motoneuron impulse activity via known synaptic pathways (*10*), these biophysical changes, shown to be intrinsic to the type B photoreceptor soma membrane, can play a causal role in producing the associatively learned behavior. Sustained illumination, unlike conditioning, dramatically reduced a delayed voltage-dependent K^+ current (I_B), but had no effect on I_A when the cell was voltage-clamped at −60 mV (*6*).

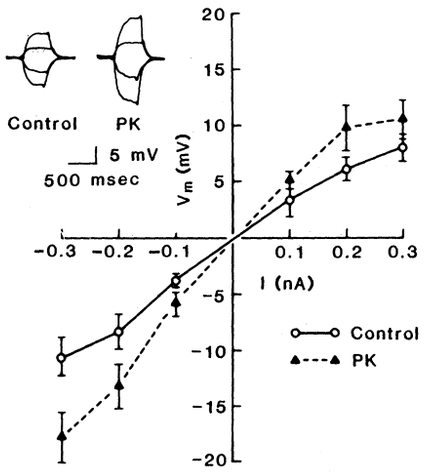

Fig. 1. Effects of iontophoretic protein kinase (*PK*) injection on dark membrane effective input resistance in type B photoreceptors. Symbols: ○, control injection of carrier solution (for each point, mean ± standard error, $N = 12$); ▲, *PK* values ($N = 10$). Inset shows representative voltage responses to current injection (±0.1 and ±0.2 nA).

In order to investigate the possible relationship of protein phosphorylation to these type B photoreceptor K^+ conductances, we have injected, under cur-

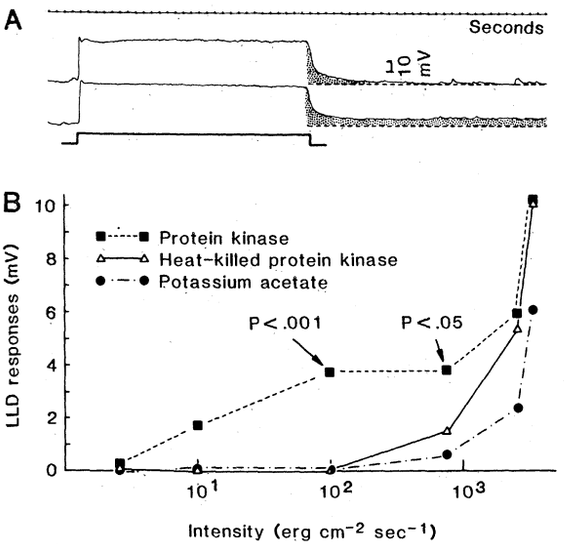

Fig. 2. Effects of protein kinase injection on LLD of the type B photoreceptor after the offset of light steps. (A) Responses to a 30-second light step (intensity 7.5×10^2 erg cm^{-2} sec^{-1}, lower trace) recorded intracellularly with potassium acetate (upper trace) and protein kinase solution (lower trace) in the microelectrodes. Recordings with potassium acetate electrodes were not significantly different from those obtained with heat-denatured protein kinase solutions. The lower trace monitors the light step. (B) Effects of protein kinase injection on the amplitude of LLD (ordinate) measured 30 seconds after the light offset as a function of different light intensities (abscissa). Values for each are the means of five or more cells from different animals. Statistical differences were tested by the Mann-Whitney *U* test. Energy values indicate the intensity (unfiltered) of the light stimulus provided by a tungsten 6V, 15W bulb (Philips, Netherlands).

rent and voltage clamp control, the catalytic subunit of cyclic adenosine monophosphate (AMP)–dependent protein kinase into type B photoreceptors of untrained animals. We found that cyclic AMP–dependent protein phosphorylation can exert a differential effect on the two voltage-dependent K^+ currents, I_A and I_B (*11*).

All experiments were performed on type B photoreceptors electrically isolated from all other cells of the nervous system by a cut through each cell's axon approximately 20 μm from the soma. This procedure eliminates all impulse activity as well as synaptic interactions without damaging the cell soma or its axonal stump (*12*). A single iontophoretic injection of the catalytic subunit of cyclic AMP–dependent protein kinase (*13*) significantly increased the effective input resistance (that is, decreased chord conductance) (*14*) of the photoreceptor dark membrane compared with input resistance before injection (Table 1 and Fig. 1). Repeated injections produced little or no further increase in effective input resistance. The increases of input resistance were not accompanied by any consistent or marked changes of resting membrane potential. These resistance changes did not reverse over the 20 to 40 minutes of intracellular recording, nor were they produced by iontophoretic injection of control solutions (Table 1) (*15*). The same protein kinase injections also enhanced the LLD after a 30-second light step [220.14 ± 99.05 percent, t (12) = 2.2, $P < .025$] (*16*). Identical iontophoretic injections of the control solution had no significant effects on depolarization [an increase of 29.9 ± 12.7 percent, t (14) = 1.7, not significant]. The effect of protein kinase injection on the LLD also did not reverse during the 20 to 40 minutes of recording.

The effects of protein kinase injection on type B cell responses to light steps were also studied with another experimental procedure (Fig. 2). The injection was accomplished by causing the voltage across the microelectrode to undergo an electrical oscillation ("ringing") of approximately 30 V peak to peak for approximately 1 second. Since the protein kinase is negatively charged at *p*H 8.6, (*13*), the 15-V negative phases of the oscillation were probably directly responsible for the actual enzyme injection. Responses of type B photoreceptors impaled with microelectrodes filled with protein kinase solution were recorded (after ringing) during and after light steps of increasing intensity following 10 minutes of dark adaptation. Control responses were also recorded (after ringing) from type B photoreceptors impaled with microelectrodes filled with heat-denatured (in boiling water for 1 minute) protein kinase solutions or potassium acetate alone.

The steady-state responses during a 30-second light step were not significantly larger for cells treated with protein kinase. However, the amplitude of the LLD following the light step was clearly different between groups. Particularly for the lower light intensities, the LLD was greater and more prolonged for the type B cells treated with protein kinase (Fig. 2) than for the other two groups. This effect on LLD did not reverse during the recording period (½ to 1 hour). The LLD of the denatured protein kinase cell group was not significantly greater than that of the potassium acetate group (Fig. 2B) for light intensities $\leq 10^3$ erg

Table 1. Effects of iontophoretic protein kinase injection into type B photoreceptors. Chord conductance values (means ± standard errors) were calculated for membrane voltage displacements (steady-state values) elicited by intracellular current injection through a single-electrode balanced bridge circuit (*14*). Voltage clamp data were peak K^+ current values. Statistical analyses were by one-tailed *t*-tests for paired samples, with α values corrected for repetitive *t*-tests; the critical *t* (9) with the α correction is 2.82. Control data were collected before, and test data after, injection; N.S., not significant.

Condition	Chord conductance						Voltage clamp data					
	N	Current (nA)	Control (nS)	Test (nS)	*t*	*P*	*N*	K^+ current	Control (nA)	Test (nA)	*t*	*P*
Protein kinase	10	−0.1	24.8 ± 0.37	15.80 ± 0.74	12.8	< .01	5	I_A	33.15 ± 3.50	26.33 ± 3.17	3.26	< .025
		+0.1	28.4 ± 0.30	17.80 ± 0.62	11.72	< .01		I_B	7.75 ± 0.93	4.18 ± 1.09	6.9	< .001
		−0.2	22.9 ± 1.08	14.07 ± 1.62	12.52	< .01						
		+0.2	29.4 ± 0.87	17.50 ± 1.60	7.3	< .01						
		−0.3	27.4 ± 1.22	15.60 ± 1.90	7.64	< .01						
		+0.3	31.2 ± 1.33	22.40 ± 1.81	6.6	< .01						
Control	12	−0.1	21.0 ± 0.71	21.30 ± 0.60	0	N.S.	3	I_A	24.80 ± 3.19	25.90 ± 3.93	0.88	N.S.
		+0.1	22.8 ± 0.66	22.20 ± 0.62	− 1.52	N.S.		I_B	9.40 ± 1.56	10.75 ± 2.25	1.31	N.S.
		−0.2	19.4 ± 1.07	20.60 ± 1.30	− 2.48	N.S.						
		+0.2	23.3 ± 1.30	23.30 ± 0.99	0	N.S.						
		−0.3	20.9 ± 1.80	22.06 ± 1.80	− 2.6	N.S.						
		+0.3	26.5 ± 0.51	30.93 ± 1.32	− 7.6	N.S.						

cm^{-2} sec^{-1}. At the brighter light intensities, differences in LLD produced by protein kinase injection were less apparent.

These results are consistent with the interpretation that protein phosphorylation, stimulated by injection of the catalytic subunit of cyclic AMP–dependent protein kinase, is associated with changes of the type B cell membrane conductances in the dark. The enhanced LLD and increased input resistance could both be consequences of the decreased voltage-dependent K^+ conductances observed for the dark type B photoreceptor membrane. Two voltage-dependent K^+ currents (*17, 18*) across the type B photoreceptor membrane (in the absence of light) have previously been determined (*6, 8, 18, 19*): an early, rapidly inactivating K^+ current (I_A) (*20*) and a late, slowly inactivating K^+ current (I_B) (*21*). To assess how changes of these individual conductances might account for the effects of protein kinase on the type B photoreceptor, we measured K^+ currents under voltage clamp (*22*) before and after protein kinase injection. A single iontophoretic injection (*23*) caused a greater decrease in I_B (46.1 percent) than in I_A (20.5 percent) (Table 1 and Fig. 3). No significant effect was observed on I_{Na^+} [a light-induced inward current (*12*)]. Control injections of carrier solution had no significant effect on either I_A or I_B (Table 1). A highly purified source of the catalytic subunit of cyclic AMP–dependent protein kinase was used for three of the five voltage clamp experiments. The purified and commercially available subunits produced identical effects on these currents.

The decrease of I_A after injection of protein kinase probably accounts for the observed increase of effective input resistance in the dark (*9*). Previous quantitative determinations show a substantial I_A and minimal I_B contribution to the type B photoreceptor conductance at its resting membrane potential (*8*). The enhancement of the LLD after injection of protein kinase, however, could result from both I_A and I_B decrement. It is also possible that the previously observed decrease of I_B (but not I_A) during the steady-state light response of the type B photoreceptor (*6*) occurs through cyclic AMP–dependent protein phosphorylation. Light-induced changes of photoreceptor protein phosphorylation have been reported (*24*). Changes in input resistance after injection of cyclic AMP–dependent protein kinase (*25*) as well as the protein kinase inhibitor (Walsh inhibitor) (*26*) have also been observed in other molluskan neurons.

That protein kinase injection reduced I_B more than I_A may be due to a difference of accessibility of the I_A channel to the exogenous protein kinase under the conditions of this study. Alternatively, I_A may be more directly regulated by a different type of protein kinase, such as Ca^{2+}-calmodulin–dependent protein kinase (*27*) (as opposed to cyclic AMP–dependent protein kinase). Two recent types of experiments support the latter possibility: (i) I_A is reduced by intracellular Ca^{2+}. This follows from the observations that intracellular injection of Ca^{2+} under voltage clamp control or induction of $I_{Ca^{2+}}$, achieved by pairing light steps with depolarizing command steps, caused prolonged reduction of I_A (*18*). In addition, elevation of intracellular Ca^{2+} after illumination has recently been demonstrated by arsenazo III differential absorption spectrophotometry (*28*). The concentration of intracellular Ca^{2+} returns to baseline with approximately the same time course and shows the same voltage dependence as the LLD (*28*). (ii) Intracellular injection of a Ca^{2+}-calmodulin–dependent protein kinase (phosphorylase kinase) into type B cells preferentially reduced I_A more than I_B (*29*). The increased phosphorylation of a protein of low molecular weight in eyes from associatively trained animals (*2*) could therefore result from increased Ca^{2+}-calmodulin–dependent protein kinase activity. The biophysical expressions of such a change of enzymatic activity might then be a reduced I_A and an enhanced LLD of the type B photoreceptor.

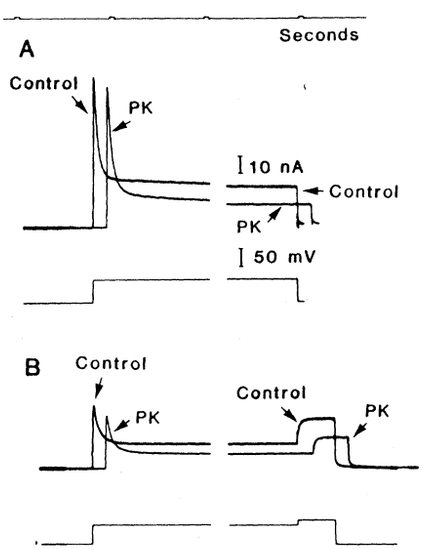

Fig. 3. Outward K^+ currents of a type B photoreceptor. Current recordings before and after protein kinase injections have been juxtaposed for ease of comparison. Prolonged lower traces indicate command voltages; traces that monitor voltage commands after protein kinase injection are not shown. Recordings are not presented for the full 10-second interval, as indicated by interruption of the traces. (A) Outward currents elicited by command steps to 0 mV from a holding potential, V_H of −60 mV (lower trace indicates +60-mV command). I_A currents are the early peak transients. Late outward K^+ currents (I_B) at 0 mV occur only at threshold of the steep part of the activation curve and are thus masked by the predominant I_A currents elicited. The peak value of I_A after protein kinase injection (*PK*) is only somewhat smaller than that before the control injection, whereas I_B (*PK*) is substantially reduced when measured 1.0 second after the onset of the command step. (B) Outward currents elicited by command steps to −10 mV from a V_H of −60 mV (prolonged lower trace indicates +50-mV command). I_B attains a maximum value approximately 1.0 second after the onset of the command step. I_B is significantly reduced after protein kinase injection (*PK*) when measured 1.0 second after the onset of the command step to −10 mV and in response to a second command step to 0 mV 30 μsec after a 5-second first command step.

Taken together, the results to date implicate cyclic AMP–dependent protein kinase in the light-induced changes of I_B and Ca^{2+}-calmodulin–dependent protein kinase in conditioning-induced changes of I_A. In addition, there may be some interactive effects between these two biochemical processes (*27*). In support of this possibility, cyclic AMP and Ca^{2+}-calmodulin have been reported to regulate the phosphorylation of the same protein (*30*).

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9. In support of this interpretation is the finding that 5 m*M* 4-aminopyridine [a specific blocker of I_A in type B photoreceptors (*8*)] produces an increase in dark membrane input resistance and a concomitant enhancement of LLD in type B

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13. The catalytic subunit of cyclic AMP–dependent protein kinase was obtained from Sigma (P-2645). The lyophilized powder, containing approximately 1 percent protein, 80 percent sucrose, and 19 percent potassium phosphate buffer, was dissolved in carrier solution, 0.95*M* potassium acetate, 0.05*M* tris, *p*H 9.8 (1390 enzyme units per milliliter). The *p*H of the dissolved protein solution is 8.6. Microelectrodes were first filled by capillarity with the protein kinase carrier solution and then backfilled with fresh protein kinase solution (1.38 unit/μl) 10 minutes before use. Since protein kinase is negatively charged at *p*H 8.6 (isoelectric point, *p*I, 7.6) [I. Uno, T. Ueda, P. Greengard, *J. Biol. Chem.* **252**, 5164 (1977)], negative currents (0.3 to 1.0 nA, 30 to 60 seconds) were used to eject it. Detectable enzyme concentrations ejected through microelectrodes into assay tubes by these currents were measured with the method of J. J. Witt and R. Roskoski, Jr. [*Anal. Biochem.* **66**, 253 (1975)].
14. Input resistance measured through a balanced bridge circuit agrees well with measurements obtained with two intracellular electrodes, one for current passage and one for voltage monitoring (*12*).
15. Control solution consisted of 0.26*M* sucrose, 0.097*M* K_2HPO_4, 0.95*M* potassium acetate, 0.05*M* tris; *p*H was adjusted to 8.6 with acetic acid.
16. After 10 minutes of dark adaptation, 30-second light steps (10^4 erg cm^{-2} sec^{-1}) were presented at 3-minute intervals. The LLD to the third step was compared to those to the fourth and fifth steps. The protein kinase was injected in darkness between the third and fourth light steps.
17. These two dark K^+ currents could be separated pharmacologically: I_A was preferentially blocked by 10 m*M* 4-aminopyridine and I_B by tetraethylammonium ion (*8*). I_B was also specifically blocked by perfusion in 10 m*M* Ba^{2+} (*18*). No residual I_{Na} or $I_{Ca^{2+}}$ were detected in the dark after treatments that would eliminate K^+ currents, enhance inward currents, or both. The I_B currents were estimated from current values measured with a command step (to 0 mV) occurring 30 μsec after a 5.0-second preliminary command step to − 10 mV. This method for I_B measurement was arrived at after numerous experiments (J. J. Shoukimas and D. L. Alkon, unpublished observations) that established I_B characteristics in the absence of I_A (after blocking the 4-aminopyridine).
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23. Fresh protein kinase solution was injected iontophoretically (anodal current, 0.5 to 2.0 nA; 30 to 60 seconds) through the voltage-recording electrode, while opposite and equal current was delivered through the current-recording electrode to prevent any net change in resting potential during injection. Peak current values for I_A and I_B before protein kinase injection were compared separately with the first responses after injection. Values reported in the text are the percent changes obtained from the average peak current values for five different cells (Table 1).
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Prorocentrin: An Extracellular Siderophore Produced by the Marine Dinoflagellate *Prorocentrum minimum*

Abstract. *Prorocentrin, a putative iron transport compound, has been extracted from the filtrates of* Prorocentrum minimum *cultures by XAD-2 resin. Production of prorocentrin can be stimulated by culturing* Prorocentrum minimum *under conditions of iron deficiency. The iron(III) complex of prorocentrin has an ultraviolet-visible absorption spectrum typical of hydroxamate siderophores.*

Marine phytoplankton, like most other living organisms, have a nutritional requirement for iron (*1*). Iron in seawater is either associated with organic chelators (*2*) or present as aggregates of the almost totally insoluble iron(III) hydroxide (solubility product constant $\approx 10^{-38}$) (*3*). Although a portion of the chelated iron(III) may be utilized by phytoplankton, the large pool of insoluble iron(III) hydroxide is presumed to be unavailable as a nutritional source.

Aquatic microorganisms—for example, bacteria (*4*) and cyanobacteria (*5–8*)—produce extracellular iron(III)-specific chelating compounds (siderophores) that enable them to solubilize and therefore acquire the iron present in the iron(III) hydroxide aggregates.

It is not clear whether eukaryotic phytoplankton acquire iron(III) in a similar manner. Spencer *et al.* (*9*) characterized a possible siderophore from a nonaxenic marine diatom, *Chaetoceros socialis*. The isolated substance had chemical properties similar to those of a hydroxamate siderophore, but definitive evidence for the origin of the compound (from the diatom and not the bacteria) was not presented. More recently McKnight and Morel (*10*) have concluded that a number of axenic eukaryotic phytoplankton are unable to produce iron(III)-specific chelators. They have suggested that the diatom *Thalassiosira weissflogii* utilizes iron(II) rather than iron(III) (*11*), because ferrous iron is more soluble in seawater and therefore it should be more nutritionally accessible. Since iron(II) is extremely labile in the marine environment, transformation of iron(III) to iron(II) at the cell membrane may be required for efficient uptake. Armstrong and Van Baalen (*7*) showed that a concentrated chloroform extract of the supernatant from an iron-depleted culture of the marine diatom *Cylindrotheca* was Csaky-positive (*12*) and that it stimulated the growth of the siderophore auxotroph *Arthrobacter flavescens* JG-9 (*13*). Circumstantial evidence for the utilization of siderophores by phytoplankton comes from numerous observations that either synthetic or natural chelators stimulate phytoplankton growth in culture and in the field, possibly by enhancing the availability of iron (*14, 15*).

To clarify whether marine eukaryotes

Table 1. Influence of culture medium on growth rate of *Prorocentrum minimum* and production of Csaky-positive compounds.

Medium and nutrient status	Growth (divisions per day)	Final yield ($\times 10^7$ cells per liter)	Csaky test* (μg)
ESNW, iron-sufficient	1.04 ± 0.08	6.27	5.9
AQUIL-Fe, iron-deficient	0.42 ± 0.01	4.01	127.2
ESNW-Fe, iron-deficient	0.75 ± 0.02	3.95	133.0

*Measured as micrograms of NH_2OH per 10^8 cells.