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Time-dependent cGMP-activated conductance of detached patches of ROS plasma membrane

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cGMP markedly increases the cationic conductance of the 'inside-out' patches of rod outer segment plasma membrane when applied to the inner side. The cGMP-activated conductance of some patches was shown to be time-dependent. The data obtained suggest that the change of cGMP concentration in the near membrane layer underlies this phenomenon rather than the change in the channel's activity. The hydrolysis and, probably, the desorption of the nucleotide are responsible for this.

cGMP-activated conductance; Phosphodiesterase; cGMP-binding site

1. INTRODUCTION

At present the evidence that cyclic guanosine monophosphate (cGMP) is involved in visual transduction (review [1]) is increasing. If cGMP is a messenger indeed, the problem of excitation of photoreceptor cells reduces itself to the question of how the photon absorption leads to the changes in nucleotide concentration. The study of properties of the system 'excised patch of rod outer segment (ROS) plasma membrane and the volume surrounded by it' may allow the elucidation of this question. We have found that the cGMP hydrolysis and its sorption-desorption may take place within the volume mentioned. The membrane patch being a sensor of the nucleotide concentration follows these processes detecting the changes in cGMP-activated conductance.

2. MATERIALS AND METHODS

The experiments were performed on the photoreceptor cells of frog (*Rana temporaria*) isolated mechanically [2]. The currents were recorded by 'patch clamp' method in the 'inside-out' configuration [3]. To measure the conductance of the system 'electrode-membrane patch', the electrode was polarized by 10 mV impulses (fig.1A). The amplitude of impulses on the current-voltage converter output was proportional to the conductance of the above system. The following solutions were used (mM): 100 NaCl; 10 KCl; 1 $MgCl_2$; 0.1 $CaCl_2$; 5 Hepes, pH 7.5. All the experiments were carried out in light and at 20–25°C.

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3. RESULTS AND DISCUSSION

The ROS plasma membrane contains cation channels activated by cGMP [4]. The application of cGMP acting from the cytoplasmic side of the ROS membrane patch increases its conductance which remains constant for at least several seconds. However, if the cGMP-induced conductance is recorded within several minutes its time-dependence is observable. In some cases after perfusion has ceased, the cGMP-induced conductance decreases with a time constant of 50–150 s reaching a lower level (fig.1C). Sometimes, the conductance decrease was followed by a return to

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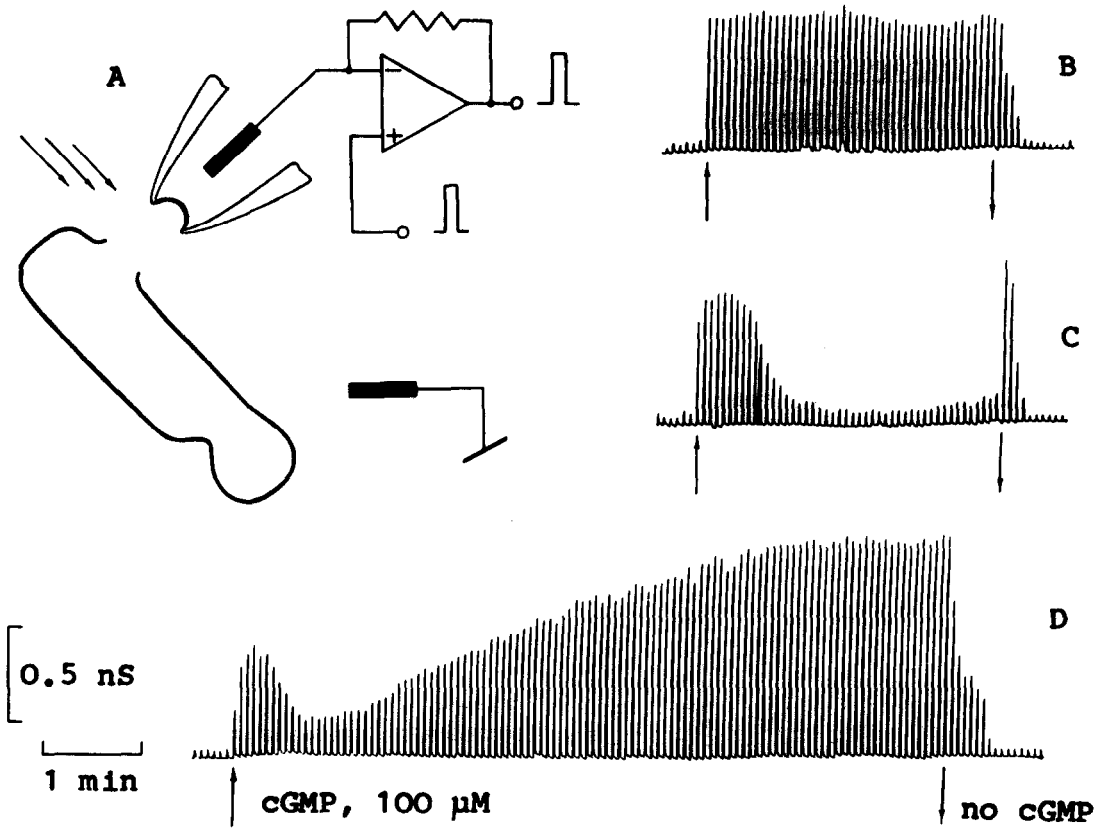

Fig.1. (A) Diagram of the system used to measure conductance. Three types of response of isolated ROS membrane patches to the cGMP application; (B) the cGMP-activated conductance is constant during the application; (C) inactivation; (D) reactivation. Recording on three different patches.

the initial level or even to a higher one (fig.1D). We term these phenomena 'inactivation' and 'reactivation', respectively.

3.1. *Inactivation is conditioned by cGMP hydrolysis*

8-Br-cGMP, a slowly hydrolyzable cGMP analog, increases the conductance of the ROS membrane patch with an efficiency that is even greater than that of cGMP. The inactivation of the 8-Br-cGMP-induced conductance is usually not observed (fig.2B). Therefore, the inactivation of the cGMP-induced conductance seems to be caused by nucleotide hydrolysis rather than by inactivation of cGMP-activated channels. This speculation is supported by the following arguments. (i) Inactivation can be prevented by application of phosphodiesterase (PDE) inhibitor, isobutylmethylxanthine (IBMX) to perfusate (fig.2C). (ii) In the presence of GTP (200 μM) or of protamine sulfate (1 mg/ml) cGMP loses its ability to increase the patch conductance. After application of IBMX it can be recorded again (fig.2D). It is noteworthy that in vitro PDE in retinal rods is activated in the presence of GTP as well as protamine sulfate [5].

In our opinion, the data presented suggest that PDE does function in the volume δV surrounded by the ROS membrane patch (fig.2). The cGMP hydrolysis by PDE underlies the decrease (inactivation) of cGMP-induced conductance.

3.2. *PDE-activity change*

The cGMP concentration in δV (fig.2A) attains

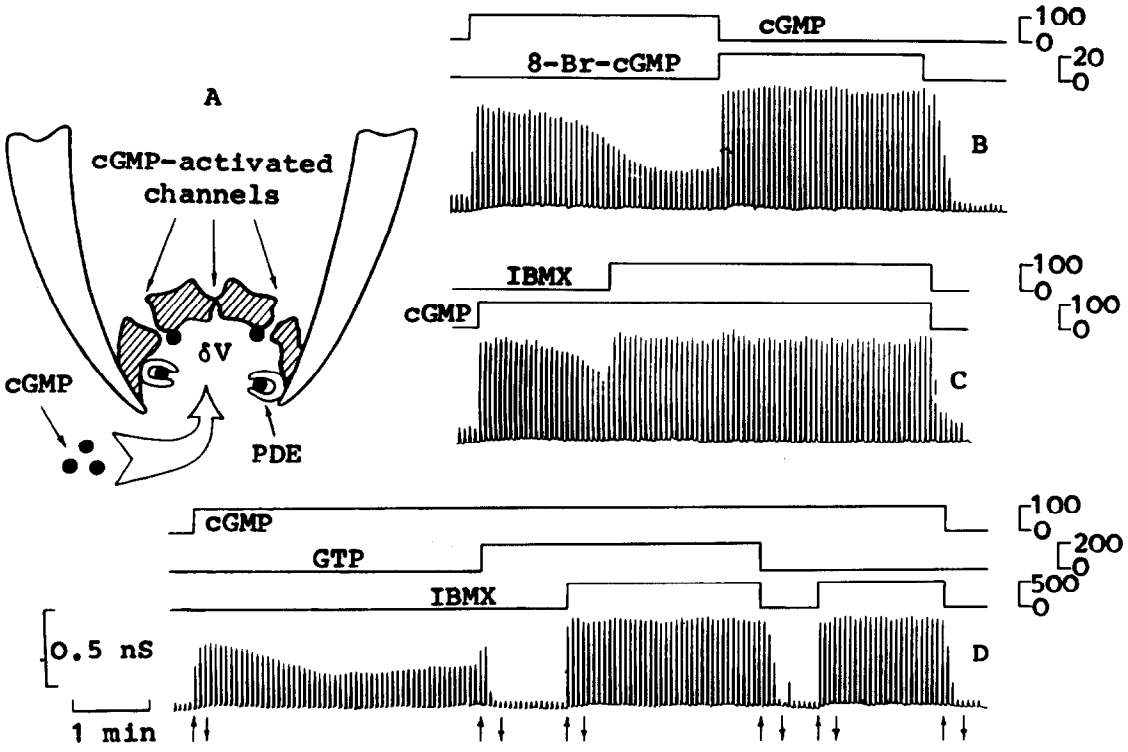

Fig.2. The effect of different drugs (μM) on inactivation of the cGMP-induced conductance. The initial and terminal moments of perfusion are marked with ↑ and ↓, respectively.

its stationary level when the cGMP hydrolysis is balanced by diffusion. Hydrolysis decreases the number of cGMP molecules in the near membrane layer without changing the time characteristic for molecule diffusion from perfusate to the membrane. cGMP diffuses within several milliseconds with a diffusion coefficient $D = 10^{-5}$ cm$^2 \cdot$s^{-1} and at patch dimensions of approx. 1 μm. Thus, at a constant hydrolysis rate and after a sudden change of the cGMP content in the perfusate, the cGMP concentration in the near membrane layer should reach its stationary level within several milliseconds.

The time typical for inactivation is 10^2 s (fig.1C). This value is about four orders of magnitude greater than that for diffusion of cGMP via δV. Therefore, it is impossible to relate the time of inactivation with the kinetics of establishing the equilibrium between diffusion and cGMP hydrolysis. Such a relation is possible on the assumption that the nucleotide hydrolysis rate slowly increases in time. The cGMP concentration in the near membrane layer will decrease following the same kinetics. At present the causes of changes in PDE-activity are unknown. Probably, the activity of PDE is controlled allosterically by cGMP itself [6].

3.3. *Is reactivation conditioned by the cGMP desorption with cGMP-binding sites?*

In some experiments the decrease of the cGMP-induced conductance was followed by its return to a higher level (reactivation, fig.1D) which was usually not observed for the 8-Br-cGMP-induced conductance. Apparently, the changes of conductance are determined by the changes of cGMP concentration in the near membrane layer but not by alteration in the activity of cGMP-activated channels.

This phenomenon may involve the changes in PDE-activity. However, one more possibility should be pointed to. In some cases the change in

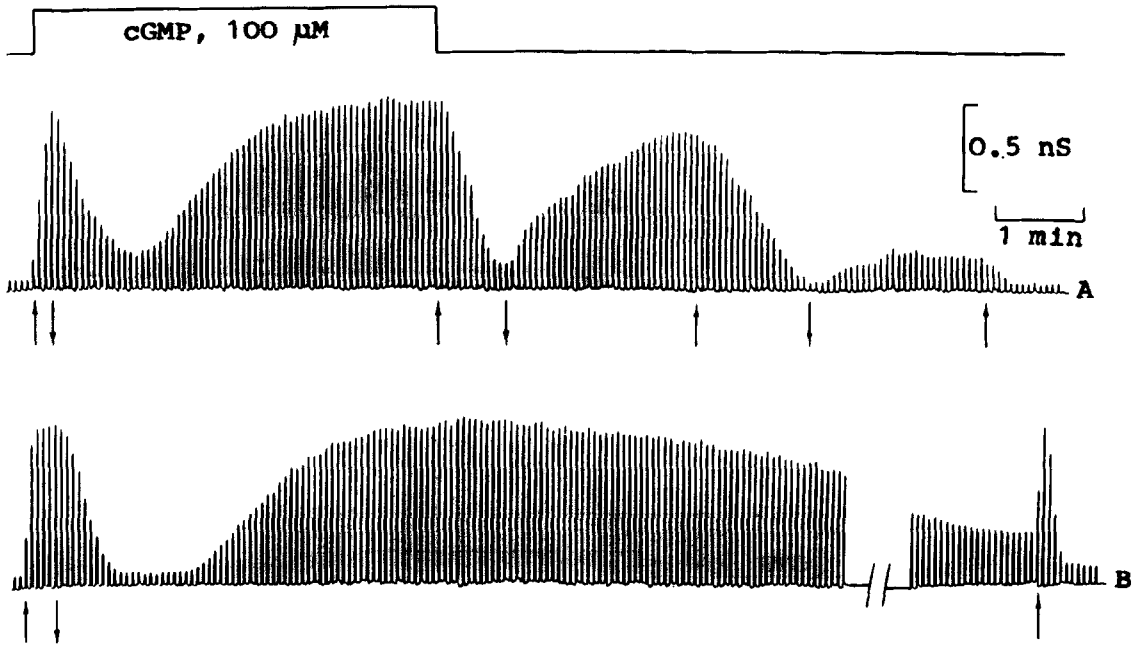

Fig.3. The cGMP desorption of the cGMP-binding sites. The initial and terminal moments of perfusion are marked with ↑ and ↓, respectively.

cGMP-induced conductance did not coincide with the kinetics of lowering the cGMP concentration in the perfusate when reactivation was observed. When the solution containing cGMP was replaced by that without cGMP, a sharp decrease of conductance of the system 'electrode-membrane patch' was observed. After the perfusion had ceased, the conductance started to increase. (Most likely the perfusion increases the rate of exchange between perfusate and the volume surrounded by membrane patch.) This recurred for several times until the conductance reached the level of leakage conductance (fig.3A). The excessive conductance, compared to the leakage one, possessed the properties of the cGMP-induced conductance. (For instance, it was inhibited by 1-*cis*-diltiazem blocking the cGMP-induced conductance [7].) This indicates that the cGMP concentration in the near membrane layer was different from zero. The phenomena observed are easy to explain if the sorption-desorption of cGMP occurs in the volume δV (fig.2A). After application of cGMP the nucleotide is sorbed by cGMP-binding sites. After complete removal of the nucleotide from the perfusate it is released from cGMP-binding sites. So the cGMP concentration in the near membrane layer becomes different from zero. This idea is supported by the following facts: (i) the cGMP concentration in ROS is several dozens of micromoles [8,9], the bulk of the nucleotide is probably bound [10,11]; (ii) the cGMP-binding sites of different types are revealed in ROS [12–15]. Proceeding from the above we conclude that the volume surrounded by membrane patch contains cGMP-binding sites.

In our experiments reactivation of cGMP-induced conductance was always accompanied by cGMP release from cGMP-binding sites (fig.3A). So, it is highly probable that desorption of the nucleotide from cGMP-binding sites is responsible for reactivation. If it is just the case, then after a period of time cGMP-binding sites will deplete and after reactivation the conductance will decrease (fig.3B). The subsequent investigations are assumed to allow the elucidation of possible mechanisms underlying the reactivation of cGMP-induced conductance. Of particular interest is the fact that GTP produces irreversible activation of PDE

(fig.2D). At present GTP is believed to act not directly but via the GTP-bound form of transducin [16]. This implies that the system 'excised patch and volume surrounded by membrane patch' should contain rhodopsin, transducin and, probably, other elements of transduction. If so, using the isolated ROS plasma membrane patches as a cGMP sensor it should be possible to follow the processes that may occur in excited photoreceptor cells.

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