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Cyclic nucleotide-activated channels in the frog olfactory receptor plasma membrane

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Patch clamp technique was used to record cyclic nucleotide-dependent current of the frog olfactory receptor cell plasma membrane. Data obtained indicate that the channels passing this current are permeable to Ca^{2+} or Mg^{2+} and moderately selective for monovalent cations according to the sequence Li^+, Na^+, $K^+ > Rb^+ > Cs^+$ and are effectively blocked by l-*cis*-diltiazem and 3',4'-dichlorobenzamil. The conductance of single cyclic nucleotide-gated channels in solutions with low Ca^{2+} and Mg^{2+} content is about 19 pS. The results demonstrate that cyclic nucleotide-activated channels of olfactory receptor cells are virtually identical to photoreceptor ones.

Olfactory receptor cell; Cyclic nucleotide-activated channel; Single channel recording

1. INTRODUCTION

Direct gating of conductance by cyclic nucleotides has been observed in patches excised from PR and OR plasma membrane [1,2]. CN-conductance of rod outer segments was studied in detail [3–11] whereas that of OR had not been completely characterized. The present work is dealing with some particular properties of the OR CN-conductance which have not been described yet.

2. MATERIALS AND METHODS

Conventional patch clamp technique was used to obtain gigaseal excised patches and to measure their electrical characteristics [12]. The experiments were performed using the frog OR (*Rana ridibunda*). Olfactory epithelium was dissociated as described in [13]; single OR cells were identified according to their characteristic morphology [14]. In this study we used: cGMP, 8BrcGMP, Hepes from Boehringer (Austria), cAMP, ATP, GTP from Reanal (Hungary) and EDTA, EGTA from Serva (FRG); salines of the following composition (mM): 100 NaCl, 1 $MgCl_2$, 0.1 $CaCl_2$, 10 Hepes, pH 7.5 (solution A); 100 NaCl, 0.5 EGTA, 0.5 EDTA (solution B); 50 $MeCl_2$, 50 glucose, 10 Hepes, pH 7.5 (solution C) where Me^{2+} is Ca^{2+}, Mg^{2+} or Ba^{2+}.

In our experiments CN-conductance was low when both sides of excised patches were bathed with solution A, while in low Ca^{2+},Mg^{2+} solution CN-conductance was essentially higher. Therefore to increase CN-conductance (i.e. to ensure experimental accuracy) patch pipettes were filled with solution B. Experimental chamber was filled with solution A prior to gigaseal formation.

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Abbreviations: cAMP, cyclic adenosine-3',5'-monophosphate; cGMP, cyclic guanosine-3',5'-monophosphate; 8BrcGMP, cyclic 8-bromoguanosine-3',5'-monophosphate; ATP, adenosine-5'-triphosphate; GTP, guanosine-5'-triphosphate; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(2-aminoethylether)-*N,N,N'*-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; DCPA, 3',4'-dichlorobenzamil; OR, olfactory receptor cell; PR, photoreceptor cell; CN-channels (conductance), cyclic nucleotide-activated channels (conductance); low Ca^{2+},Mg^{2+} solution, solution containing 10^{-7} M Ca^{2+} and Mg^{2+} or less

3. RESULTS

We succeeded in obtaining the gigaseal inside-out patches from the cilia, dendrite or cell body regions and in recording CN-conductance. These observations agree with the data by Nakamura and Gold [2]. The highest rate of success was achieved in the experiments with cell bodies; therefore the results presented were mainly obtained on cell body patches.

3.1. *Single CN-channels*

The OR CN-conductance was blocked by Ca^{2+} and Mg^{2+} ions as is seen in PR patches [2]. Removal of divalent cations permitted recording of the single CN-channels in OR excised patches. Responses of the inside-out patches to cGMP or cAMP in low Ca^{2+},Mg^{2+} solutions are shown in Fig. 1A. Patch current fluctuations in the presence of cyclic nucleotides differ from those recorded in their absence (Fig. 1B–D) and obviously manifest a single channel activity behaviour. The latter provided the possibility to estimate the value of single channel conductance being equal to 19 ± 5 pS.

3.2. *The selectivity of CN-conductance*

The selectivity of conductance described was characterized according to the shift of cGMP-

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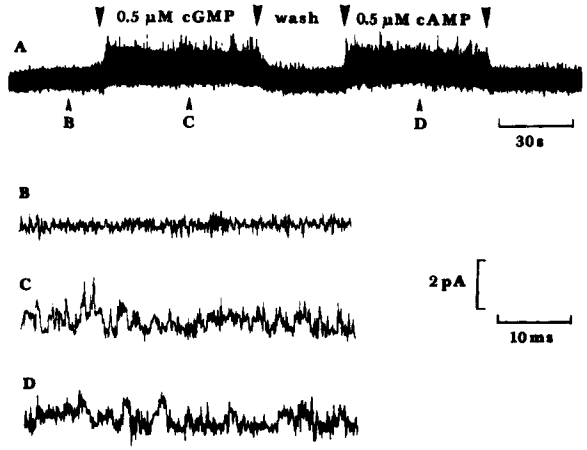

Fig. 1. (A) Perfusion of the inside-out patches from OR cell body with cGMP or cAMP containing solutions induces single channel events. (B–D) High-resolution recordings of patch current fluctuations in the absence (B) and in the presence (C, D) of cyclic nucleotides; band width is 0–2000 Hz; patch membrane potential is −50 mV. Both sides of membrane fragment were bathed with solution B.

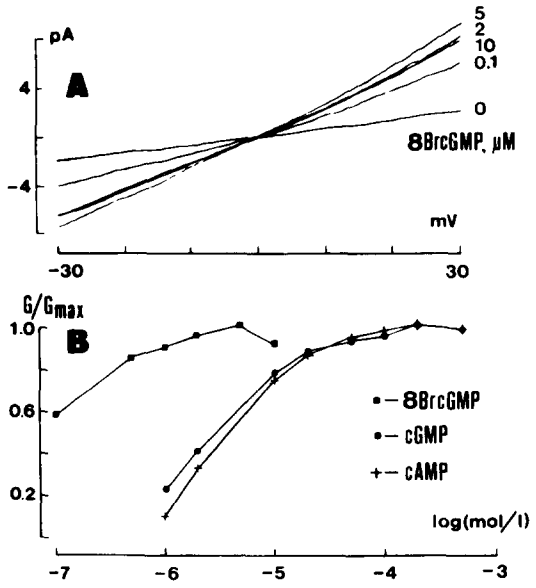

Fig. 2. (A) I–V curves of an excised patch measured in the presence of different concentrations of 8BrcGMP; both sides of membrane fragment were bathed with solution B. (B) Normalized conductance plotted against agonist concentration.

dependent current reversal potential induced by equimolar substitution of NaCl in solution B bathing the intracellular side of excised patches by other alkaline or divalent metal chlorides. While using divalent cations inside-out patches were perfused with solution C.

Two patches, in which the conductance was sufficiently high and stable to provide appropriate accuracy of measurements yielded the following permeability sequences:

$$Li^+ \geqslant K^+ \geqslant Na^+ > Rb^+ > Cs^+$$

and

$$K^+ \geqslant Na^+ \geqslant Li^+ > Rb^+ > Cs^+$$

Substitution of NaCl by KCl or LiCl shifted the reversal potential by about 1–3 mV which is close to accuracy limit of our measurements (± 1 mV). This might explain the discrepancy in the sequences obtained. In any case, OR CN-channels are certainly more permeable to Li^+, K^+, Na^+, than to Rb^+, Cs^+. For divalent metals the following sequence was obtained:

$$Ca^{2+} > Na^+ > Mg^{2+} > Ba^{2+}$$

3.3. *The dependence of CN-conductance on agonist concentration*

The conductance of OR excised patches varied with agonist concentration in a dose-dependent manner (Fig. 2A). The ligand specificity of the conductance described shown in Fig. 2B, where typical normalized patch CN-conductance is plotted against 8BrcGMP, cGMP or cAMP concentrations. Similarly to PR CN-conductance [15] the OR conductance exhibits higher affinity to 8BrcGMP than to cGMP per se; in contrast to PR [1,8] cAMP in OR patches is approximately as effective as cGMP, which was already noted by Nakamura and Gold [2]. Data obtained allowed us to estimate EC_{50} values for 8BrcGMP, cGMP and cAMP in the moderate concentration range being equal to

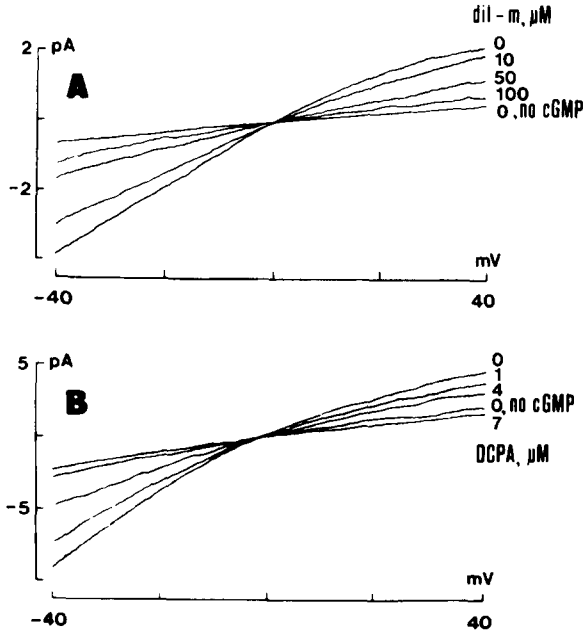

Fig. 3. (A and B) I–V curves of an excised patch measured in the presence of different concentrations of 1-*cis*-diltiazem (A) or DCPA (B). Intra- and extracellular sides of membrane fragment were bathed with solutions A and B, respectively.

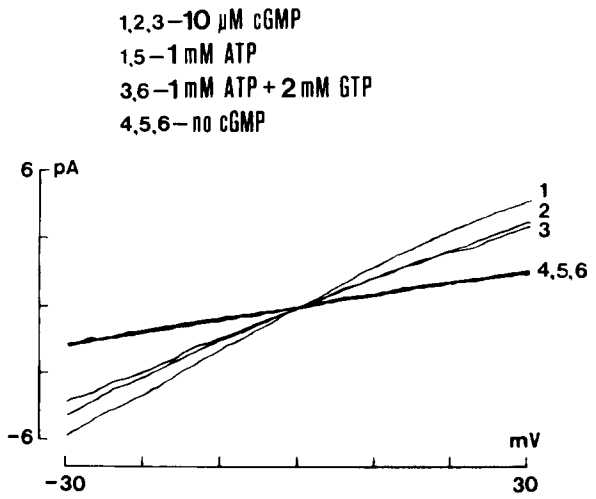

Fig. 4. Effects of ATP and GTP on I–V curves of an excised patch. Intra- and extracellular sides of membrane fragment were bathed with solution A and B, respectively.

0.8 μM, 2.1 μM and 3.4 μM, respectively. It should be noted that the CN-conductance was inhibited at high agonist concentrations (Fig. 2) similarly to what was observed in PR patches [11].

3.4. *Effects of blockers*

Derivative of amiloride, DCPA [16], and 1-*cis*-diltiasem [17] are highly effective blockers of PR CN-conductance. We have examined these drugs and found that they block CN-conductance of OR as well. Typical effects of diltiasem and DCPA on the conductance in patches are presented in Fig. 3A and B. Based on these data, we estimated EC_{50} values for diltiasem and DCPA as 47 μM and 3.7 μM, respectively.

3.5. *The effects of ATP and GTP on CN-conductance*

Recently ATP and GTP were demonstrated to be modulators of PR CN-conductance [9]. In our experiments OR CN-conductance was also found to depend on the presence of nucleoside triphosphates in the solutions bathing the intracellular side of patches. In the absence of cyclic nucleotides the changes in the patch conductance were negligible when ATP and/or GTP were applied (Fig. 4, curves 4, 5, 6). ATP (0.1–1 mM) increased CN-conductance (Fig. 4, curves 1, 2) whereas several millimoles of GTP inhibited the ATP effect (Fig. 4, curve 3). Thus, in the experiments described ATP-GTP antagonism was observed, just as in the case of PR [9].

4. DISCUSSION

Our data indicate that properties of cyclic nucleotide-activated channels in OR and PR patches are very similar and first of all in respect to their single channel conductance and selectivity, sensitivity to DCPA or diltiasem, responsiveness to ATP and GTP, Ca^{2+} and Mg^{2+}-induced blockage. Affinity to various agonists of CN-conductance in OR differs appreciably from that in PR. Nevertheless, both kinds of channels may be considered as virtually identical.

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