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Adenyl Cyclase as a Link between Photon Capture and Changes in Membrane Permeability of Frog Photoreceptors

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ABSTRACT **Tomita has shown by electrophysiological measurements that the photoreceptors of the vertebrate retina are depolarized (excited) by darkness and hyperpolarized (inhibited) by light. Excitation is accompanied by an increase, and inhibition by a decrease, in the sodium-ion permeability of the receptor cell.**

The retinal-rod outer segments of the frog contain an adenyl cyclase that is active in darkness and inactivated by light. This cyclase has a specific activity ten-times higher than the activity in previously described tissues. It is suggested that cyclic AMP is an intermediate in the light- and dark-induced changes in sodium permeability in the photoreceptor cell.

The initial active event in all known photoreceptor systems is the absorption of light by the chromophore 11-*cis* retinal (1). Photon capture in vertebrate photoreceptors leads to a decrease in sodium conductance of the receptor cell membrane and a consequent increase in membrane potential (2). The chain of events between the absorption of light and the conductance change is unknown. We report here an experiment on vertebrate photoreceptors which suggests that adenyl cyclase acts as a switch that is turned off by light and that adenosine 3′:5′-cyclic monophosphate (cAMP) mediates the observed increase in sodium conductance.

EXPERIMENTAL PROCEDURE

In a typical experiment, we adapted sixteen *Rana pipiens* to darkness overnight. The next morning, we removed the retinas in absolute darkness with the aid of an infrared image converter (sniperscope). All subsequent operations, except deliberate light adaptations, were performed either in absolute darkness or with dim-red illumination and at ice temperature. After each step in the isolation procedure, we examined the fractions using phase-contrast microscopy. Retinas were collected in ice-cold 47.6% sucrose and agitated vigorously for 1 min with a mechanical vibrator. The sample was centrifuged at 100,000 × *g* for 90 min at 4°C. The rod outer segments were driven upwards and formed a viscous paste on the top of the tube. The top layer was collected with a spatula and yielded about 200 μl of an admixture of rods and sucrose. 40 μl of double-distilled water was then added and the mixture was sonicated at 120 watts in two 3-sec bursts. Before sonication, we saw a dense suspension of rods accompanied by a small quantity of noncellular debris under the phase-contrast microscope. After addition of distilled water, the outer segments and the rod disks had swelled. After sonication, we saw neither outer segments nor disks, but only amorphous debris.

Six 15-μl aliquots of the sonicated material were pipetted into 0.5-ml culture tubes; three tubes were exposed to (room) fluorescent light until the color changed from a red purple to an opalescent yellow (less than 1 min).

All samples were assayed in complete darkness at 30°C, using a reaction volume of 25 μl and an incubation time of 10 min. Adenyl cyclase activity was measured with 1.6 mM [8-^{14}C]ATP (45 Ci/mol), 5.3 mM aminophylline, 2.9 mM $MgSO_4$, 32 mM glycylglycine, and an ATP-regenerating system (2 mM phosphocreatine and 80 μg of creatine phosphokinase). The reaction was stopped with 0.1 M EDTA and the tubes were then exposed to light. Tubes that had never been exposed to light still retained their unbleached rhodopsin; they bleached completely in the room light. The reaction tubes were centrifuged at 9,000 × *g* for 30 min; 10-μl aliquots were spotted on PEI cellulose thin layers, which were developed in a descending direction with methanol–1 M ammonium acetate 75:30 for 9 hr and with *n*-butanol–acetic acid–water 2:1:1 for 12 hr. Unlabeled marker cAMP was visualized under near-ultraviolet light; labeled cAMP was harvested from the glass plate by shaving the thin layers. The harvested material was sonicated in a toluene–2,5-diphenyloxazole cocktail and Cabosil was added to prevent settling of the dispersed PEI cellulose. The cAMP was measured (3, 4) in a Beckman CPM-200 liquid-scintillation spectrometer. All determinations were in triplicate and agreed within ± 5%.

RESULTS

We measured adenyl cyclase activity in both light- and dark-adapted rod outer segments. The specific activity of adenyl cyclase in the dark-adapted sample was about ten times higher than the activity found in the brain, heretofore the richest source of cyclase known. The light-adapted sample had about 15% of the activity in the dark-adapted sample (Table 1). Rat brain adenyl cyclase was prepared from light- and dark-adapted brains (5) and assayed by the same procedure. The enzymic activity of this preparation was independent of illumination (Table 1).

DISCUSSION

Location of adenyl cyclase

These data indicate the presence of adenyl cyclase in the rod outer segments for the following reasons: (*1*) Morphologically, our purified material contains rod outer segments as the only identifiable element. (*2*) The specific activity of our material is higher than for any other known tissues, therefore the activity can not reasonably be ascribed to contamination. (*3*) The enzymic activity of adenyl cyclase in our preparation is regulated by light. (*4*) The cyclase activity in the rod outer segments is inactivated by light. This parallels the physiologi-

TABLE 1. *Adenyl cyclase activity in light- and dark-adapted frog photoreceptors*

Preparation	Adenyl cyclase specific activity*	
	Dark adapted	Light adapted
Frog-rod outer segments	78.4	11.9
Rat brain†	8.1	8.2

The protein concentration of the rat-brain (membrane) enzyme suspension was 35 mg/ml, while the protein concentration of the rod outer segment suspension was 3 mg/ml (13).

* Adenyl cyclase activity is expressed as nmol of cAMP per mg of protein per 10 min.

† The rat-brain enzyme (washed membrane particles) was prepared as described (5).

cal data of Tomita, which demonstrate that the vertebrate photoreceptors are inhibited by illumination. It should be mentioned that although the vertebrate photoreceptors are inhibited by light, it has been known since 1938 (6) that illumination results in a rich variety of response patterns in the optic nerve. These responses result from a transformation of the initial inhibition within the retina so that the optic-nerve response reflects an interplay of excitatory and inhibitory influences. The significance of this mechanism is reviewed by Hartline (7) and Ratliff (8). Most invertebrate photoreceptors differ from those of vertebrates in that they are excited by light. We expect by analogy that invertebrate adenyl cyclases would be activated by light.

Residual adenyl-cyclase activity in the light-inactivated rod outer segments

There are three likely sources of the residual activity. *1.* Incomplete photoinactivation of rod adenyl cyclase. *2.* Mechanical unmasking of enzymic activity resulting from trauma to the constraining regulatory component of cyclase during sonication. *3.* Non-rod enzymic activity associated with contaminating neural elements. Because we found little morphological evidence of contamination and in view of the high specific activity, the third possibility seems less likely.

The organization remaining in the sonicated material is sufficient to preserve normal photoregulation, yet enough disruption has occurred to permit access of labeled substrate to catalytic sites.

Suggested mechanism

Tomita and his colleagues have shown by electrophysiological experiments that the vertebrate photoreceptors are inhibited by light, and that their conductance to sodium ion is decreased by light (2). In other words, the vertebrate photoreceptors are active in the dark and inhibited by light. We have shown that frog-rod adenyl cyclase is active in the dark and inactivated by light. Our experiments suggest that the cyclase is mediating the effect of light on sodium conductance. The morphology of the vertebrate photoreceptor is compatible with this view, since a large ATP reservoir is available in the form of a mitochondrial pool in the inner segment, immediately adjacent to the rod disks. Noell has shown, by experiments that involve iodoacetate poisoning, that photoreceptor function is coupled to ATP availability (9).

The data further suggest that the relationship between rhodopsin and rod adenyl cyclase is analogous to the switching function served by the hormone-receptor moiety coupled with adenyl cyclase in other tissues, and that cAMP is the effector that regulates sodium conductance in this, and perhaps in other, systems.

While in all other known cyclase systems the regulating hormone is a small peptide or a catecholamine that arrives at the target tissue through the circulation, in the rod outer segment, light functions as the regulatory hormone. In the vertebrate photoreceptor the regulator, light, acts to turn the system off by causing rhodopsin to assume a configuration that produces inactivation of contiguous cyclase molecules.

Leaf has previously suggested that the observed increase in active sodium transport, produced by vasopressin action on the toad bladder, is primarily associated with cAMP-induced changes in sodium conductance at the mucosal surface (10). More recently, Civan has presented additional experimental evidence for this theory based on the measurement of voltage–current relationships in toad bladder. He postulates that the vasopressin effect results from increased active sodium transport that is made possible by increased sodium permeability [opening up of parallel channels (11)]. In the vertebrate photoreceptors, Sillman, Ito, and Tomita have shown that the receptor potential (distal PIII) is unaffected at first by ouabain poisoning; that is, that the receptor potential is caused by a change in sodium permeability rather than a primary effect on active transport (12). It is this fact that links adenyl cyclase to sodium permeability. Therefore, a relation of the adenyl-cyclase system to permeability changes in all sensory receptors must be seriously considered.

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