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Direct action of cGMP on the conductance of retinal rod plasma membrane

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In order to identify the intracellular transmitter in the phototransduction process in the retinal rod, the action of cGMP, 2′,3′cGMP, cAMP, GMP and Ca^{2+} on the isolated inside-out patches of the plasma membrane of retinal rods of the frog (*Rana temporaria*) was studied. cGMP applied at the intracellular membrane surface markedly increased the conductance of patches. The action of cGMP took place in the absence of nucleoside triphosphates and, hence, was not mediated by protein phosphorylation. The dependence of cGMP-induced component of conductance on cGMP concentration was S-shaped, with half-saturation within 10–30 μM and a Hill coefficient of about 1.7–1.8. cAMP, 2′,3′cGMP, GMP (1 mM) did not exhibit any action on the membrane. Ca^{2+} did not affect the patch conductance in the absence of cGMP. In the presence of cGMP, lowering Ca^{2+} concentration from 10^{-3} to 10^{-8} M decreased the cGMP-dependent component of conductance by 20–30%. The approximate value of the elementary event underlying the cGMP-induced conductance estimated from the magnitude of the variance of the cGMP-induced current is within 100–250 fS. We suppose that the cGMP-activated channels found by us provide the light-sensitive conductance of the rod plasma membrane in vivo and that cGMP is the intracellular transmitter acting in the phototransduction process.

Introduction

It has been shown that the coupling between the processes of light absorption by a rhodopsin molecule and the change of plasma membrane conductance is to be performed by means of an intracellular transmitter [1]. There are two current views on the nature of the transmitter. According to the first one, Ca^{2+} is released into the cytoplasm when light is absorbed by rhodopsin and diffused to plasma membrane, where they block the light-dependent ionic channels [2]. In another scheme, cGMP keeps these channels open and the light activates a phosphodiesterase that reduces cGMP concentration (see review [3]). Both views are supported by indirect experimental data. Changes in the intracellular concentration of Ca^{2+} produced by various methods lead to changes in membrane current and voltage, consistent with the Ca^{2+} model of excitation [4–10]. When the intracellular concentration of cGMP is increased by extracellular application or intracellular microinjection of cGMP or phosphodiesterase inhibitors, the membrane potential becomes less negative and light-sensitive current across the rod membrane increases [10–16].

cGMP and Ca^{2+} might be interrelated messengers. Cohen et al. [17] and Polans et al. [18] showed that the lowering of extracellular Ca^{2+} concentration elevated the level of cGMP in the rod. George and Hagins [19] reported that cGMP activated Ca^{2+}-transport into rod outer segment disks. Such interrelations present principle difficulties in revealing the phototransduction mechanism, as in the intact rod the change in concentration of one hypothetical messenger immediately

affects the concentration of the other.

We believed that progress in the investigation of mechanisms of regulation of rod cellular membrane conductance might be achieved taking advantage of the patch voltage clamp technique in a modification allowing the investigation of 'inside-out' membrane patches [20,21], since inthis case one can control the composition of the solution bathing the intracellular membrane surface. In the present work this approach was used to study the action of possible intracellular messengers, Ca^{2+} and cyclic nucleotides, on the conductance of the plasma membrane of rod outer segment.

Some results of this work have been published earlier in a preliminary form [22].

Methods

The techniques for preparation of trypsin-treated cells, isolation of patches and electric measurements were as presented earlier [22,23]. Briefly, a frog (*Rana temporaria*) retina was treated with trypsin (5–10 mg/ml, 20–30 min, 20°C) in solution A (see Table I). After treatment, the retina was placed in a small volume of the same saline and gently shaken and the solution containing detached rod outer segments and whole rods was pipetted into a perfusion chamber. The present work was performed with inside-out fragments obtained by a normal method [21]. Only those patches containing no high-conductivity anion channels described by us earlier [23] were used. The trypsin treatment was carried out in the dark. All other procedures were performed under normal laboratory lighting. Thus, the photoreceptors before patch isolating were in the light-adapted state. The isolation of membrane patches and electrical measurements were performed under a light microscope.

In some experiments we used retinas not treated by proteolytic enzymes. In this case, the yield of morphologically intact photoreceptor cells was very low. Therefore, only isolated rod outer segment were used in these experiments. Generally, when working with trypsin-treated cells, we did not observe significant differences between whole rods and isolated rod outer segments, although rods were characterized, as a rule, by higher values of the cgMP-induced effect.

The composition of solution is given in Table I. If not otherwise stated, the microelectrodes were filled with solution A and all the drugs were applied in 'intracellular' solution B bathing the intracellular side of the membrane. The electrical potential at the extracellular membrane surface was always taken for zero.

Results

cGMP increases the conductance of the system microelectrode – a patch of the rod outer segment plasma membranes

Using the photoreceptor cells from trypsin-treated retinae we succeeded in obtaining 169 GΩ-sealed patches of the rod outer segment plasma membrane sufficiently stable for the solution in the chamber to be changed several times. Of these, for 92 patches, application of cGMP at the intracellular membrane surface produced an increase of the conductance of the system 'elec-

TABLE I

COMPOSITIONS OF SOLUTIONS USED (mM)

pH 7.5. In some trials, solutions with high (1 mM) or low (10 nM) Ca^{2+} concentration were used. Low Ca^{2+} concentration was obtained by adding EGTA up to 0.384 mM, according to Ref. 24.

Solution	NaCl	KCl	LiCl	RbCl	CsCl	Sodium phosphate	$MgCl_2$	$CaCl_2$	Glucose
A	90	10	0	0	0	10	2	0.1	0
B	0	100	0	0	0	10	2	0.1	0
C	0	10	90	0	0	10	2	0.1	0
D	0	10	0	90	0	10	2	0.1	0
E	0	10	0	0	90	10	2	0.1	0
F	45	5	0	0	0	5	2	0.1	110

trode-patch' and, if the solutions at both membrane surfaces were not identical, a shift of the baseline, which, in principle, could be determined not only by the increase in conductance, but also by the change in its ionic selectivity. The record presented in Fig. 1 illustrates these effects. Either in the absence of cGMP or in its presence the current fluctuated in a random manner without jumps, which could correspond to single-channel activity (Fig. 2). Therefore the conductive properties of the system were determined from their integral voltage-current relationships.

The action of cGMP was completely reversible (see Fig. 1). The sensitivity of patches from isolated rod outer segment as well as those from rod outer segment of whole cells to cGMP remained unchanged for 10–30 min (it was sufficient for 5–15 solution exchanges) and then started to decrease. Each experiment was preceded and followed by the controls of basal conductance and of sensitivity to cGMP. For this purpose the current-voltage relations in solution A without cGMP and at [cGMP] of 100 μM were recorded. About a half of cGMP-sensitive patches obtained from trypsinized cells showed complete stability and reversibility of the cGMP effect for 10–15 min and could be used for quantitative experiments.

cGMP really increases the membrane conductance, but not the seal conductance

The data presented above do not permit any confident conclusion that cGMP really increases the membrane conductance. From these data it follows that the cytoplasmic surface of the rod outer segment cellular membrane contains cGMP-binding sites. One might imagine that the binding of cGMP by these sites changes their conformation in such a way that eventually it causes the change in the mechanical properties of the whole membrane patch that might result in the change of conductance of the seal between the electrode and membrane. To exclude this possibility we have compared the ionic selectivities of seal conductance and of the cGMP-dependent one.

For this purpose, the values of the reversal potential for the leakage current (measured in the absence of cGMP) and for the cGMP-induced component of the current have been compared. Such an approach enabled us to compare the data obtained from different patches showing different conductances.

The examples of voltage-current relations are shown in Fig. 3. It is revealed that both the cGMP-induced and seal conductances were preferentially cationic ones, because, when the NaCl gradient was doubled, the reversal potentials were positive (Fig. 3a). The values of reversal potential for the cGMP-dependent component of current did not depend on the absolute size of the cGMP-induced conductance (Fig. 4) being equal to 16.5 $\pm$ 0.3 mV (mean $\pm$ S.D., eight determinations). It indicates the practically perfect selectivity of the cGMP-dependent conductance for Na^+ in these conditions. On contrary, the reversal potentials for

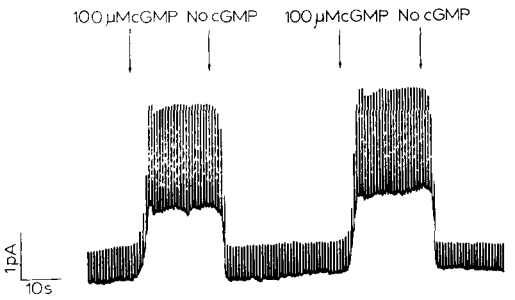

Fig. 1. Chart recording of the action of cGMP on an isolated patch of the rod outer segment plasma membrane. The command voltage comprised 10 mV 300 ms pulses. The trace represents the current flowing through the patch. cGMP was applied to the bathing solution B.

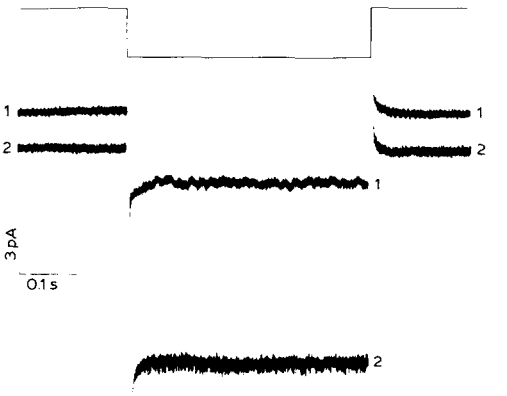

Fig. 2. The responses of the patch current to a 30 mV hyperpolarizing voltage pulse. Curve 1, no cGMP; curve 2, 100 μM cGMP.

the leakage current and, hence, the selectivity of the leakage conductance substantially depended on its absolute value (Fig. 4). This result reveals the different natures of cGMP-induced and leakage conductances.

Additional evidence in favour of this conclusion arose from the study of selectivity of these conductances for different cations. On substitution of NaCl in the solution bathing the intracellular side of the membrane by KCl, LiCl, RbCl or CsCl (Table I, solutions B–E), the values of reversal potentials for the cGMP-induced current did not depend on the magnitude of this current and were equal to 2.7 ± 0.7, 2.2 ± 2.1, 5 ± 1 and 6.0 ± 2.2 mV (mean ± S.D., 8, 8, 4 and 4 determinations, respectively, see the voltage-current relation in Figs. 3b–d). These results indicate that the cGMP-controlled conductivity units are mod-

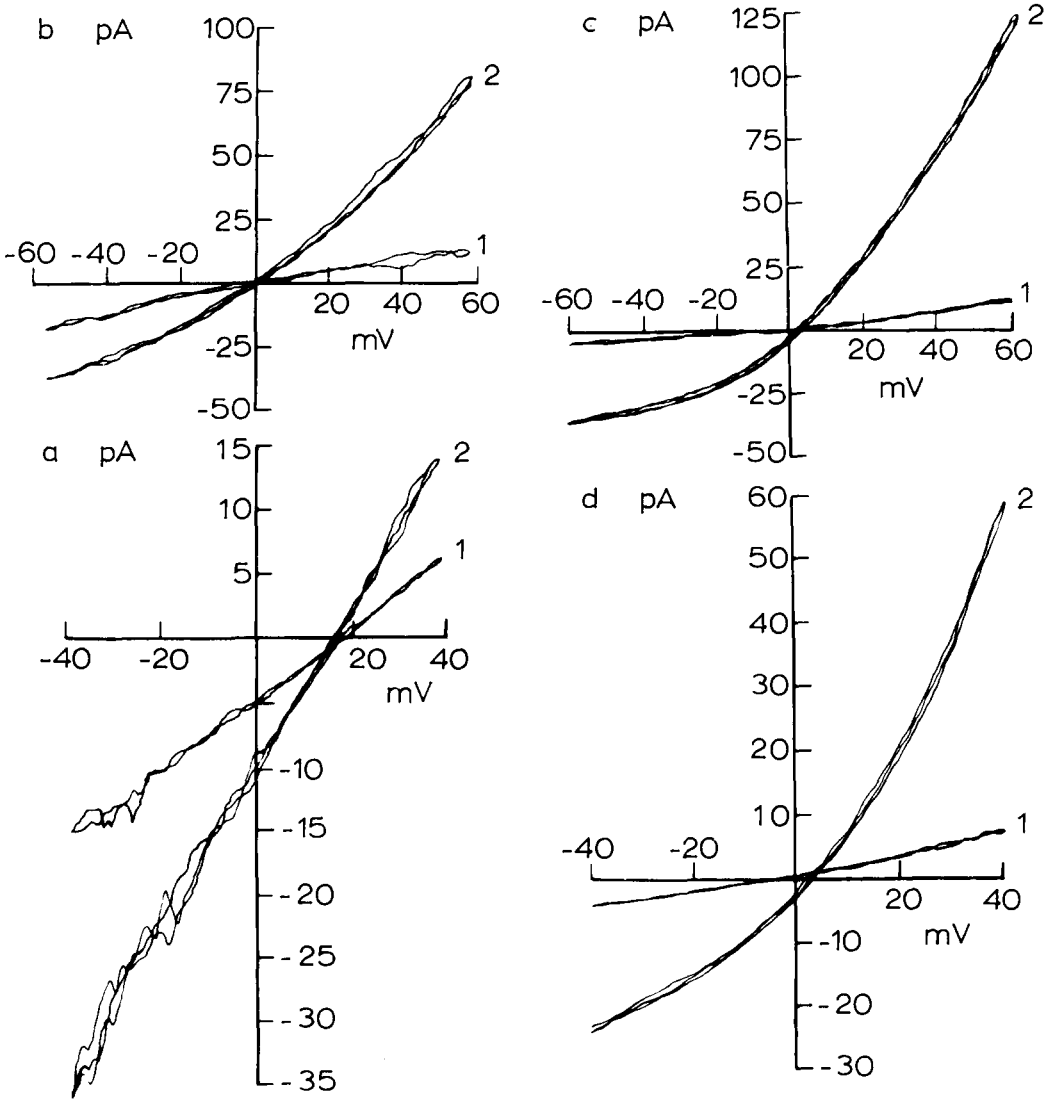

Fig. 3. Voltage-current relations of patches of the rod outer segment plasma membrane. The relations were recorded using several cycles of a triangle voltage (+ 5 mV·s^{-1}). The relations presented in parts b, c were obtained with one and the same patch, the others with different patches. The curves designated 1 and 2 represent the relations obtained correspondingly without and with cGMP (100 μM), added in the solution bathing the intracellular surface of patches. Composition of solutions: (a) solution F; (b) solution A; (c) solution B; (d) solution E.

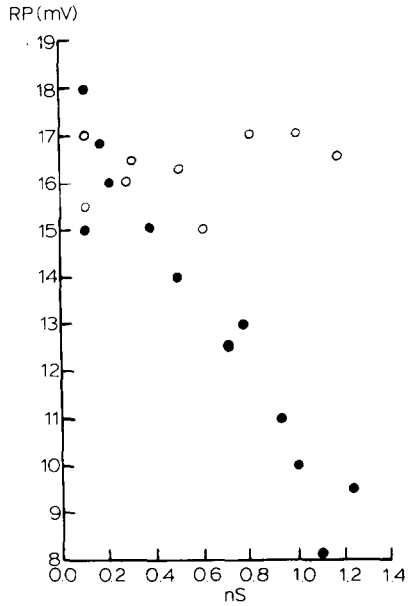

Fig. 4. The dependence of values of reversal potential for leakage current (●) and cGMP-induced component of the current (○) measured when the NaCl gradient was doubled (the intracellular patch surface was bathed with solution F) on the magnitude of conductance of the system electrode-patch. All the points represent the results of measurements performed with different patches.

erately selective for monovalent cations according to the sequence $Na^+ > Li^+$, $K^+ > Rb^+$, $Cs^+ \gg Cl^-$.

The reversal potentials of the leakage current in solutions B–E depended on the absolute value of the conductance. The higher the leakage conductance, the lower its selectivity. The experiments carried out when the seal resistance was above 1 GΩ have shown the reversal potentials in solutions B–E to be -3.6 ± 2.2, 0 ± 1.4; -4.9 ± 2.0 and -5 ± 2.0 mV, respectively (mean ± S.D., 12, 8, 6 and 6 determinations). These results correspond to the sequence Rb, Cs, K > Li, Na. In all the trials, the reversal potentials in solutions B, D, E were negative. Hence, the electrode-membrane contact was always less permeable to Na^+ ions than to Rb^+, Cs^+, K^+.

Thus, the leakage conductance and that induced by cGMP differ substantially in ion selectivity. Hence, cGMP really increases the conductance of the membrane but not of the micropipette-membrane contact.

The induction by cGMP of cationic conductance in patches of plasma membrane of rod outer segment is not an artifact induced by trypsin treatment

The treatment of cells by proteolytic enzymes is usually used in order to isolate single cells and to obtain gigaohm contacts between the cell surface and the electrode. However such a treatment might, in principle, change the properties of cellular membrane. Therefore, attempts to obtain gigaohm contacts with cells not treated with trypsin and to study the action of cGMP on the conductance of inside-out membrane patches if such cells have been undertaken.

Over the course of more than 200 trials, we obtained 41 rod outer segment membrane patches with seal resistances greater than 1 GΩ. 21 of these responded to cGMP application by a reversible increase in conductance and the cGMP-induced component of conductance in experiments on one and the same patch revealed cationic selectivity according to the sequence $Na^+ > K^+ > Rb^+$, Cs^+.

The membranes of nontrypsinized cells formed much less stable contacts with the measuring micropipette than those of treated ones. In addition, the cGMP-dependent conductance in this case degraded more rapidly in comparison with the case of treated cells. Of 21 patches from untreated cells which responded to cGMP, only one showed stability of leakage conductance and of the size of

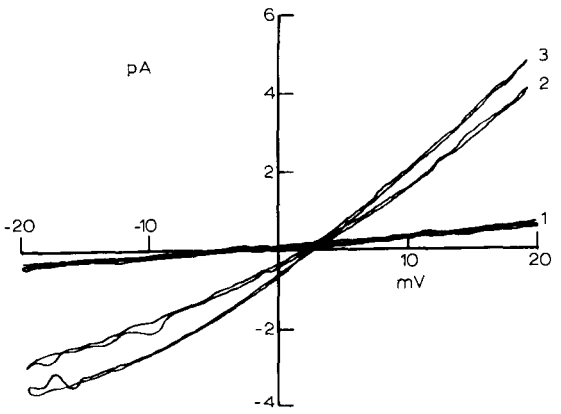

Fig. 5. Effect of cGMP and Ca^{2+} on a patch of the rod outer segment plasma membrane (the patch was excised from a non-trypsinized cell). Curve 1, 10 nM or 0.1 mM or 1 mM Ca^{2+}, no cGMP; curve 2, 10 nM Ca^{2+}, 100 μM cGMP; curve 3, 1 mM Ca^{2+}, 100 μM cGMP.

the cGMP-induced effect sufficient for quantitative measurements. The voltage-current relations obtained from this patch are presented in Fig. 5.

Thus the experiments with untreated cells could give us mainly qualitative information. In any case it may be concluded that the cGMP-activated conductance is not an artifact produced by enzymatic damage of the membrane.

The action of cGMP on the conductance of plasma membrane of rod outer segment is not mediated by protein phosphorylation

At present, any action of cyclic nucleotide is considered to be mediated by activation of protein kinases phosphorylating protein targets (see, for example, a review, Ref. 25). Phosphorylation is impossible in the absence of nucleoside triphosphates as donors of the phosphate group. In our experiments, the cGMP-induced increase of conductance took place in the absence of nucleoside triphosphates. The addition of 1 mM ATP + 1 mM GTP did not modify the effect of cGMP. Analysis of cGMP preparation purity by thin-layer chromatography has shown the possible admixture of nucleoside triphosphates not to exceed $1:10^6$. Thus, their concentration in 100 μM cGMP solution could not exceed 10^{-10} M. This is not enough for the protein kinase functioning. The effect of cGMP could not also be associated with the presence of endogenic nucleoside triphosphates. If it were, then the phenomenon of conductance increase would be observed only on the first application of cyclic nucleotide, but not on subsequent ones, when triphosphates would be washed out as a result of the solution exchange (the completeness of the solution exchange in the perfusion chamber could be easily controlled by the restoration of basal conductance when the cGMP-containing solution was washed out). At the same time, the reaction towards cGMP could be observed in some experiments up to 10–15 times.

Thus we are dealing with a direct action of cGMP on the cellular membrane, not mediated by protein phosphorylation.

Calcium ions do not markedly change the conductance of rod outer segment plasma membrane

We have tested the action on the patches of rod outer segment membrane of solutions with Ca^{2+} concentrations from 10^{-9} to 10^{-3} M. The results of a typical experiment are shown in Fig. 5. Ca^{2+} in the absence of cGMP did not change the conductance of the patches to any practical extent. The cGMP-induced component of conductance was relatively decreased when the Ca^{2+} concentration was lowered, this effect being completely reversible. No differences were revealed in the sensitivity to Ca^{2+} of trypsinized and non-trypsinized membranes.

The dependence of conductance of patches of rod outer segment plasma membrane on cGMP concentration

As voltage-current relationships for the cGMP-dependent component of current show some rectification, the conductance was calculated by the magnitude of the cGMP-dependent current flowing across the patches at a holding voltage equal to 20 mV.

Since the cGMP-induced current was always completely saturated at 300 μM cGMP, the corresponding value of the conductance was taken as unity and the conductance at lower cGMP concentrations was expressed in relative units. This allowed us to compare the data obtained with different patches.

Fig. 6a shows the dependence of cGMP-induced conductance on cGMP concentration for seven patches. This dependence is characterized by a non-hyberbolic shape and a marked scatter of experimental points. Fig. 6b shows the same data on Hill's coordinates. The straight line in the figure has a slope corresponding to a Hill coefficient of 1.8. It is seen from Fig. 6b that the points for every patch can be satisfactorily fitted by a straight line corresponding to a Hill coefficient of 1.7–1.8 and that the uncertainty of results for different patches considerably exceeds that for one and the same patch. Therefore it may be concluded that the cause of scattering of experimental data is the irreproducibility of experimental conditions for different patches. Judging by the results presented, the dependence of the conductance of patches of the rod outer segment plasma membrane upon cGMP concentration reaches half-saturation within 10–30 μM cGMP. This dependence is S-shaped and can be characterized by a Hill coefficient of 1.7–1.8.

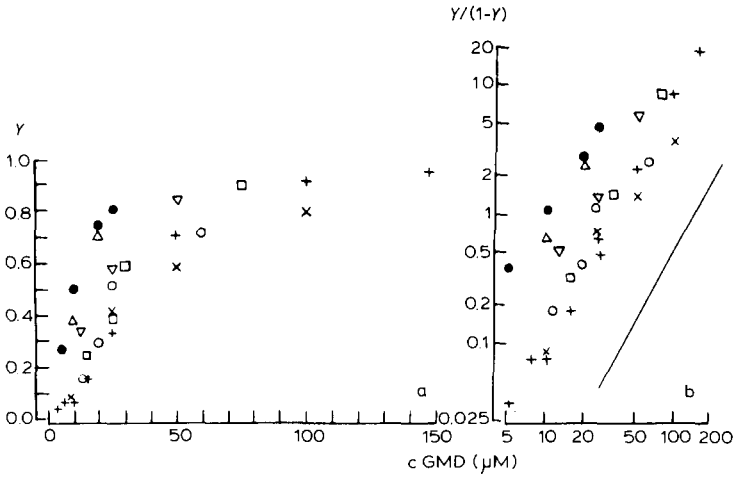

Fig. 6. (a) Dependence of cGMP-induced component of patch conductance, expressed in the normalized form (Y) on cGMP concentration. The cGMP-induced conductance at 300 μM cGMP is taken as unity. The data points represent single measurements on seven different patches. +, 10 nM Ca^{2+}, solution A; ×, ○, □, ●, 0.1 mM Ca^{2+}, solution A; ▽, △, 0.1 mM Ca^{2+}, solution B. (b) The same dependence in Hill coordinates (log $Y/(1-Y)$, log[cGMP]). The slope of the straight line corresponds to a Hill coefficient of 1.8.

A high specifity of cGMP action

cAMP, 2',3'cGMP and GMP (1 mM) did not affect the membrane conductance and did not compete with cGMP (50 μM). Thus, cGMP is the specific inductor of cationic conductance of the rod outer segment plasma membrane.

cGMP acts only on membranes of rod outer segment

We succeeded in obtaining 10 GΩ-sealed inside-out patches of the rod inner segment membrane. In no cases did cGMP elevate the conductance of these patches.

cGMP acts only at the cytoplasmic membrane surface

A set of experiments was performed with cGMP (100 μM) in a measuring electrode. Under these conditions the conductance of patch always revealed the ion selectivity characteristic for the leakage conductance as soon as the application of cGMP at the intracellular membrane surface produced the conductance increase, its ion selectivity being typical for that of the cGMP-induced conductance described above. Hence, cGMP acts only at the intracellular membrane surface.

TABLE II

NOISE-TO-CURRENT RATIOS FOR cGMP-DEPENDENT CURRENT

The holding potential is equal to −30 mV.

Patch	$\Delta\sigma^2$ $(pA)^2$	ΔI (pA)	$\Delta\sigma^2/\Delta I$ (fA)	g (fS)
1	1.4	204	6.9	230
2	0.035	10.5	3.3	110
3	0.030	10.0	3.0	100
4	0.70	168	4.2	140
5	0.34	75	4.5	150
Mean ± S.D.				146 ± 22

On the size of the elementary event underlying the cGMP-induced conductance

As was mentioned above, we failed to recognize current steps which could correspond to single-channel opening-closing events. A usual approach in such a situation is to estimate the size of a single event by the value of the variance of the current (see, for example, a review by Stevens [26]). If we assume that the channel under study possessed only two states, the open and the closed ones, then the conductance may be calculated from the equation

$$g = \frac{\Delta\sigma^2}{\Delta I V(1-p)}$$

where ΔI, $\Delta\sigma^2$, V and p stand for the change in the mean current across a patch on cGMP application, the change in variance of membrane current, the membrane potential and the probability of finding the channel in the open state, respectively.

If p is unknown, the expression $\Delta\sigma^2/\Delta i \cdot V$ is as a rule used as an evaluation of the conductance.

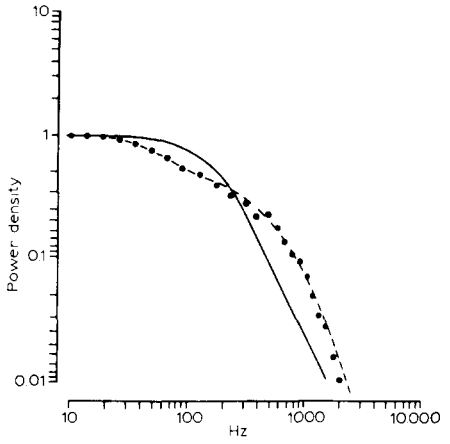

Fig. 7. Points, normalized power density spectrum for cGMP-dependent component of current, 50 μM cGMP, the holding voltage is equal to -30 mV. Dotted curve, the approximation of the spectrum by two Lorentzians with half-power frequencies of 46 and 420 Hz and contributions of 0.64 and 0.36, correspondingly; continuous curve, the best least-squares-approximation with a single Lorentzian. The bandwidth of the recording arrangement was 0–4.4 kHz. For computer treatment the data were sampled at 100-μs intervals.

Such estimations for the cGMP-activated channels for the holding potential of -30 mV at saturating cGMP concentration (50–200 μM) showed that the elementary event underlying the cGMP-sensitive conductance is within 100–250 fS (see Table II).

Statistical properties of the cGMP-dependent current

As the cGMP-dependent current did not reveal any inactivation during a long period (minutes and more), it was possible to deduce its steady-state power density spectrum. Such a spectrum is presented in Fig. 7. The spectrum could be approximated by two Lorentzians (the representation by a single Lorentzian suggested by us in a preliminary communication [22] seems to be an oversimplification) with half-power frequencies of 46 and 420 Hz, the contributions being of 0.64 and 0.36, respectively.

Discussion

Our experiments have shown approximately half of patches of the rod outer segment plasma membrane to be sensitive to cGMP. The existence of insensitive patches may be accounted for by several factors. Firstly, we have found that the cGMP-dependent conductance units degrade over the course of experiments. The insensitive patches may be the ones with the cGMP-dependent conductance already degraded. Secondly, cGMP-modulated units might be not uniformly distributed over the rod outer segment surface. Thirdly, at least some of them are vesicles (the exact yield of these was not determined).

The question arises whether the cGMP-dependent conductance studied represents the light-sensitive one in vivo. To answer this question the properties of the light-dependent conductance of intact rods and the ones of cGMP-induced conductance of isolated patches should be compared.

On the amounts of the cGMP-dependent and light-dependent conductancies in rod membrane and concentration of free cGMP in rod cytoplasm

The typical magnitude of the cGMP-induced conductance at saturating cGMP concentration is 0.5–1 nS for a patch sucked in an electrode of 1 μm diameter. Judging by the data of Sakmann and Neher [27] the most probable (but rather rough) estimation of the area of the membrane patch sucked into such electrode is 10 μm^2. Assuming the area of the frog rod outer segment plasma membrane to be about 1000 μm^2, we obtain its cGMP-dependent conductance as being of the order of $((0.5–1)/10) \cdot 1000 = 50–100$ nS.

The dark current generated by a toad rod in normal conditions is about 20 pA [9,15,28] at a membrane potential of -35 to -45 mV (see for example [29]). The reversal potential for the dark current is about zero [30] or slightly negative [41]; hence, the driving force for the dark current is approximately equal to the membrane potential. Thus the light-regulated conductance in normally functioning rod is 20 pA/40 mV = 0.5 nS, i.e., at least two orders less than the cGMP-induced one.

Some external influences (low Ca^{2+}, high cGMP) drastically increase the light-modulated component of conductance of the rod outer segment plasma membrane, activating the light-sensitive channels. In low Ca^{2+} (10^{-6} M) the dark current increases to 400pA [9]; at the same time the membrane potential falls to -7 to -8 mV [8].

Hence in such conditions the rod possesses a light-sensitive conductance of at least 400 pA/7–8 mV = 50–60 nS.

Taking into account that the estimation of the amounts of the cGMP-dependent and light-dependent conductances is very rough and is performed for different species, one may conclude that there is no incompatibility between them.

The question arises as to what mechanism may keep inactive about 99% of the light-sensitive conductance of the rod plasma membrane. On the suggestion that the light-sensitive channels are identical to the cGMP-activated ones, the simplest explanation is that the concentration of free cGMP in rod cytoplasm is low. It follows from the data presented in Fig. 6 that 1% activation of the cGMP-dependent conductance occurs at cGMP concentration of the order of 1 μM. The assumption that cGMP in rod outer segment exists preferentially in a bound form and therefore the majority of the light-sensitive channels are inactive, even in the dark, has been already put forward by Cobbs and Pugh [16]. As these authors we believe that, based on this suggestion, it is easy to interpret the results reported by Kilbride and Ebrey [32] and Govardovskii and Berman [33], who did not observe the changes of cGMP level in intact retina on illumination. Really, the change of order of 1 μM could not be detected because the total concentration of cGMP in rod outer segment is much higher (50–100 μM). The fact that illumination markedly decreased the level of cGMP in detached rod outer segment [34] may mean that the mechanism controlling the quantity of free cGMP in cytoplasm does not normally function in such preparation.

The ionic selectivity

Earlier, the common view was that most of the current through the light-sensitive conductance of retinal rod can be carried only by sodium ions [35,36]. But recently it has been shown that this conductance is permeable also to Li^+, K^+, Rb^+, Cs^+, Tl^+ [37–40] and that the disappearance of photoresponses in the absence of Na^+ observed in early works is probably the result of inactivation of a Na^+-Ca^{2+} countertransport system [37,40]. So, the light-sensitive conductance units are permeable for all the small cations for which the cGMP-dependent units are permeable.

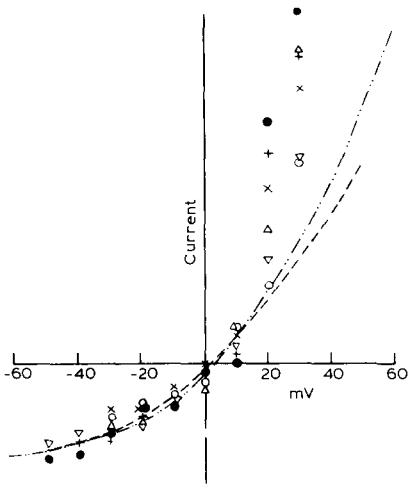

Fig. 8. The current-voltage relations of the light-dependent current of toad rods (data points from Ref. 26, each symbol corresponds to an experiment with a single cell) and of the cGMP-dependent current for two patches (–·–·–, – – – – –). The data were scaled to coincide at −30 mV.

The voltage-current relations

Fig. 8 shows the current-voltage relations of the cGMP-dependent current for two patches of rod outer segment membrane which exhibited especially large sensitivity to cGMP and stability of the cGMP effect (curves) and the same relation for the light-suppressed current of toad rod obtained by Bader et al. [30] (points). It is seen that these relations coincide within the accuracy of experiments in the range of −50 + + 10 mV. The discrepancies are observed only on substantial depolarization in non-physiological for the rod range of voltage.

The sensitivity to cyclic nucleotides

The light-sensitive conductance of the rod plasma membrane depends on the intracellular concentration of cGMP, but not of cAMP [10,14]. That is the case of the cGMP-activated conductance of isolated membrane patches too.

The unit event size

The light-dependent conductance for rods of the lizard, axolotl and turtle is characterized under normal conditions by the magnitude of a single event of about 50–100 [31,41], 100 [42] and 600 [43] fS, correspondingly. The analogous estimation

for the cGMP-activated conductance gives 100–250 fS. being fairly consistent with these data.

Spectra of membrane current fluctuations

Gray and Attwell [42] and Bodoia and Detwiler [41] have recently obtained whole-cell voltage clamp recordings on isolated normally functioning photoreceptor cells. Power spectral analyses of these record revealed both low- and high-frequency components of the light-sensitive noise. The low-frequency (under 1 Hz) component which was first characterized by Baylor et al. [44] is though to be due to thermal isomerization of rhodopsin and other biochemical noise produced at an early stage of the transduction process. The high-frequency component of the noise was attributed to the functioning of the light-sensitive ion channels. It was described by a simple Lorentzian with a half-power frequency of 62 Hz for lizard rod [41] and 90 Hz for that of axolotl [42]. These values are rather close to the half-power frequency of the low-frequency component of fluctuations of the cGMP-dependent current in our experiments (46 Hz). As for the high-frequency component of the cGMP-dependent current, the corresponding component of the light-sensitive current could hardly be revealed in the works mentioned, since the experimental records therein were as a rule low-pass filtered at 160–500 Hz and the high-frequency range (400–500 Hz) was not thoroughly studied.

Thus one may conclude that the noise characteristics of the cGMP-dependent and light-sensitive currents are not incompatible.

Some general considerations

Taking into account the similarities between the characteristics of the light-sensitive conductance of the rod outer segment membrane and those of the cGMP-activated conductance of isolated patches of the rod outer segment membrane, it seems quite possible that the cGMP-dependent conductive units described by as are responsible for the light-sensitive conductance in vivo. If this is so, it is cGMP which is the transmitter regulating the permeability of the rod outer segment plasma membrane. The effect of Ca^{2+} on the rod response may in our opinion have two causes. Firstly, it is possible that Ca^{2+} comprises such a transmitter, too, but the corresponding Ca^{2+}-sensitive sites governing the ionic channels are inactivated in our preparation. Secondly, the effect of Ca^{2+} might be realized through its action on cGMP metabolism or distribution of cGMP between free and bound pools.

The direct (not mediated by protein phosphorylation) action of cGMP on the membrane conductance is, in our opinion, a striking phenomenon which has not been described earlier. The evident advantage of such a 'direct' mechanism should be its fast response time. This is essential for retinal rods where the latency of the response to intense illumination is about 0.7 ms [45]. Such a short latency means that the rate constant for the reaction of dissociation of cGMP from the light-sensitive channels should be at least of the order of 10^3 s^{-1}. As for the rate constant of the reaction of association of cGMP with these channels, it should be at least about 10^8 $s^{-1} \cdot M^{-1}$ in order to provide an effective equilibrium constant of about 10^{-5} M as determined by us for the cGMP-dependent channels (see Fig. 6). Of course, such estimations are rather rough, because the allosteric nature of the interaction of cGMP with the channels is neglected, but they seem to give some idea on the scale of rates of reactions controlling the light-sensitive channels of the rod membranes.

The data recently obtained by Koch and Kaupp [46] imply that cationic channels directly activated by cGMP exist in the disc membranes too. Their characteristics are close to those of the cGMP-dependent channels described in the present work. This demonstrates the similarity between the structures and functional properties of the cytoplasmic and disc membranes of the rod.

In such a way, retinal rods have become the first biological system in which a cyclic nucleotide is shown to regulate a cellular process in a direct way.

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References

1 Baylor, D.A. and Fuortes, M.G.F. (1970) J. Physiol. 207, 77–92

2 Hagins, W.A. (1972) Annu. Rev. Biophys. Bioeng. 1, 131–158
3 Hubbel, W.L. and Bownds, M.D. (1979) Annu. Rev. Neurosci. 2, 17–34
4 Hagins, W.A. and Yoshikami, S. (1974) Expt. Eye Res. 18, 299–305
5 Brown, J.E., Coles, J.A. and Pinto, L.G. (1977) J. Physiol. 269, 707–722
6 Hagins, W.A. and Yoshikami, S. (1977) in Vertebrate Photoreception (Barlow, H.B. and Fatt, P., eds.), pp. 97–120, Academic Press, New York
7 Lipton, S.A., Ostroy, S.E. and Dowling, J.E. (1977) J. Gen. Physiol. 70, 747–770
8 Bastian, B.L. and Fain, G.L. (1982) J. Physiol. 330, 307–330
9 Yau, K.-W., McNaughton, P.A. and Hodgkin, P.A. (1981) Nature 292, 502–505
10 Walloga, G. (1983) J. Physiol. 341, 341–357
11 Lipton, S.A., Rasmussen, H. and Dowling, J.E. (1977) J. Gen. Physiol. 70, 771–791
12 Capovilla, M., Cervetto, L. and Torre, V. (1983) J. Physiol. 343, 277–294
13 Brown, J.E. and Walloga, G. (1981) in Current Topics in Membranes and Transport, Vol. 7 (Bronner, S. and Kleinzeller, A., eds.), pp. 369–380, Academic Press, New York
14 MacLeish, P.R., Schwartz, E.A. and Tachibana, M. (1984) J. Physiol. 348, 645–664
15 Matthews, H.R., Torre, V. and Lamb, T.D. (1985) Nature 313, 582–585
16 Cobbs, W.H. and Pugh, E.N. Jr. (1985) Nature 313, 585–587
17 Cohen, A.I., Hall, I.A. and Ferrendelli, J.A. (1978) J. Gen. Physiol. 71, 595–612
18 Polans, A.S., Kawamura, S. and Bownds, M.D. (1981) J. Gen. Physiol. 77, 41–48
19 George, J.S. and Hagins, W.A. (1983) Nature 303, 344–348
20 Horn, R. and Patlak, J.E. (1980) Proc. Natl. Acad. Sci. USA 77, 6930–6934
21 Hamill, O.P., Marty, A., Neher, E., Sakman, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100
22 Fesenko, E.E., Kolesnikov, S.S. and Lyubarsky, A.L. (1985) Nature 313, 310–313
23 Kolesnikov, S.S., Lyubarsky, A.L. and Fesenko, E.E. (1984) Vision Res. 24, 1295–1300
24 Caldwell, P.C. (1970) in Calcium and Cellular Function (Cuthbert, A.W., ed.), pp. 11–16, St. Martin's Press, New York
25 Rasmussen, H. and Goodman, D.B.P. (1977) Physiol. Rev. 57, 421–509
26 Stevens, C.F. (1977) Nature 270, 391–396
27 Sakman, B. and Neher, E. (1983) in Single Channel Recording (Sakman, B. and Neher, E. eds.), pp. 37–51, Plenum Press, New York
28 Baylor, D.A., Lamb, T.D. and Yau, K.-W. (1979) J. Physiol. 288, 589–611
29 Bastian, B.L. and Fain, G.L. (1979) J. Physiol. 207, 493–520
30 Bader, C.-R., MacLeish, P.R. and Schwartz, E.A. (1979) J. Physiol. 296, 1–26
31 Detwiler, P.B., Conner, J.D. and Bodoia, R.D. (1982) Nature 300, 59–61
32 Kilbride, P. and Ebrey, T.G. (1979) J. Gen. Physiol. 74, 415–426
33 Govardovskii, V.I. and Berman, A.L. (1981) Biophys. Struct. Mechan. 7, 125–130
34 Woodruf, M.L., Bownds, D., Green, S.H., Morrisey, J.L. and Shedlovsky, A. (1977) J. Gen. Physiol. 69, 667–679
35 Sillman, A.L., Ito, H. and Tomita, T. (1969) Vision Res. 9, 1443–1451
36 Korenbrot, J.I. and Cone, R.A. (1972) J. Gen. Physiol. 60, 20–45
37 Bastian, B.L. and Fain, G.L. (1982) J. Physiol. 207, 331–347
38 Woodruf, M.L., Fain, G.L. and Bastian, B.L. (1982) J. Gen. Physiol. 80, 517–536
39 Yau, K.-W. and Nakatani, K. (1984) Nature 309, 352–354
40 Hodgkin, A.L., McNaughton, P.A. and Nunn, B.J. (1985) J. Physiol. 358, 447–468
41 Bodoia, R.D. and Detwiler, P.B. (1985) J. Physiol., in the press
42 Gray, P. and Attwell, D. (1985) Proc. R. Soc. (Lond.) 223, 379–388
43 Schwartz, E.A. (1977) J. Physiol. 272, 217–246
44 Baylor, D.A., Matthews, G. and Yau, K.-W. (1980) J. Physiol. 309, 591–621
45 Penn, R.D. and Hagins, W.A. (1972) Biophys. J. 12, 1073–1094
46 Koch, K.-W. and Kaupp, U.B. (1985) J. Biol. Chem. 260, 6788–6800