Inactivation of cGMP-dependent conductance of rod outer segment plasma membrane induced bij cGMP

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Integral cGMP-dependent currents as well as activity of single cGMP-activated channels in plasma membrane of rod outer segment (ROS) were recorded using the patch-clamp method. The dependence of integral currents on cGMP concentration is shown to be bell-shaped. Decrease in cGMP-dependent conductance at high cGMP concentration results from a decrease in channel opening probability. Thus, cGMP in high concentrations inactivates cGMP-dependent conductance.

cGMP-dependent channel; Rod outer segment; Channel operation kinetics

1. INTRODUCTION

Recently cationic channels activated by cyclic nucleotides were found in plasma membranes of photoreceptor and olfactory cells [1–3]. Their properties allow one to classify these channels as a new species of ionic channels. The data presented in this paper provide a deeper insight into interaction between an agonist and ionic channel.

2. MATERIALS AND METHODS

The experiments were carried out with the rods from *Rana temporaria* and *Xenopus laevis*. In the present study we used cGMP, 8BrcGMP, Hepes from Boehringer (Austria) and EDTA, EGTA from Serva (FRG). The solutions were of the following composition (mM): solution A – NaCl 100, $MgCl_2$ 2, $CaCl_2$ 0.1, Hepes 10, pH 7.5; solution B – NaCl 100, EDTA 0.3, EGTA 0.3, Hepes 10, pH 7.5.

The methods used to provide gigaseals and those for measuring the electrical parameters of the patches excised from the rod plasma membranes were described earlier [1,4,5]. The integral cGMP-dependent currents were studied using excised patches of ROS plasma membranes bathed with solution A from both sides. Single cGMP-dependent channels were recorded using the patches from RIS membranes [6], in this case solution B was applied.

3. RESULTS

3.1. *Dependence of conductance of excised ROS membrane patches on 8BrcGMP concentration*

While studying the concentrational dependences we have observed in a number of experiments that high cGMP concentrations (>10 mM) cause inhibition of cGMP-dependent conductance. It should be noted that in the experiments with high cGMP concentrations membrane patches were often unstable. In order to obviate nonspecific effects of high nucleotide concentrations on the membrane, we used 8BrcGMP as an agonist which is approximately 10 times more effective than cGMP [7]. Concentrations of 8BrcGMP were usually within 0–2 mM.

We observed 3 types of concentrational dependences of 8BrcGMP-induced conductances of ROS membrane patches (10 experiments in all). In 3 cases we observed a monotonous dependence (fig.1A, curve 1); in the other 3 cases a bell-shaped dependence with a slight decrease in conductance at high nucleotide concentration was found (curve 2); and in the remaining 4 experiments we registered complete inhibition of membrane patch conductance at millimolar concentrations of 8BrcGMP (curve 3). It should be noted that just those membrane patches where the strongest inhibition was observed at high nucleotide level were most sensitive to 8BrcGMP at low concentrations. Such a correlation allows us to suggest that apparently monotonous concentrational dependence observed within the range of 0–2 mM actually was of a bell-shaped character if one broadens the range of concentration. We failed to test this hypothesis experimentally since high concentrations of BrcGMP caused damage
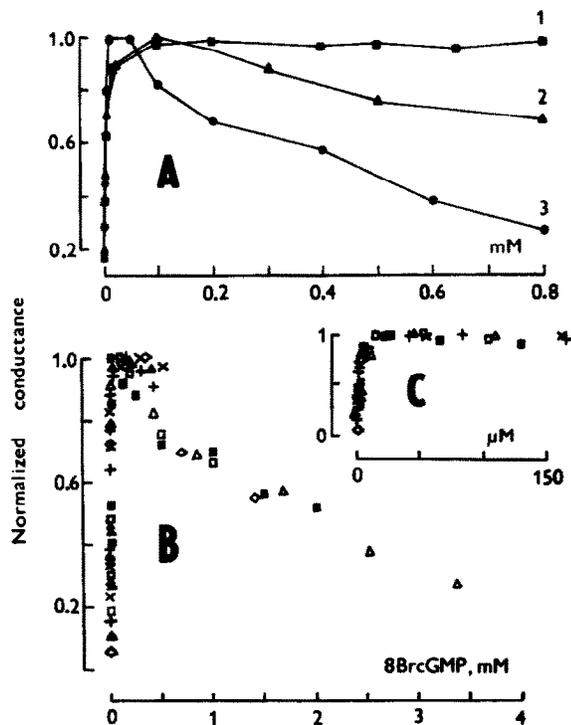
Fig.1. (A) Three types of dependence of ROS membrane patches conductance on 8BrcGMP concentration. (B and C) Matching of 6 different concentrational dependences after scaling concentrational axis.

Fig.3. Fluctuations of the current through single cGMP-dependent channel at various 8BrcGMP concentrations, range of milliseconds. Membrane voltage is −70 mV.

3.2. Activity of single cGMP-dependent channels

Under physiological conditions the unitary conductance of single cGMP-dependent channel is of about 0.1 pS [1]. Upon removal of divalent cations in 0.1 NaCl the conductance increases up to 25 pS and the single channel currents tend to be well resolved [8,9]. The density of these channels in RIS is much lower than in ROS, so the probability of recording the activity of a single cGMP-dependent channel is rather high [6]. That is why the experiments described below were carried out on plasma membrane patches excised from the rod inner segment.

Fig.2 shows an example of single channel activity at different 8BrcGMP concentrations. It is seen that the channel activity enhances with increasing agonist concentration, but above 400 μM 8BrcGMP induces complete inhibition. Dose-dependent disappearance of cGMP-dependent current fluctuations is not incidental since they become restored while decreasing agonist concentrations and are eliminated again when agonist concentrations exceed 400 μM. Visually the suppression of channel activity is manifested as the appearance of silent intervals the duration of which equals a few seconds but rises with increasing 8BrcGMP concentration.

In accordance with the concentrational dependences shown in fig.1, we observed 3 types of behaviour of single cGMP-dependent channels upon variations of 8BrcGMP concentration. Besides the experiments (3 patches) described above, we also observed slightly pronounced inhibition of channel activity at high agonist concentrations (1 patch). In this case the open state probability (P_o) was less than that at moderate 8BrcGMP concentrations; however, even at 2 mM the channel remained activatable. In two patches P_o rose monotonously with the increase of 8BrcGMP concentration.

of membrane patches. However, this idea can be indirectly supported by the fact that within low nucleotide concentration ranges all the dependences can be matched by appropriate scaling of the concentrational axes (fig.1B,C).

A bell-shaped concentrational dependence can be due to several reasons. At high agonist concentrations the unitary conductance of the cGMP-dependent channel as well as the number of activatable channels or open state probability may decrease. To find the appropriate answer direct registration of single cGMP-dependent channels is of indispensable value.
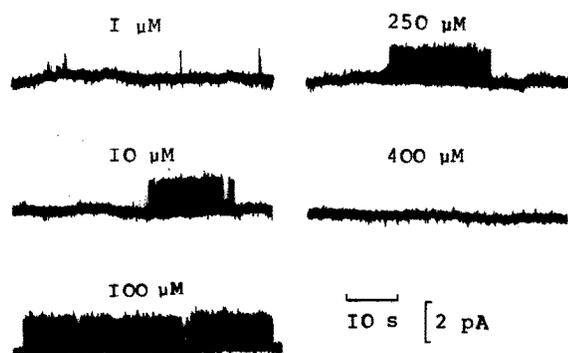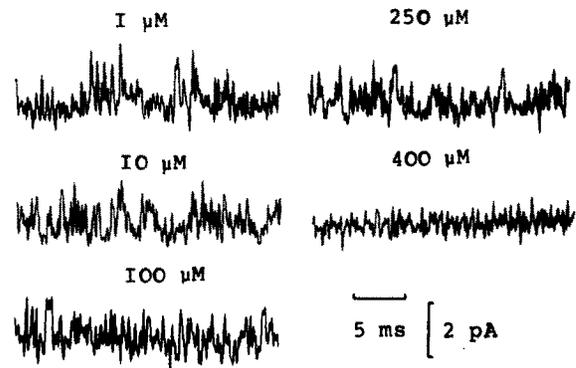

Fig.2. Current fluctuations through single cGMP-dependent channel at various 8BrcGMP concentrations, range of seconds. Membrane voltage is −70 mV.

4. DISCUSSION

Thus, in a number of experiments inhibition of ac-

tivity of cGMP-dependent channels in frog rod plasma membrane is observed at high agonist concentrations. This is manifested by the appearance of prolonged (a few seconds in duration) silent intervals in channel activity. At the same time the probability of single channel open state decreases considerably.

It should be noted that the effects described here are specific to cGMP since GMP did not produce any inhibitory effect on cGMP-dependent conductance in control experiments.

There seem to exist several ways to explain the effects observed. (i) The samples of the nucleotides used in our experiments could contain contaminants of certain heavy metals the presence of which is extremely difficult to control. However, we observed inhibition of cGMP-dependent channel activity in EDTA and EGTA buffers; this fact makes this supposition hardly probable. (ii) The samples of cGMP could also contain some amounts of other nucleotides. In control experiments we added other nucleotides to cGMP samples in concentrations of their probable contamination (1–5% of cGMP content). No essential effects were observed: in any case they produced no inhibitory effect on cGMP-dependent conductance. (iii) The inhibitory effect of cGMP-dependent conductance is produced by the agonist itself. We think that this explanation is the most reasonable for the results described, the more so, as similar behaviour at high agonist concentrations was observed for acetylcholine receptor [10,11]. The difference is that inhibition of acetylcholine receptor was never complete.

Inhibition of acetylcholine receptor was explained by blockage of an open channel by the agonist molecule at high ligand concentrations [12,13]. The hypothesis of the open channel blockage is supported by: (i) the dependence of channel conductance on acetylcholine concentration; (ii) appearance of flickering at high agonist concentrations; (iii) specific alterations in channel kinetics [12–14].

The question arises whether an agonist blocks cGMP-dependent channels. To answer this question we need more experimentation and thorough analysis of the data, especially on time intervals. The results of the present study do not show any pronounced changes in the open-close kinetics within bursts at variations of 8BrcGMP concentration (fig.3).

In conclusion it should be noted that the data described here have no physiological implications since the total cGMP concentration in ROS is equal to about 100 μM [15] and the level of free cGMP is even less. Nevertheless, these findings should be taken into account while interpreting the data obtained in the experiments with cGMP microinjection into visual cells, when local nucleotide concentration may be very high.

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