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17. Statistically significant comparisons are described in the text. For more intergroup comparisons, a Bonferroni correction could be applied to critical *P*.

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# Blockade of Visual Excitation and Adaptation in *Limulus* Photoreceptor by GDP-β-S

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**Light causes both depolarization and adaptation to light in *Limulus* ventral photoreceptors. Both visual excitation and adaptation were blocked by guanosine 5′-*O*-(2-thiodiphosphate) (GDP-β-S), a metabolically stable analog of guanosine 5′-diphosphate (GDP). However, GDP-β-S did not block the excitation caused by injection of inositol 1,4,5-trisphosphate into the cell. These results suggest a molecular cascade of visual excitation and adaptation: Light isomerizes the visual pigment rhodopsin, which in turn activates a guanyl nucleotide–binding protein. The binding protein then stimulates production of inositol 1,4,5-trisphosphate, which causes release of calcium from the endoplasmic reticulum.**

BECAUSE OF THEIR LARGE SIZE, THE ventral photoreceptors of *Limulus* have been a favorite preparation for the investigation of the molecular events underlying phototransduction in microvillar photoreceptors. Independent lines of evidence suggest that both a guanine nucleotide–binding protein (N) and a phospholipase C may be involved in phototransduction in these cells. (i) Agents that activate guanine nucleotide–binding proteins excite ventral photoreceptors by depolarizing them in a manner similar to light (*1–4*). These findings led to the suggestion that illumination of ventral photoreceptors isomerizes the visual pigment rhodopsin, which in turn activates N and thereby leads to membrane depolarization (*2, 4*). (ii) The intracellular messenger inositol 1,4,5-trisphosphate ($IP_3$), which is released from phosphatidylinositol 4,5-bisphosphate ($PIP_2$) by the enzyme phospholipase C (*5*), can excite and adapt ventral photoreceptors when injected intracellularly (*6, 7*). Thus, phospholipase C may play a role in visual transduction.

A growing body of evidence suggests that receptor-mediated production of $IP_3$ involves a guanine nucleotide–binding protein (*5, 8*). In enzyme cascades such as hormonal activation of adenylate cyclase and light-triggered hydrolysis of cyclic guanosine monophosphate, information flows from the receptor to N and then to the enzyme that is regulated (*9, 10*). Application of these ideas to phototransduction in ventral photoreceptors suggests a model for the visual cascade. Photoisomerization of rhodopsin activates N, which in turn activates phospholipase C, thereby causing the production of $IP_3$. One would predict that block of an early step of the cascade would leave later stages unaffected. Thus, blockade

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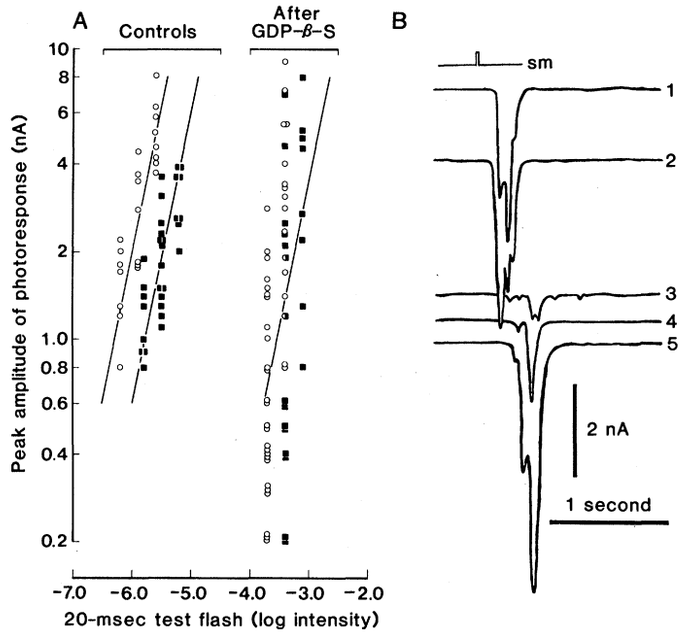


Fig. 1. Voltage-clamp recordings of light-induced current in ventral photoreceptors before and after injection of GDP-β-S. The methods for preparation, intracellular recording, voltage clamping, and pressure injection have been described (*25, 26*). Cells were impaled with two microelectrodes, a current-passing electrode filled with 2.5*M* KCl and a voltage-measuring electrode filled with 20 m*M* GDP-β-S, 100 m*M* potassium aspartate, 10 m*M* Hepes, *p*H 7.0. In some experiments 0.001% Triton X-100 was added to the GDP-β-S solution to facilitate pressure injection (*16*). GDP-β-S was from Boehringer Mannheim (Indianapolis). (A) A log-log plot of the peak amplitude of the light-induced current as a function of light intensity for two different cells (closed squares, open circles). Light intensities are relative to the maximum available from the light source. The solid lines have a slope of 1. The injected volume of GDP-β-S–containing solution was between 1% and 10% of the cell volume (*26*) giving a final concentration of 0.2 to 2 m*M* in the cell. For 16 cells injected with GDP-β-S, the range of desensitization was between 2 and 4 log units with a mean of 2.6 log units. When nine cells were injected with 20 m*M* GDP (three of the nine also received 0.001% Triton X-100), they showed no loss in sensitivity. The amount of desensitization with GDP-β-S is similar to that observed previously (*3*). Records 1 and 2 of (B) are responses to a 20-msec flash of log intensity −5.6 obtained before injection of GDP-β-S. After injection, records 3, 4, and 5 were obtained with a flash of log intensity −2.9. The flash is indicated by the stimulus monitor (sm). The light-induced currents are plotted downward in (B) with the inward currents negative. The data in (B) are from a different cell than those in (A).

at N would cause inhibition of light-induced excitation and adaptation without any effect on the response of the cell to activation at a later stage. To test this, ventral photoreceptor cells were injected with guanosine 5′-*O*-(2-thiodiphosphate) (GDP-β-S) to inhibit N (*11*), and the light-induced current was measured under voltage clamp conditions (Fig. 1). The desensitization of the photoreceptor by GDP-β-S was indicated by the finding that it took about 400 times (2.6 log units) more light to produce the same amplitude current after injection as it did before injection (Fig. 1A). Similar results were found for 16 cells injected with GDP-β-S, whereas 9 cells injected with GDP showed no densensitization (Fig. 1). Two aspects of the desensitization induced by GDP-β-S were different from desensitization produced by light. After light adaptation, the variability of the threshold response amplitude decreases and the latencies of threshold responses decrease (*12*). In contrast, after injection of GDP-β-S, the variability of the threshold response amplitude increased (Fig. 1A) and the latencies of threshold responses increased (Fig. 1B).

If my model is correct, the desensitization produced by GDP-β-S should have no effect on the cells' response to injected $IP_3$. To test this, cells were impaled by two pipettes, one containing $IP_3$ and the other containing GDP-β-S; the response of the cell to light and $IP_3$ before and after injection of GDP-β-S was measured (Fig. 2). The light intensity was constant throughout the experiment, thus the effect of GDP-β-S was to

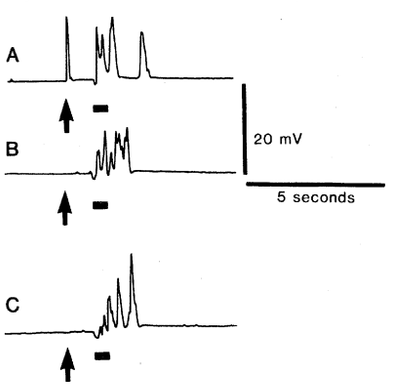


Fig. 2. Effect of GDP-β-S on the light response and the $IP_3$-induced response. The cell was impaled with two micropipettes, one containing GDP-β-S (Fig. 1) and the other 100 μ*M* $IP_3$, 100 m*M* potassium aspartate, 10 m*M* Hepes, *p*H 7.0. The cell was stimulated by a 20-msec light flash of log intensity −4.6 (arrows). The cell was also stimulated by injection of $IP_3$ (bars below the traces). (A) The response to light and $IP_3$ before injection of GDP-β-S. Between (A) and (B) the cell was injected with GDP-β-S. (B and C) The responses of the cell to the same stimuli as in (A). (B) was obtained 5 minutes and (C) 25 minutes after the injection of GDP-β-S. GDP-β-S selectively blocked the light response; the response to injected $IP_3$ was unaffected.

suppress the light response (Fig. 2). In contrast, the response to $IP_3$ was unaffected, as predicted by the model. This result was consistent with the activation of N prior to the production of $IP_3$ during photoreceptor excitation. However, this experiment did not address the question of whether activation of N is involved in adaptation of the photoreceptor.

Because inhibiting N with GDP-β-S desensitizes the response to light (Figs. 1 and 2), it is difficult to use GDP-β-S to show that activation of N is involved in adaptation of the light response. However, I could monitor the sensitivity of the cell by measuring the response to $IP_3$, which is not affected by GDP-β-S (Fig. 2), because both the $IP_3$-induced response and the light response are desensitized by light (*6, 7*). Thus, the $IP_3$ response was used to test the ability of light to adapt the cell, both before and after the injection of GDP-β-S (Fig. 3). A flash was chosen with an intensity sufficient to completely suppress the response to $IP_3$ (Fig. 3A). About 4 minutes after the adapting light, the response to $IP_3$ recovered (Fig. 3B). After the record shown in Fig. 3B, the cell was injected with GDP-β-S; this caused a reduction in the response to light and a reduction in the ability of light to suppress the $IP_3$ response (Fig. 3C). A second injection of GDP-β-S after the record in Fig. 3C resulted in almost complete suppression of the light response and in a loss of the ability of the light flash to suppress the response to $IP_3$. Thus, it would appear that GDP-β-S blocked the ability of light to adapt the cell. Because adaptation in ventral photoreceptors is mediated by a rise in intracellular calcium (*12–14*), the experiment suggests that calcium release by light was suppressed by GDP-β-S.

My experiments are consistent with a model in which information flows from rhodopsin to N to phospholipase C during light-induced excitation and adaptation of ventral photoreceptors. This cascade may only be part of the complete transduction pathway because calcium buffers block both $IP_3$-induced excitation and adaptation (*15*), but only block light-induced adaptation and not light-induced excitation (*13*). The implications for visual excitation of these findings with calcium buffers have been discussed (*16*). The model predicts that ventral photoreceptors and, by analogy, other microvillar photoreceptors should exhibit light-activated, guanosine triphosphate–dependent production of $IP_3$. The light-induced changes in $IP_3$ should be large enough and occur rapidly enough to participate in excitation and adaptation of the photoreceptor.

Different components of the model have been demonstrated in a variety of microvillar photoreceptors, although there is no photoreceptor for which the entire biochemical pathway has been shown to exist. For example, light-activated guanosine triphosphatase activity, which is exhibited by N, is present in membranes from the eyes of cephalopods (*17*) and the fly *Musca* (*18*). Also, a light-induced increase in $InsP_3$ has been observed in the ventral eye of *Limulus* and the squid eye (*7, 19, 20*), and light causes a drop in the incorporation of $^{32}P$ into $PIP_2$, the precursor of $IP_3$, in the octopus eye (*21*) and in squid photoreceptor membranes (*22*). In addition, phosopholipase C activity is concentrated in the retinular cells from the head of *Drosophila*, but is absent from the head of the "no receptor potential" (norp A) mutant (*23*), which also

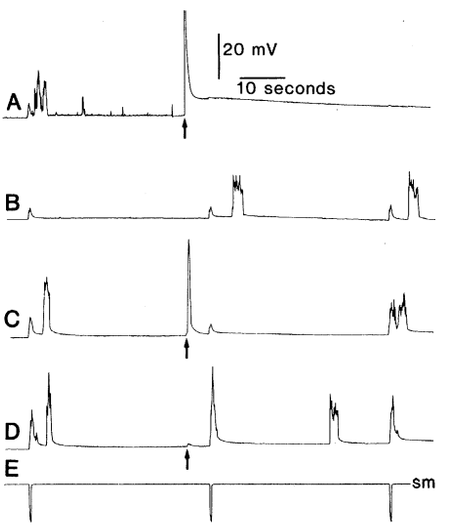


Fig. 3. Effect of GDP-β-S on the ability of light to adapt the ventral photoreceptor. The cell was impaled with two micropipettes, one containing GDP-β-S, the other $IP_3$ (Figs. 1 and 2). (A) to (D) show the responses of the cell to the $IP_3$ injections that occurred during the times shown by the stimulus monitor (sm) in (E). In (A), (C), and (D) the cell was stimulated by an adapting flash of 20-msec duration and a log intensity of −2.0 (arrows). The record in (A) shows that before injection of GDP-β-S the adapting flash caused a profound desensitization of the photoreceptor, completely suppressing the response to injected $IP_3$. It took about 4 minutes for the cell to recover its sensitivity to $IP_3$ (B). There was a 190-second interval between (A) and (B). Between (B) and (C) the cell was injected with GDP-β-S. This reduced the response to light [note that the light response in (A) went off the scale of the recorder] and also reduced the ability of light to desensitize the response to $IP_3$ (C). A second injection of GDP-β-S occurred between (C) and (D). This further reduced both the response to light and the ability of light to desensitize the response to $IP_3$ (D). After injection of GDP-β-S and after light adaptation, the amplitude and time course of the response to $IP_3$ appears to have changed (B to D). Because of the inherent variability in the amplitude and time course of the $IP_3$ response, I could not establish if this was truly an effect of GDP-β-S.

exhibits increased incorporation of $^{32}P$ into $PIP_2$ (*24*).

Invertebrates seem to use the same biochemical principles for phototransduction as vertebrates. In rods, photoexcited rhodopsin activates a cascade of chemical reactions, the first step of which is activation of a guanine nucleotide–binding protein (*10*). My model for *Limulus* suggests that the protein activated by photoexcited rhodopsin is also a guanine nucleotide–binding protein. Thus, one of the functions of rhodopsin in both vertebrates and invertebrates may be to activate guanine nucleotide–binding proteins.

My results lead me to propose the following cascade of events leading to calcium release in ventral photoreceptors. Photoisomerization of rhodopsin activates N, which stimulates phospholipase C. This activation of phospholipase C leads to the production of the intracellular messenger $IP_3$, which then causes the release of calcium from the endoplasmic reticulum. GDP-β-S inhibits both visual excitation and adaptation by blocking N, thereby disrupting the flow of information from rhodopsin to later stages of the cascade.


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27. A brief abstract describing this work has appeared previously [A. Fein, D. W. Corson, R. Payne, *Biol. Bull.* **167**, 526 (1984)]. Supported by NIH grants EY 01362 and EY 03793. I thank D. W. Corson, J. Lisman, and R. Payne for criticisms of an earlier version of this manuscript. $IP_3$ was a gift of R. F. Irvine.

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# Cytotoxicity of Human *p*I 7 Interleukin-1 for Pancreatic Islets of Langerhans


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**Activated mononuclear cells appear to be important effector cells in autoimmune beta cell destruction leading to insulin-dependent (type 1) diabetes mellitus. Conditioned medium from activated mononuclear cells (from human blood) is cytotoxic to isolated rat and human islets of Langerhans. This cytotoxic activity was eliminated from crude cytokine preparations by adsorption with immobilized, purified antibody to interleukin-1 (IL-1). The islet-inhibitory activity and the IL-1 activity (determined by its comitogenic effect on thymocytes) were recovered by acid wash. Purified natural IL-1 and recombinant IL-1 derived from the predominant *p*I 7 form of human IL-1, consistently inhibited the insulin response. The *p*I 6 and *p*I 5 forms of natural IL-1 were ineffective. Natural and recombinant IL-1 exhibited similar dose responses in their islet-inhibitory effect and their thymocyte-stimulatory activity. Concentrations of IL-1 that inhibited islet activity were in the picomolar range. Hence, monocyte-derived *p*I 7 IL-1 may contribute to islet cell damage and therefore to the development of insulin-dependent diabetes mellitus.**


INTERLEUKIN-1 (IL-1) IS A FAMILY OF peptide hormones with a wide range of biological properties, including the ability to alter immunologic, neuroendocrine, and metabolic functions (*1*). Interleukin-1 activates a broad spectrum of cell types, such as T and B lymphocytes, neutrophils, hepatocytes, muscle cells, fibroblasts, chondrocytes, osteoclasts, and hypothalamic cells. Although the primary sources of IL-1 are blood monocytes and tissue macrophages, IL-1 is also elaborated by vascular endothelium, skin keratinocytes, gingival and corneal epithelial cells, renal mesangial cells, and brain astrocytes (*2*). The IL-1 produced by any of the latter cell types, or by tissue macrophages as a result of a localized immune reaction, probably exerts its primary effects within discrete anatomical regions. There is indirect evidence that a localized autoimmune reaction participates in the development of type 1 (insulin-dependent) diabetes mellitus (IDDM) (*3*), and infiltration by mononuclear cells is the hallmark of the histologic process affecting the islets of Langerhans (*4*). Cytotoxic macrophages that destroy cultured islet cells have been found in the mouse (*5*). Supernatants of activated human blood mononuclear cells were shown to inhibit insulin secretion and islet insulin content by a direct cytotoxic effect (*6*). Screening of partially purified lymphocyte and monocyte mediator molecules (cytokines) indicated that IL-1 contributed to this effect. We now report that this inhibition of islet cell function appears to be mediated only by the *p*I 7 form of IL-1.

The effect of IL-1 and other cytokines on the function of rat islet cells in vitro was tested as previously described (*6*). The islets were isolated from collagenase-treated pancreatic tissues and cultured for 7 days before cytokines were added. We determined by radioimmunoassay how much insulin was secreted during six subsequent days of cul-


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