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Regulation of cGMP-dependent conductance in cytoplasmic membrane of rod outer segments by transducin

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A preparation of the photoreceptor G-protein, transducin, containing mainly the T_α-subunit in a GTP-γ-S-bound form, has been used for perfusion of the intracellular surface of excised patches of rod outer segment cytoplasmic membrane from frog retina. The preparation has been shown to result in the complete suppression of the cGMP-activated ionic conductance of the cytoplasmic membrane patch. The effect is entirely reversible after the protein has been washed out and is not observed in the absence of cGMP. The degree of conductance inhibition depends on the protein concentration, half-maximal inhibition occurring at 1 μM T_α-GTP-γ-S.

Rod outer segment; Transducin; cyclic GMP; Membrane conductance; Patch-clamp

1. INTRODUCTION

At present cGMP-specific phosphodiesterase is widely believed to play a key role in the mechanism of phototransduction of rods in vertebrates [1-3]. The model of phototransduction is based upon the theory of a light-activated two-stage enzyme amplifier cascade regulating the cGMP concentration in rods [4]. As shown in experiments with isolated inside-out patches of rod plasma membrane, cGMP is capable of activating the cationic conductance of the membrane [5-7]. Thus, in this model cGMP serves as a transmitter.

However, thus far, there is no direct evidence of light-induced changes in cGMP concentration in the intact photoreceptor cell and available data are rather conjectural [8-13]. Analysis of other possible schemes of phototransduction not involving hydrolysis of cyclic nucleotide thus appears to be reasonable. We have previously discussed one such scheme postulating a role of cGMP sorption-desorption in the phototransduction mechanism [14], however, this is not the only possibility.

Experimental data suggesting that regulation of the conductance of ionic channels may occur as a result of their interaction with G-proteins have been reported [15]. According to some indirect evidence, transducin oligotrimer appears to dissociate into subunits and T_α-GTP is likely to be released into the aqueous space of the cell after activation by photoexcited rhodopsin and exchange of GDP for GTP [4,16-19]. The number of transducin molecules activated by one photoexcited rhodopsin molecule ranges from 500 to 7000, as shown by various authors [13,20,21]. If one supposes that T_α-GTP interacts with cGMP-activated channels (Davis and co-workers [13] seem to be the first to assume that photoreceptor channels might be the targets of activated transducin) and causes suppression of the ionic current through them, there is no longer a need for modulation of the cGMP level in photoreceptors to explain the light-dependent decrease in conductance of cGMP-activated channels.

For these reasons, we were prompted to under-

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Abbreviations: ROS, rod outer segment; T_α, $T_{\beta\gamma}$, α- and $\beta\gamma$-subunits of transducin, respectively; GTP-γ-S, guanosine 5'-*O*-[γ-thio]triphosphate

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take a study of the action of transducin on the conductance of ROS cytoplasmic membranes. We show here that a preparation of transducin obtained in GTP-γ-S bound form suppresses the conductance of cGMP-activated channels in patches of rod plasma membranes.

2. MATERIALS AND METHODS

Rod outer segments were isolated from frog retina (*Rana temporaria*) by means of a step sucrose gradient as in [22]. Soluble ROS proteins were washed three times with buffer A (10 mM Hepes, pH 7.4, 100 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride). The membranes were then washed twice with buffer B (10 mM Hepes, pH 7.4, 110 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride) with 20 μM GTP-γ-S. In this procedure, the α-subunit of transducin was the major species eluted. The concentration of T_α was calculated on the basis of protein level and T_α content of preparations, the latter being determined after SDS electrophoresis of transducin preparations and after scanning of stained gels. The protein solution was concentrated in a Centriflo CF 25 (Amicon) up to 1.5 mg protein/ml. Protein concentration was determined according to Bradford [23] using bovine serum albumin as a standard. SDS gel electrophoresis of proteins was carried out according to Laemmli [24] using 10% gels. The cGMP-activated conductance of excised ROS cytoplasmic membrane patches was recorded via the patch-clamp technique as in [25]. All experiments were carried out with 8-Br-cGMP, a cGMP analog which is more resistant to hydrolysis by phosphodiesterase.

Hepes, GTP-γ-S and 8-Br-cGMP were obtained from Boehringer, other chemicals being of the highest grade from Sigma.

3. RESULTS AND DISCUSSION

Transducin isolated from frog ROS (see section 2) contains a nonhydrolysable GTP analog, GTP-γ-S, firmly bound to the α-subunit [17]. Electrophoretic analysis showed the preparation to contain 75–80% T_α and 20–25% $T_{\beta\gamma}$ (fig.1). Impurities due to other proteins were not observed when the gels were stained with Coomassie.

Fig.2 shows the protocol followed in one of the experiments where the action of a transducin preparation on the cGMP-dependent conductance of excised cytoplasmic membrane patches was studied. The experiments were carried out at a Br-cGMP concentration leading to half-maximal increase in conductance. Transducin is observed to cause the complete and rapid suppression of BrcGMP-induced conductance. The effect is entirely reversible after washing out protein from the

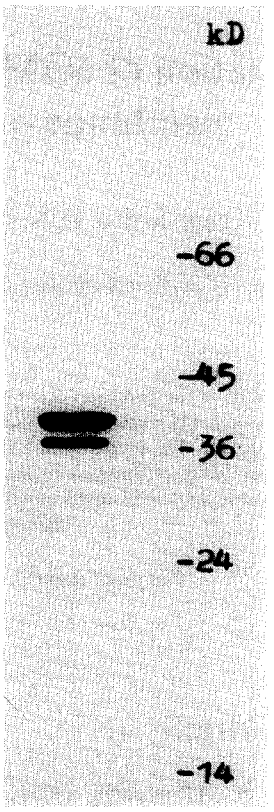

Fig.1. SDS electrophoresis of the preparation of transducin from frog ROS enriched in α-subunit used here. Coomassie G-250 staining. Gel scanning shows 75–80% T_α and 20–25% $T_{\beta\gamma}$ to be present in the preparation. Admixtures of other proteins are not revealed.

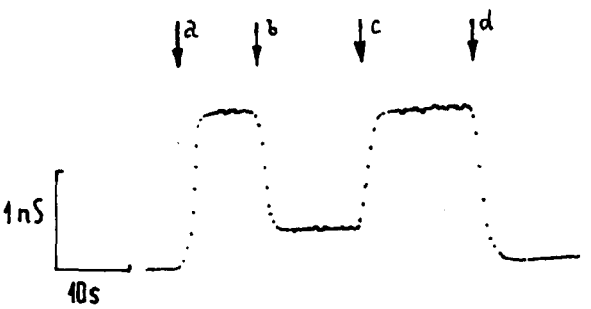

Fig.2. Effect of transducin-GTP-γ-S on Br-cGMP-induced conductance. Points at which replacement of perfusion solutions was carried out are indicated by arrows: a, buffer B replaced by 5 μM BrcGMP; b, 5 μM BrcGMP + 1 μM T_α – GTP-γ-S + 5 μM GTP-γ-S; c, 5 μM BrcGMP; d, buffer B. All solutions prepared using buffer B. Micropipette was filled with the same buffer.

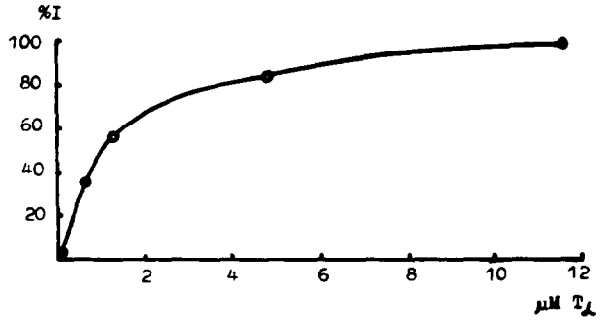

Fig.3. Dependence of inhibition of 8-Br-cGMP-induced conductance of an excised patch on concentration of T_α-GTP-γ-S. Degree of inhibition (I) determined using: $I = (A_{max} - A_T)/A_{max}$, where A_{max} and A_T denote the cGMP-induced conductance of an excised patch in the absence and presence of transducin, respectively.

chamber with BrcGMP. In control experiments it was found that 5 μM GTP-γ-S did not change the conductance and that transducin had no effect on the conductance of the excised patch in the absence of BrcGMP.

The degree of conductance suppression depends on transducin concentration (fig.3). Half-maximal inhibition of the cGMP-induced conductance takes place (with different transducin preparations and various cytoplasmic membrane patches) at 1–3 μM T_α. As the preparation contains an admixture of the $\beta\gamma$-subunits of transducin, it is still unclear as to which of the oligomer subunits is inhibitory.

The data of Davis et al. [13] suggest that the bleaching of one rhodopsin molecule in *Bufo marinus* rod activates about 7000 transducin molecules per s. Using the same object, Baylor et al. have shown [26] the half-maximal electrical response to be observed upon absorption of 73 $h\nu/\mu^2 \cdot$ per s. According to these data, one may readily estimate the transducin concentration in rods on generation of half-maximal amplitude of the photoresponse to be 1 μM. This value is in good agreement with the T_α concentration obtained in our experiments and causing half-maximal inhibition of cGMP-dependent conductance. Such coincidence allows one to assume that in rods the regulation of cGMP-dependent conductance of cytoplasmic membranes by light may occur directly via an activated α-subunit of transducin. Thus, it is not expected that the cationic conductance of cytoplasmic membranes is determined by opposite effects of the two mediators: T_α and cGMP.

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