INCORPORATION OF ION CHANNELS FROM BOVINE ROD OUTER SEGMENTS INTO PLANAR LIPID BILAYERS

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ABSTRACT Membranes vesicles, prepared from bovine rod outer segments were fused with planar lipid bilayers. Two different ion channels were identified by recording currents from single channels. Both types of channels were selective for sodium rather than potassium and were impermeable to chloride ions. Unit conductances were 20 and 120 pS, respectively, in 150 mM sodium chloride. The channel with the larger unit conductance was sensitive to the transmembrane potential. This channel rapidly activated within <10 ms after a voltage jump to a more negative membrane potential and then inactivated after several seconds. The duration of the active period and the properties of the channel depended on the amplitude of the voltage jump. The channel of smaller unit conductance did not show any voltage-dependent activation or inactivation. Both types of channels were insensitive to light in the planar bilayer system. Channels incorporated into planar bilayers on a Teflon sandwich septum or on the tip of a glass micropipette gave similar results.

INTRODUCTION

Vertebrate photoreceptors respond to light with a transient hyperpolarization of their plasma membrane. It has been suggestd that an intracellular messenger substance exists that acts as a mediator between the photoexcitation of rhodopsin in the disk membrane and the closing of sodium channels in the plasma membrane of the outer segment. Calcium ions (Yoshikami and Hagins, 1971), cyclic GMP (i.e., Liebman and Pugh, 1979; Bownds, 1981), and protons (Mueller and Pugh, 1983) have been tentatively proposed as intracellular transmitter substances. Although there exists some circumstantial evidence for each of these substances, none has been unequivocally identified as an intracellular messenger. The light-sensitive sodium conductance is blocked by extracellular (Yoshikami and Hagins, 1973; Yau et al., 1981) and also intracellular calcium (Brown et al., 1977; Oakley and Pinto, 1981, 1983). The number of the hypothetical light-sensitive channels and the size of the unit conductance have been estimated from signal-to-noise considerations (Cone, 1973; Yoshikami and Hagins, 1973) and from the analysis of the current fluctuations of the plasma membrane (Detwiler et al., 1982). Yoshikami and Hagins (1973) estimated that there are 3,000 channels per outer segment, whereas Detwiler et al. (1982) estimated that there are 20,000 channels. A unit conductance (Λ) of 0.1 pS has been estimated for the light-sensitive channel (Detwiler et al., 1982). The ion selectivity of the light-sensitive conductance is different from other known sodium conductances (Yau et al., 1981; Yau and Nakatani, 1984*a*, *b*).

Little is known about the permeability of the disk membrane (Uhl et al., 1980) and no channel protein(s) of the disk membrane has been identified, isolated, or characterized so far.

Proteins having properties like those of ion channels from membrane fragments of rod outer segments have been previously incorporated into planar bilayers (Montal et al., 1977, 1981; Hanke et al., 1981*a*; Boheim et al., 1982). Montal and co-workers identified light-sensitive channels after adding partially purified rhodopsin-lipid complexes to planar bilayers. However, they could not unequivocally identify the source of these channels. Previously, we reported that two different channels from membranes of purified bovine rod outer segments were incorporated into planar bilayers (Hanke et al., 1981*a*; Boheim et al., 1982). In particular, the ion selectivity, the effects of voltage on the opening and closing events, and the activation/inactivation behavior of these channels will be investigated. To identify the origin of the channels in the outer segment more clearly, we applied a new technique to this preparation, in which bilayers form on the tip of patch-pipettes (Hanke et al., 1984*b*).

MATERIALS AND METHODS

Reagents

Soybean phosphatidylethanolamine (S-PE) was prepared by column chromatography. 1-stearoyl-3-myristoyl-glycero-2-phosphatidylcholine (1,3-SMPC) (Eibl, 1984), which had a main phase transition temperature t_c of 30°C, was a generous gift of H. Eibl. 1-palmitoyl-2-myristoyl-glycero-3-phosphatidylcholine (1,2-PMPC, t_c = 27°C), was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and cholesterol was purchased from Fluka A. G. (Buchs, Switzerland). All lipids were tested for purity using thin layer chromatography. All other reagents were at least of analytical grade.

Preparation

Intact bovine rod outer segments were prepared in dim red light according to Schnetkamp et al. (1977). Briefly, retinas were dissected from cattle eyes and gently vortexed in an isolation medium (600 mM sucrose, 20 mM Tris-HCl at pH 7.4, 10 mM glucose, 1 mM $CaCl_2$, 0.1 mM EDTA, and 5% Ficoll 400 [wt/vol]). The crude homogenate was subjected to centrifugation on a continuous sucrose density gradient. The band containing intact rod outer segments was removed from the gradient and washed in a medium containing 600 mM sucrose and 20 mM Tris-HCl at pH 7.4. Rod outer segments were stored in a concentrated suspension (100–200 μM rhodopsin) in standard medium containing 600 mM sucrose, 20 mM Tris-HCl (pH 7.4), and 5% Ficoll 400. Membrane vesicles were prepared from intact rod outer segments after sonication in a medium containing 200 mM NaCl, 600 mM sucrose, and 20 mM Tris-HCl at pH 7.4. The suspension was sonicated for 30 s at 40 W using a model B-1 sonifier (Branson Sonic Power Co., Danbury, CT). The suspension was immersed in an ice water bath to prevent heating during sonication. Aliquots of the sonicated suspension were rapidly frozen and stored in liquid nitrogen.

Formation of Planar Lipid Bilayers

All aqueous solutions were made with double distilled water (2-stage quartz destill) and passed through Satorius Minisart filters (Satorius, Göttingen, Federal Republic of Germany) (SM 16529, 0.2 μm). Lipid solutions were made from lipid mixtures of various composition (see figure legends for details) by dissolving 10 mg of lipid in 1 ml of a pentane/ethanol mixture (9:1, vol/vol).

Planar lipid bilayers virtually free of solvent were formed on holes (ϕ = 150 μm) in thin sandwich Teflon septa according to Montal and Mueller (1972) and Schindler and Feher (1976). 10–50 μl of the lipid solution was spread on the surface of the aqueous solution on both sides of the Teflon septum. The water surfaces were below the level of the hole. The solvent was allowed to evaporate for 5–15 min. Then the water surfaces were raised above the hole in both compartments by adding aqueous solution. The Teflon septum was pretreated with hexadecane/pentane, (1:9, vol/vol) to increase the stability of the bilayer.

The instrumentation and principles of recording electrical signals from planar bilayers have been described in detail elsewhere (Boheim et al., 1981; Hanke and Miller, 1983). Data were stored on a FM tape recorder (Racal Store 4D, Racal-Thermionic Limited, Southampton, England) and analyzed on a computer (model 11/23; Digital Equipment Corp., Marlboro, MA) as described in the Results section.

The composition of the salt solution was identical in both compartments of the recording chamber and is given in the figure legends. The ionic strength of the solution in the measuring chamber was similar to that of the sonication medium (~150 mM), except that sucrose was not present in the solution in the measuring chamber. The interior of the vesicles was therefore hyperosmotic with respect to the external medium. Fusion of membrane vesicles with the planar lipid bilayer occurred spontaneously after adding the vesicles (20–50 μl; rhodopsin concentration ~100–150 μM) to the *cis*-side compartment. Fusion events and incorporation of ionic channels were monitored by continuously recording the transmembrane current at a fixed voltage. After the first fusion event occurred, the *cis*-side compartment was perfused with a solution identical to that on the *trans*-side to reestablish symmetrical conditions and to decrease the probability of further fusion events. The *trans*-side of the bilayer was kept at virtual ground potential.

Experiments were either performed in dim red light or in bright day light. In the first set of experiments, reconstitution and recording of current fluctuations from channels, were done in dim red light. Subsequently, the bilayer was exposed to daylight for 1–10 min. After illumination channel fluctuations were recorded again. In a second set of experiments, membrane vesicles were illuminated before reconstitution into planar bilayers.

Electrical Recording from Bilayers on the Tip of Glass Micropipettes

Glass pipettes, on whose tips bilayers formed, were pulled on a puller (David Kopf Instruments, Tujungar, CA) and then fire polished (Hamill et al., 1981). The tip diameter of the pipettes was ~1 μm. Bilayers were formed at this tip from a monolayer on the surface of a vesicle suspension (Hanke et al., 1984*b*; Coronado and Latorre, 1983; Suarez-Isla et al., 1983; Schuerhlolz and Schindler, 1983; Wilmsen et al., 1983). The vesicle suspension was made by sonicating a preparation of rod outer segments to which 5 mg of lipid (1,3-SMPC/S-PE, 50:50) per ml solution had been added. A freeze-thaw cycle was applied to the vesicle suspension before use. A monolayer in equilibrium with the subphase is spontaneously formed on the air-water interface of a vesicle suspension (Pattus et al., 1978*a*, *b*). Repetitive movement of a glass pipette tip through the surface monolayer allowed the bilayer to form across the pipette tip.

RESULTS

General Description of Channels

Two different ion channels were detected after vesicles that were prepared from rod outer segments were fused with a planar bilayer (Fig. 1 *a* and *b*). The planar bilayer was composed of 1,3-SMPC and S-PE (1:1) in both experiments. All experiments were carried out at room temperature (~22°C).

One channel type (Fig. 1 *a*) had a unit conductance of 20 pS in 150 mM sodium chloride. The conductance remained unchanged when chloride ions were replaced by sulfate, which demonstrates that the channel is not permeable to chloride. To determine the selectivity of this channel for sodium over potassium, currents were measured under asymmetrical salt conditions. The ion concentrations at the *cis*-side of the chamber were 120 mM NaCl, 20 mM KCl, and 1 mM $CaCl_2$ (buffered as described in the figure legends) and at the *trans*-side, 20 mM NaCl, 120 KCl, and 1 mM $CaCl_2$. The current-voltage relation of the open channel under these conditions is given in Fig. 3 *b*, together with that in NaCl alone. A selectivity ratio of -6:1 for sodium/potassium was calculated from the extrapolated reversal potential (V) of 25 mV using the Goldman equation (the permeabilities for all other ions were taken to be zero). In addition, the conductance of the same channel in a solution containing only 150 mM KCl was 2 pS, which is in good agreement with the selectivity calculated from biionic potentials. For simplicity, we refer

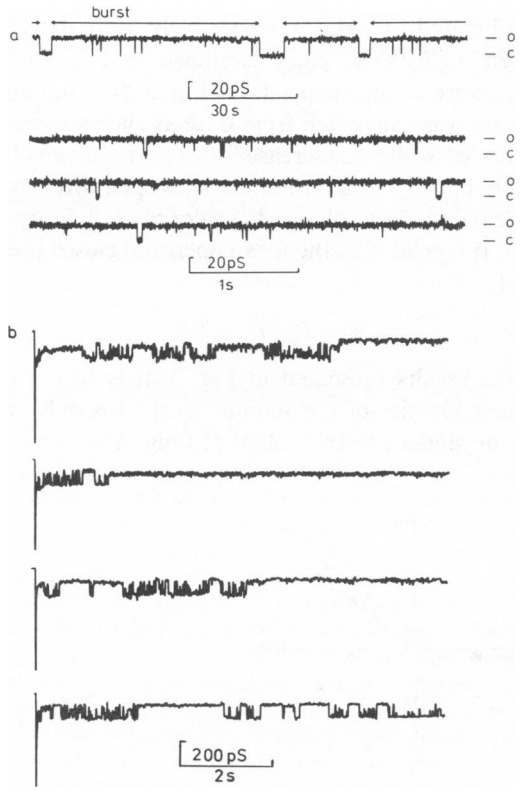

FIGURE 1 *a* shows fluctuations of the conductance of a sodium selective channel after membranes from rod outer segments were reconstituted into planar bilayers according to the method of Montal and Mueller (1972) from 1,3-SMPC/S-PE (1:1 molar ratio). The temperature was 22°C and the applied voltage was 115 mV. The composition of the salt solution was 150 mM NaCl and 10 mM Tris-Cl, pH 7.2. The mean unit conductance of the channel is 20 pS. It is evident from the traces that the channel is fluctuating in bursts (*upper* trace). The *lower* traces show the intraburst transitions on an expanded time scale. *b* shows four traces of conductance fluctuations of a cationic channel under the same conditions as given in *a*. Because the channel is inactivated at constant voltage, voltage jumps from 0 to −50 mV were applied. The capacitive transient at the beginning of each trace indicates the onset of the voltage. All four traces show bursts that activate and inactivate. The mean unit conductance of the channel is 120 pS.

to this channel as the sodium channel in the following sections. It showed a pronounced bursting behavior, and the probability of being in the open state within a burst was high.

The second channel type (Fig. 1 *b*) had a much larger unit conductance of 120 pS in 150 mM sodium chloride. Its conductance in a solution of 150 mM potassium chloride was 65 pS. From experiments under asymmetric conditions as described for the sodium channel, we calculated a selectivity of 1.6:1 for sodium/potassium (the reversal potential [V] was ~8 mV). Similar values were obtained in solutions that contained no chloride ions; thus this channel is not permeable to clhoride ions. We will refer to this channel as the cationic channel. It showed a pronounced activation and inactivation behavior after the voltage was stepped to a more negative value. Four individual examples of this activation-inactivation behavior are shown in Fig. 1 *b*. The onset of the transmembrane potential is indicated by the transient of the capacitive current at the beginning of each trace. The channel was activated within ~10 ms and then was inactivated within seconds. From a detailed analysis of the innerburst kinetics of the channel (see Fig. 5 *b* and *c* and text below), we know that the channel is mainly closed at zero potential.

The kinetic behavior of the cationic channel was highly voltage dependent, and the channel was mostly observed after a rapid change of the voltage to a more negative value. Sometimes no current fluctuations occurred after appropriate voltage jumps. Occasionally long-lasting current fluctuations did occur lasting up to minutes after an activating voltage jump. No channel fluctuations were observed when the voltage was changed towards the positive direction.

Statistical Analysis of the Sodium Channel

The sodium channel did not activate or inactivate within the time scale of our experiments. This channel was, therefore, analyzed under constant voltage conditions. Fig. 2 shows the opening and closing behavior of an individual sodium channel at different voltage values.

Fig. 1 *a* (first trace) illustrates that channel fluctuations occurred in bursts. In this paper we will limit the analysis of this channel to the parameters that describe its intraburst behavior. Occasionally, the kinetic behavior of the channel changed dramatically. For example, the probability of being in the open state within a burst (P_0) sometimes changed during the course of an experiment by >50%. The traces in Fig. 2 represent the predominant behavior. Only traces of current fluctuations similar to those shown in Fig. 2 have been analyzed.

Unit Conductance. A histogram of the current amplitudes of the single channel fluctuations is shown in

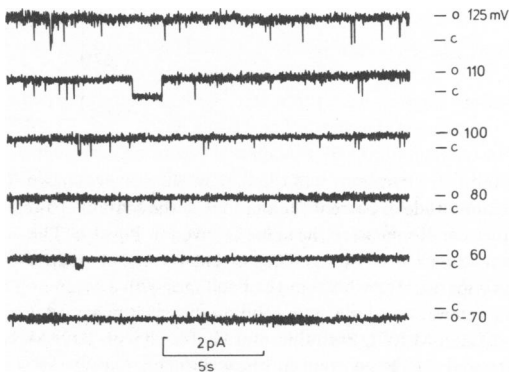

FIGURE 2 The voltage dependence of the intraburst kinetics of the sodium channel is shown. The experimental conditions are the same as those given in Fig. 1 *a*. Traces of current fluctuations are shown at different voltages. The applied voltage and the channel states (*o*, open; *c*, closed) are indicated at each trace.

Fig. 3*a*. The histogram exhibits a bimodal distribution with well-defined open and closed states. The mean current flowing through one channel at a given voltage has been calculated from such histograms. The current-voltage characteristic of an open channel was constructed from values of the mean current (Fig. 3 *a*) at different voltages (Fig. 3 *b*). The sodium channel has an ohmic behavior within the voltage range of our experiments (Fig. 3 *b*) in symmetrical salt solution. The unit conductance is 20 pS in 150 mM NaCl.

Kinetic Behavior. The lifetimes of both the open and closed state within the bursts were distributed exponentially (Fig. 3 *c*). The mean lifetimes of the open (T_0) and closed (T_c) states, respectively, were determined from semilogarithmic plots as shown in Fig. 3 *c*. Both lifetimes were voltage dependent (Fig. 3 *d*). Each lifetime in Fig. 3 *d* was calculated from data as shown in Fig. 3 *c*. T_0 decreased while T_c increased at high positive voltages. The probability (P_0) that the channel is open within a burst was calculated. The voltage dependence of P_0 is shown in Fig. 3 *e*. It is related to the mean open and closed lifetimes by Eq. 1.

$$P_0 = T_0/(T_0 + T_c). \quad (1)$$

From the results presented in Fig. 3, it is seen that the innerburst kinetics of the sodium channel is only weakly voltage dependent in the potential range that we investi-

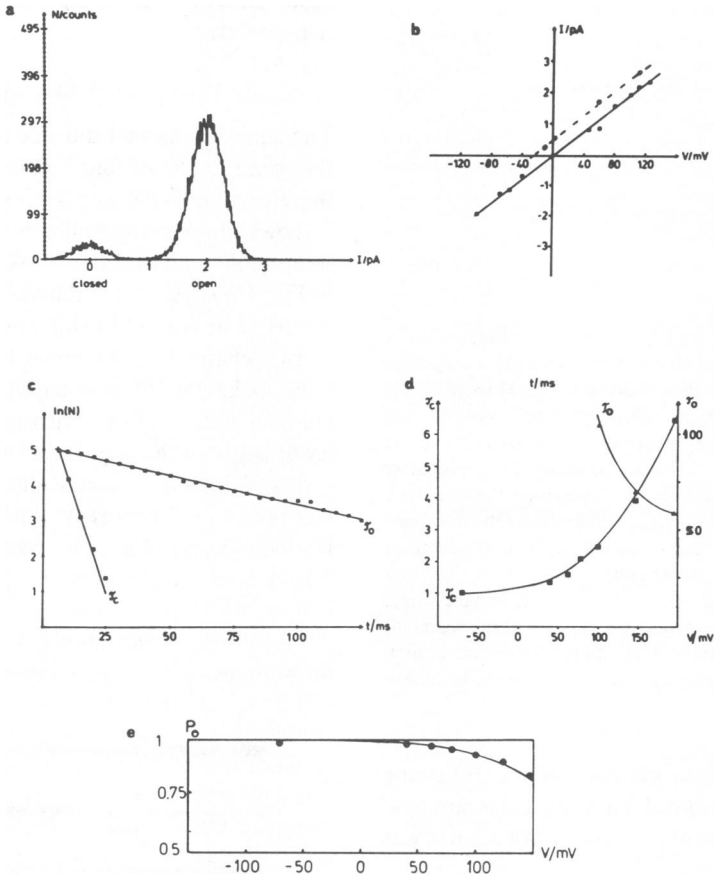

FIGURE 3 Parameters of the sodium channel are shown. The analysis was restricted to the gating behavior within bursts. (*a*) A histogram of the amplitude of current fluctuations of the sodium channel is shown where the sampling rate was 1 kHz and the applied voltage was 100 mV. Other conditions were the same as given in Fig. 1 *a*. The histogram is bimodal with a well-defined closed state, $I = 0$ pA, and a well-defined open state, $I = 2$ pA. (*b*) The current-voltage characteristic was constructed from histograms shown in *a* (-□-). The channel has an ohmic behavior over the whole range of voltages with a mean unit conductance of 20 pS in symmetrical salt solution. In addition, the current-voltage characteristic under asymmetric conditions is given (-●-), *cis* side 120 mM NaCl and 20 mM KCl and the *trans* side contained 20 mM NaCl and 120 mM KCl. Both sides were buffered with 10 MM Tris-Cl at pH 7.2 and 1 mM $CaCl_2$. (*c*) Semilogarithmic plots of the lifetimes of the open and the closed events of the sodium channel are shown. The conditions are the same as those given in Fig. 1 *a*. The membrane potential was 125 mV. The mean lifetimes, T_0 and T_c were 61 and 4 ms, respectively, and were obtained from single exponentials fitted to the lifetime distributions. (*d*) The voltage dependence of the mean open (▲) and closed (■) state lifetimes are demonstrated. The lifetimes were calculated as shown in Fig. 3 *c*. Current fluctuation traces were filtered (low-pass filter [300 Hz]) to suppress the effects of flicker and noise on the calculated lifetimes (Methfessel and Boheim, 1982). With increasing voltage, the lifetime of the open state becomes smaller and that of the closed state becomes larger. Both voltage dependences are not very steep. (*e*) The voltage dependence of the mean open-state probability P_0 is shown. The conditions are the same as in Fig. 1 *a*. The channel is closed by increasing positive voltages. An effective gating charge (two-state model) of $z = -0.5$ was calculated from the slope of the voltage dependence.

gated in this paper. The interburst kinetics was also voltage-dependent. The invtervals between bursts become longer and the bursts became shorter at high positive voltages.

Calcium Dependence of the Sodium Channel. No significant changes in the properties of the sodium channel were found when the concentration of calcium was changed from 1 μM to 1 mM in the bath solution on either side of the bilayer.

Statistical Analysis of the Cationic Channel

The cationic channel exhibited a prominent sequence of activation and inactivation after the voltage was jumped from 0 mV to a more negative value (Figs. 1 *b* and 4 *a*). The properties of the channel, therefore, could not be analyzed at constant voltage. Instead successive voltage jumps were applied. An example of the experimental protocol is shown in Fig. 4. Trace *a* shows a current recording taken while the membrane voltage was changed repetitively as shown below (Fig. 4 *b*). Fig. 4 *c* shows an averaged superposition of 50 individual recordings similar to those shown in Fig. 4 *a*. The voltage was stepped from 0 to −30 mV. Although the activation of the current fluctuations could not be resolved kinetically, the time course of the inactivation showed a distinct voltage dependence.

A characteristic time constant (τ_i) can be determined for the inactivation process, τ_i is the amount of time it takes for $\bar{P}_0$ to decrease from its maximum by the factor $1/e$. Although the time course of the inactivation is not monoexponential as in the present case, this definition gives a useful measure of the inactivation time. This time constant of the inactivation process was 12 s for the present example.

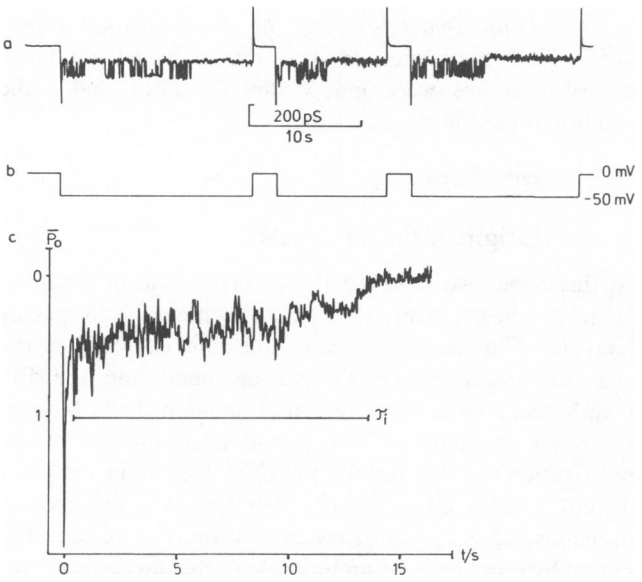

FIGURE 4 The activation and inactivation behavior of the cationic channel are shown. Traces were recorded at conditions like those given in Fig. 1. Trace *a* represents a continuous current recording while the voltage was changed repetitively. The sequence of voltage jumps is shown in trace *b*. The time-dependent mean probability $P_0(t)$ of a single channel being in the open state as shown in trace *c* is obtained by averaging 50 traces of current fluctuations like those shown in *a*. The example shown in *c* was taken at voltage jumps from 0 to −30 mV. A characteristic time constant (τ_i) of 12 s was obtained. For definition of τ_i see text.

Unit Conductance. The mean unit conductance of the cationic channel was determined as was the conductance of the sodium channel. The channel behaved ohmically in symmetrical salt solution and had a unit conductance of 120 pS in 150 mM sodium chloride.

Kinetic Behavior. Most of the kinetic parameters have been calculated from traces of current fluctuations that were activated by jumps to different negative potentials. However, occasionally the channel did not inactivate and long-lasting current fluctuations were observed. During such an active period, it was possible to slowly change the voltage over a wide range of voltages, including even changes towards positive values, before the channel closed permanently. Fig. 5 *a* shows recordings at different voltages during such a rare, long active period. For negative potentials the kinetics parameters within a burst were determined from recordings as shown in Figs. 1 *b* and 4 *a* by jumping the potential to different values; for positive voltages the kinetic parameters were determined from recordings similar to those shown in Fig. 5 *a*, during exceptionally long-lasting bursting events.

T_0 and T_c of the intraburst events are given as a function of the voltage in Fig. 5 *b*. The probability (P_0) of finding a channel in the open state within a burst depended on the applied voltage (Fig. 5 *c*). An effective gating charge (z) of −2 was calculated according to a two-state model. The characteristic time constant of the inactivation process determined according to Fig. 4 *c* is shown to be voltage dependent in Fig. 5 *d*. It became smaller with increasing negative voltages. The activation process could not be resolved. It is evident that the asymmetry of the voltage dependence of the kinetic parameters for both channel types displayed the same orientation. They were different, however, with respect to their reference voltage $V_{1/2}(P_0 = 0.5)$. Furthermore, the kinetic behavior of the cationic channel occasionally changed in the same abrupt way as did the sodium channel.

Substructure of the Conductance of the Cationic Channel. Different sublevels of the conductance were observed at high (Fig. 6 *a*) and low (Fig. 6 *b*) calcium concentrations. Usually the substructure of the channel was studied in a medium of high calcium concentration (2 mM) because the channel did not inactivate under these conditions and therefore the effects could be studied more conveniently. The sublevels are indicated by arrow marks. Sometimes the channel converted (indicated by *V*s) from a state of high conductance to a state of low conductance

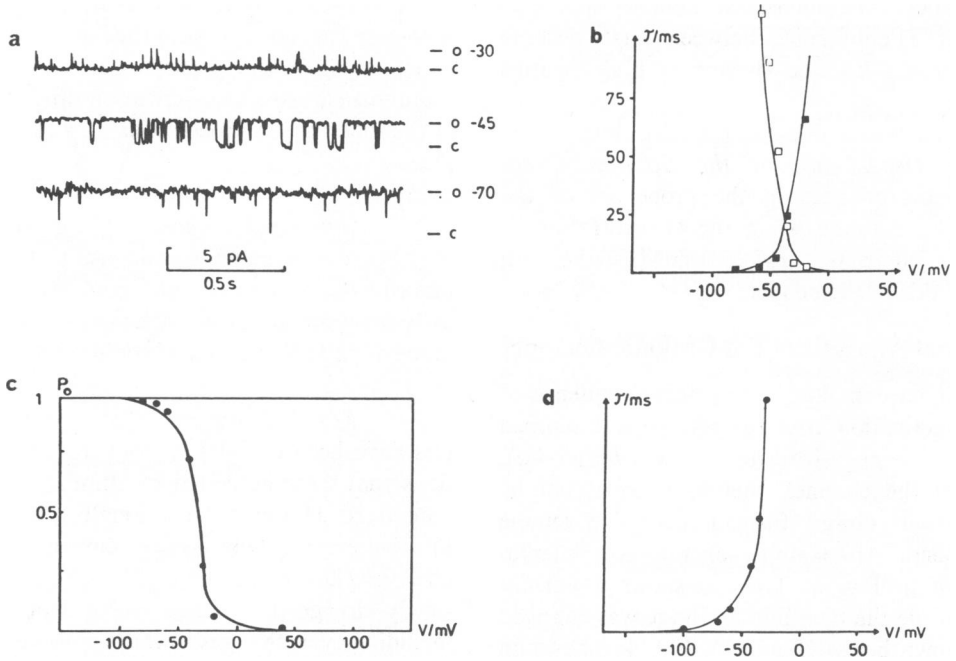

FIGURE 5 Parameters of the cationic channel are demonstrated. (*a*) Traces of current fluctuations of a cationic channel are shown at three different voltages. The traces are parts of a single, exceptionally long-lasting activity period after activation by a voltage jump. Long-lasting fluctuations like that presented here are very rare events. Calculated values of the parameters in *b* and *c* at positive voltages were obtained from these few recordings. At negative voltages the kinetic parameters were calculated from traces like those presented in Figs. 1 *b* and 4 *a*. The applied voltage and the states are indicated at each trace separately. The experimental conditions are the same in Fig. 1 *a*. (*b*) The mean lifetimes of the open (□) and closed (■) states within bursts are given as function of the applied voltage. (*c*) Mean open state probability P_0 within bursts is shown. The channel opens towards negative voltages with an effective gating charge, $z = -2$. (*d*) The characteristic time constant τ_i of the inactivation process is shown as function of the voltage. It becomes smaller with increasing negative voltage.

(Fig. 6 *a* [lower two traces] and Fig. 6 *b*). In the state of low conductance, the cationic channel did not inactivate at low calcium concentrations (Fig. 6 *b*).

Pharmacology of the Sodium Channel and of the Cationic Channel. Neither channel was sensitive to tetrodotoxin (TTX) of concentrations up to 1 μM on both sides of the bilayer.

Reconstitution of Channel Proteins into Bilayers on the Tip of Patch-Clamp Glass Pipettes. We reconstituted channel proteins from a preparation of bovine rod outer segments into planar lipid bilayers by using a second technique. Bilayers were formed on the tip of fire polished glass pipettes after the pipettes were inserted repetitively into the bath solution through a monolayer that had spontaneously formed at the air-water interface of a vesicle suspension. Fig. 7 shows a drawing demonstrating this technique and a trace of current fluctuations of cationic channels, which were activated by a voltage jump. In most of these experiments the cationic channel was detected, whereas the sodium channel was rarely seen. The properties of the cationic channel observed in reconstitution experiments on the patch pipette were identical to those observed in standard fusion experiments.

Light Dependence of the Reconstituted Channels. The properties of the reconstituted channels described above were independent of light under the conditions used in the experiments.

DISCUSSION

Origin of the Channels

In this paper we reported the incorporation of channels from purified bovine rod outer segments into planar bilayers. The most interesting question is, whether the channels originate from the plasma membrane, the disk membrane, fragments of the inner segment that adhere to the outer segment, or from small contaminants of the preparation by other cellular material. Rod outer segments do not contain mitochondria; therefore, the presence of mitochondria is a suitable measure of the level of contamination by other cellular material. We assayed the preparation of rod outer segment for mitochondria in two different ways. We looked for succinate-stimulated oxygen consumption and for uncoupler-stimulated calcium efflux from this preparation. Neither method revealed any membrane pool of mitochondrial origin. From these controls we conclude that our preparation was not significantly contaminated by other cellular material. In the reconstitution experiments the cationic channel is observed more fre-

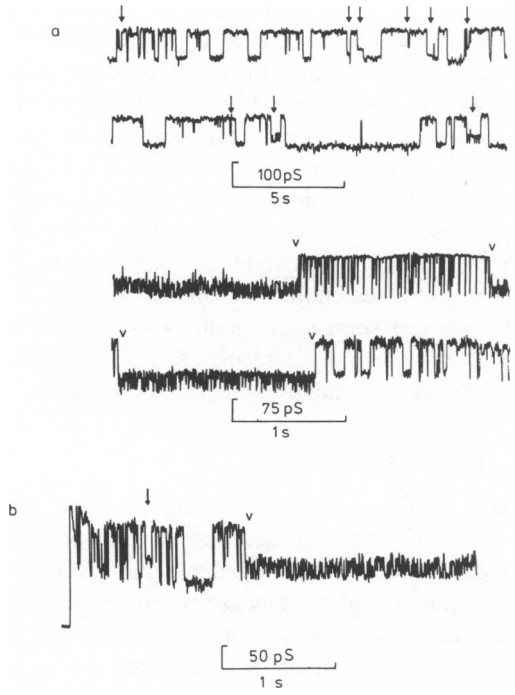

FIGURE 6 Substructure of the conductance of the cationic channel is shown. The concentration of calcium was 2 mM in *a* and 1 μM in *b*. Other conditions were like those given in Fig. 1 *a*. In *a* the voltage is kept constant at 30 mV because the channel slowly inactivates under these conditions. The upper two traces of *a* show single sublevel events indicated by arrow marks. The lower two traces of *a* show randomly occurring changes (marked by *V*s) in the channel fluctuation pattern. The channel changes its conductance from 120 to ~40 pS, together with a dramatic change in the gating kinetics. At low calcium the channel exhibits the same substructure (*b*) after a voltage jump was applied from 0 to −50 mV but, in addition, it loses its ability to inactivate. For better comparison (*b*) has been written upside down. This conversion of the channel gating occurs only in a small sample of channel events.

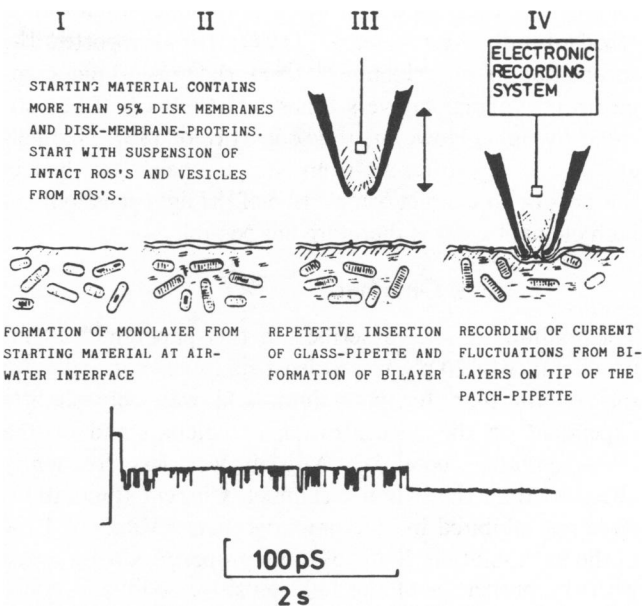

FIGURE 7 Current fluctuations of the cationic channel in a lipid bilayer formed on the tip of a patch pipette are shown. The upper part of this figure shows a drawing scheme of the technique. The trace below was recorded by applying this technique under conditions identical to those given in Fig. 1 *a*. The voltage was changed from 0 to −75 mV. The conductance of the channel is 125 pS.

quently than the sodium channel. Disks constitute ~95% of the total membrane area of rod outer segments. Therefore, it is likely, although not conclusive that the cationic channel may originate from the disk membrane. However, the selective fusion of particular populations of vesicles with a planar bilayer may result in the preferential incorporation of one channel type. Consequently, channels from a minor membrane population may be observed disproportionally more often than those from another population. This argument, however, does not apply as strongly to the incorporation of channels into a bilayer on the tip of glass pipettes. Most likely the protein composition of the monolayer on the surface of the vesicle suspension is similar to that of the membrane vesicles in the suspension. Therefore, the bilayer on the pipette tip should reflect the composition of the material in the suspension. In both types of incorporation experiments the cationic channel was predominantly found. We interpreted this to indicate that the cationic channel originated from the disk membrane. The sodium channel was less frequently detected. These results may indicate that the sodium channel probably originates from the plasma membrane of rod outer segments. Disks are formed as invaginations of the plasma membrane at the basal part of the outer segment near the connecting cilium. It is, therefore, possible that the membrane of a fraction of the disks still contains channel proteins of the plasma membrane. Furthermore, during sonciation vesicles may fuse together thereby exchanging the protein inventory of their membrane. As a result, it is too soon to attribute the origin of the channels to either the plasma or disk membrane. We do believe, however, that patch-clamp experiments on bovine rods will help to better localize and characterize these channels.

Light Dependence of the Channels

No effect of light on the incorporated channels was found. During sonication of intact rod outer segments and subsequent insertion into planar bilayers, the entire content of the rod outer segments, including any hypothetical transmitter, was diluted to large extent. Furthermore, the environment of the reconstituted proteins was quite different from the native membrane. For these reasons we did not expect the ion channels to be light sensitive in these experiments. However, if the translocation of ions is an intrinsic property of rhodopsin itself, conductances might be light dependent in the reconstituted system. Rhodopsin is the major protein component of the disk membrane and consequently, should be the predominant protein that is incorporated into the bilayers by reconstitution experiments. Our results indicate that either rhodopsin is not an

ion channel itself or that it loses its light sensitivity upon reconstitution. Montal et al. (1977, 1981) reported the reconstitution of σ channels from rhodopsin-lipid complexes into planar bilayers. These channels could be activated by light. However, the properties of these channels are completely different from the channels described in this paper and a direct comparison of the light sensitivity of both channel types is therefore not possible.

Sodium Channel

The sodium channel described in this paper had a unit conductance of 20 pS, was at least six times more selective for sodium than for potassium, and was only slightly dependent on the concentration of calcium and on the transmembrane potential. Also, it was less frequently observed than the cationic channel. Current fluctuations were not inhibited by micromolar concentrations of TTX in the bath solution. Some of these properites do not agree with the properties of the light-sensitive sodium conductance observed in standard electrophysiological experiments. This latter conductance seems to be more selective for sodium than for potassium (Yau et al., 1981), it was regulated by calcium (Oakley and Pinto, 1983; MacLeish et al., 1984), and the unit conductance was estimated to be low (~100 fS; Detwiler et al., 1982). However, recently the idea of a classical sodium channel in rods has been questioned. Yau and Nakatani (1984*a*, *b*) reported that the light-sensitive conductance in rods is also permeable to other monovalent and even divalent cations. Alternatively, the light-sensitive sodium conductance in rods is not affected by TTX or saxitoxin (STX) (Fain and Lisman, 1981), similar to the sodium channel in the reconstituted system.

Either the reconstituted channel differs from the sodium conductance that operates in the intact plasma membrane or some critical factors that determine the calcium sensitivity and the unit conductance are lost during reconstitution. Moreover, all standard electrophysiology has been performed on rod cells of the toad, rat, or tiger salamander. No electrophysiological data are available for bovine rod outer segments. This makes a direct comparison difficult. At present, we cannot attribute any particular function to the reconstituted sodium channel in a rod outer segment.

Cationic Channel

The cationic channel is predominantly found in both types of incorporation experiments. Its opening and closing behavior is highly voltage dependent. The channel is mainly closed at constant voltage. It could be activated for short periods by a stepwise change of the transmembrane potential to a more negative value in a medium of low calcium levels (μM), but no activation was observed with positive voltage steps. The cationic channel was weakly selective for sodium over potassium (2:1). The channel is probably located in the disk membrane. The properties of this channel are quite different from cationic channels found in other cells. For example, calcium-activated potassium channels are usually more selective for potassium than for sodium and do not exhibit an inactivation behavior like the channel described in this paper (Latorre et al., 1981; Moczydlowski and Latorre, 1983; Methfessel and Boheim, 1982). Colquhoun et al. (1981) described a cationic channel from cardiac cells that has similar permeabilities for Na and K ions and that is activated by 1–5 μM concentrations of calcium. The unit conductance of this channel type was somewhat smaller (30–40 pS) than the one described here and the gating of the channel was only weakly dependent on the transmembrane voltage.

CONCLUSION

Some basic features of two different channels from rod outer segments have been described. However, the functional significance of these channels and their contributions in shaping the photoresponse remain unsolved. It will be particularly interesting to know whether the gating of these channels is regulated by substances that have been proposed as intracellular messengers (i.e., calcium, protons, or cyclic nucleotides) in visual excitation. In addition, attributing the origin of channels to either the disk or plasma membrane can only be suggested at this point, more experimentation using the patch-clamp technique and using preparations of purified disk and plasma membranes is still necessary. This work is in progress in our laboratories (Hanke and Kaupp, manuscript in preparation).

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