Calcium–Hydrogen Exchange in Isolated Bovine Rod Outer Segments†

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ABSTRACT: We have measured Ca–H exchange in rod photoreceptors with different preparations of rod outer segments isolated from bovine retinas (ROS). One preparation contained ROS with an intact plasma membrane (intact ROS), and in the other preparation, the plasma membrane was leaky to small solutes (leaky ROS) and the cytoplasmic space was freely accessible to externally applied solutes. Addition of Ca^{2+} to Ca^{2+}-depleted ROS (both intact and leaky) resulted in uptake of Ca^{2+} that was accompanied by the release of protons when catalytic amounts of the ionophore A23187 were present. This ionophore mediates Ca–H exchange transport across ROS membranes and serves to gain access to the intracellular compartment where Ca–H exchange appears to take place. Two protons were ejected for each calcium ion taken up. Conversely, when protons were added to Ca^{2+}-enriched ROS, Ca^{2+} was released in the presence of A23187. The majority of this Ca–H exchange was observed only when A23187 was present in both intact and leaky ROS. We conclude that Ca–H exchange occurs predominantly in the intradiskal space and at the surface of the disk membrane rather than across the disk membrane. These exchange binding sites can accommodate 10 mol of Ca^{2+}/mol of rhodopsin at physiological pH. We were unable to detect any Ca^{2+} release when a proton gradient was rapidly established across the disk membrane in the absence of A23187. These results are discussed in relation to the hypothesis that protons produced by the light-induced hydrolysis of cGMP cause the release of Ca^{2+} into the cytoplasm of rod photoreceptor cells.

Rod photoreceptor cells respond to light by reducing the Na^+ conductance of the plasma membrane [for a review on membrane conductances in rod cells, see Fain & Lisman (1981)]. It is generally believed that this change results from the interaction between the Na^+ conductance and a diffusible substance, the concentration of which is controlled by light. Both Ca^{2+} and cGMP have been suggested as candidates for this diffusible transmitter, but little is known about the mechanism of their action [for reviews, see Hagins (1972), Hubbell & Bownds (1979), Liebman & Pugh (1982), and Kaupp & Schnetkamp (1982)]. The most direct support for the notion that Ca^{2+} acts as an intracellular messenger is the observation that illumination of rods causes Ca^{2+} release from the cell interior into the extracellular space (Gold & Korenbrot, 1980; Yoshikami et al., 1980). Absorption of a single photon in a rod cell can cause the release of 10^3–10^4 Ca^{2+} ions (Gold & Korenbrot, 1980; Yoshikami et al., 1980). The case for cGMP is advanced from the observation that bleaching of the visual pigment rhodopsin results in the activation of a rod phosphodiesterase. In vitro, absorption of a photon by one of the rhodopsin molecules in a rod outer segment (ROS) may trigger the hydrolysis of more than 10^4 molecules of cGMP within 1 s (Yee & Liebman, 1978).

Three recent studies report possible links between cGMP and Ca^{2+} in rods. In the first, cGMP is found to increase the Ca^{2+} permeability of isolated disk membranes from which peripheral proteins are removed (Caretta & Cavaggioni, 1983). In the second, cGMP is reported to stimulate uptake of Ca^{2+} into a light-sensitive pool within disks (George & Hagins, 1983). In the third, protons are suggested to form a link between cGMP hydrolysis and Ca^{2+} release (Mueller & Pugh, 1983). This hypothesis states that protons produced by the light-stimulated hydrolysis of cGMP liberate Ca^{2+} from binding sites in the disks by means of ion exchange. The resulting increase of the free intracellular Ca^{2+} concentration is thought to block the Na^+ conductance.

In this paper we report the effects of protons on the calcium content of ROS disks isolated from bovine retinae. The experiments were carried out in the presence and the absence of the ionophore A23187, which exchanges Ca^{2+} and protons (Pfeiffer & Lardy, 1976). Application of A23187 enabled us to distinguish between the properties of Ca^{2+} binding to disk membranes and those of Ca^{2+} transport through disk membranes.

Materials and Methods

All experiments and procedures were carried out in darkness or in dim red light.

Preparations. Bovine ROS were isolated and purified according to published procedures (Schnetkamp et al., 1979; Schnetkamp & Daemen, 1982) with the following modifications. Intact ROS, in which the plasma membrane functions as a permeability barrier to small solutes, were prepared from fresh retinas in two ways. Ca^{2+}-enriched ROS were prepared by incubation of the crude retina homogenate for 1 h at 10 °C in the isolation medium [600 mM sucrose, 5% (w/v) Ficoll 400, 10 mM glucose, 1 mM $CaCl_2$, 0.2 mM EDTA, and 20 mM Tris-HCl at pH 7.4]. Ca^{2+}-depleted ROS were prepared by the same procedure except that 50 mM NaCl and 0.5 mM EGTA replaced $CaCl_2$ and EDTA. After this incubation the ROS were purified by gradient centrifugation and washing as described (Schnetkamp et al., 1979; Schnetkamp & Daemen, 1982).

Leaky ROS, in which the plasma membrane is permeable to small solutes and the cytoplasmic space is accessible to

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externally applied solutes, were prepared in the two forms as described above from retinas that had been frozen and thawed.

Ca^{2+}-depleted ROS contained less than 0.1 mol of total Ca^{2+}/mol of rhodopsin, while Ca^{2+}-enriched intact ROS contained 4.5–5 mol of total Ca^{2+}/mol of rhodopsin and Ca^{2+}-enriched leaky ROS contained 2.5–3 mol of total Ca^{2+}/mol of rhodopsin. All ROS preparations were stored at 4 °C as concentrated suspensions (about 200 μM rhodopsin) in a solution containing 600 mM sucrose, 5% Ficoll 400, 20 mM Hepes, and 8.8 mM arginine (pH 7.5).

The results shown in this paper are representative of data from at least 10 ROS preparations.

Proton Measurements. Changes in free proton concentration were followed spectrophotometrically with the pH indicator bromcresol purple. The presence of ROS membranes did not change the spectral properties of bromcresol purple, and the dye does not bind significantly to ROS membranes (Bennett, 1980). In one case we compared the pH changes indicated by bromcresol purple with those measured with a pH electrode and found an excellent agreement. We also used the pH indicator phenol red at a pH of about 7.5. The results were very similar to those observed with bromcresol purple, although a slow constant drift in pH was more apparent at the basic pH. Time-resolved changes in absorption were recorded with an Aminco DW2a spectrophotometer in the dual-wavelength mode. The difference in absorbance at 590 nm and at 660 nm was recorded, with a bandwidth of 10 nm. The final concentrations in the cuvette were 600 mM sucrose, 20 μM bromcresol purple, 0.5 mM Hepes, 0.22 mM arginine, and 5–8 μM rhodopsin. In some cases gramicidin D and A23187 were added to a final concentration of 1–2 μM with 1 mM stock solutions in ethanol. Similar additions of mere ethanol were without effect. The pH of the solution was adjusted to about 6.4 with HCl or KOH. The suspension was mixed continuously during the recordings with a small magnetic stirring bar. The pH-indicating changes in absorption were calibrated by adding known amounts of HCl and KOH to the suspension. All solutions were flushed with argon before use to remove dissolved CO_2.

Calcium Measurements. Changes in free calcium concentration were followed spectroscopically with the Ca^{2+} indicator arsenazo III. The validity of the use of arsenazo III in our experimental system has been documented before (Kaupp et al., 1979). Time-resolved changes in absorption were recorded with an Aminco DW2a spectrophotometer in the dual-wavelength mode. The difference in absorbance at 650 nm and at 750 nm was recorded, with a bandwidth of 10 nm. The final concentrations in the cuvette were 600 mM sucrose, 20 mM Hepes, 8.8 mM arginine (pH 7.5), 50 μM arsenazo III, and 10–15 μM rhodopsin. The free Ca^{2+} concentration in the suspension ranged between 1 and 10 μM. Ca^{2+}-indicating changes in absorption were calibrated by adding known amounts of Ca^{2+} or EDTA to the suspension.

We used the following protocol to measure the binding of Ca^{2+} in Ca^{2+}-depleted ROS under conditions that equilibrate free Ca^{2+} concentrations in all ROS compartments. ROS were incubated for 10 min at room temperature in a solution containing 600 mM sucrose, 1 μM A23187, 1 μM gramicidin D, 10 mM Mes (adjusted to pH 6.4 with arginine), various Ca^{2+} concentrations (0–1 mM $CaCl_2$), and 20 μM rhodopsin. The ionophores were added to the ROS from 1 mM stock solutions in ethanol. The final ethanol concentration was 0.2%, and the addition of the same amount of mere ethanol was without any effect. Subsequently, the ROS were sedimented in a Beckman microcentrifuge (2 min, 12 000 rpm), and the Ca^{2+} concentrations in the supernatant were determined with arsenazo III. A calibration curve was obtained from samples treated in the same way except for the omission of ROS.

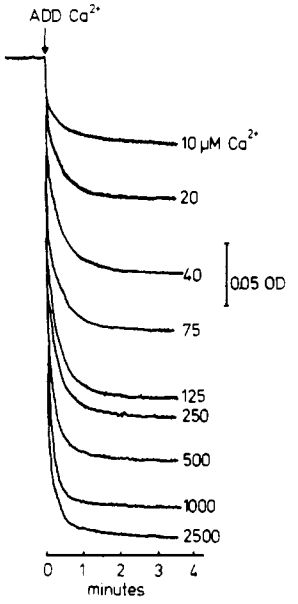

FIGURE 1: Dual-wavelength time scan of Ca^{2+}-induced proton release in Ca^{2+}-depleted intact ROS. Intact Ca^{2+}-depleted ROS (final concentration 5.1 μM rhodopsin) were suspended in 600 mM sucrose, 0.5 mM Hepes, 0.22 mM arginine, 20 μM bromcresol purple, 1 μM A23187, and 1 μM gramicidin D, adjusted to pH 6.4 with KOH or HCl. At the arrow Ca^{2+} was added under continuous stirring to the indicated total Ca^{2+} concentrations (μM). The wavelengths were 590 nm (absorbance maximum of bromcresol purple) and 660 nm (reference wavelength) with a slit width of 10 nm. A decrease in the absorbance difference of the pH-indicating dye bromcresol purple by 0.1 OD represented a decrease of the pH by about 0.1 unit. It should be noted that a substantial part of the buffering capacity of the suspension was due to the external buffer Hepes. Temperature: 22 °C.

RESULTS

Ca^{2+}-Induced Proton Release from ROS Disk Membranes. Ca^{2+}-depleted ROS in the presence of the ionophore A23187 take up maximally 9 mol of Ca^{2+}/mol of rhodopsin (27 mM total Ca^{2+}) at physiological pH, nearly all of which is bound to some site(s) inside the ROS (Schnetkamp, 1979). The uptake of Ca^{2+} in the presence of A23187 and gramicidin was accompanied by a rapid acidification of the unbuffered suspension medium, as monitored by the pH indicator bromcresol purple (Figure 1). The presence of gramicidin did not affect the Ca^{2+}-induced proton release per se, but it abolished a slow and persistent pH drift and enabled a more accurate quantification of the observed proton release. This pH drift was probably caused by leakage of K^+ from ROS accompanied by proton uptake. After each addition of Ca^{2+} the number of protons released was determined by titration with KOH and HCl.

The maximal proton release observed in Figure 1 amounted to 5×10^8 protons per outer segment, equivalent to an internal proton concentration of 50 mM (i.e., bound plus free protons in the overall volume of ROS). A proton release of such magnitude must have been electrically compensated for, most likely by an influx of Ca^{2+}. A23187 is an electroneutral Ca–H exchanger (Pfeiffer & Lardy, 1976). Nearly all internal calcium ions and protons involved seem to be bound to and released from binding sites inside ROS (Kaupp & Schnetkamp, 1982; Schnetkamp, 1979). Therefore, we measured Ca^{2+} binding in ROS (Figure 2, open triangles) and compared

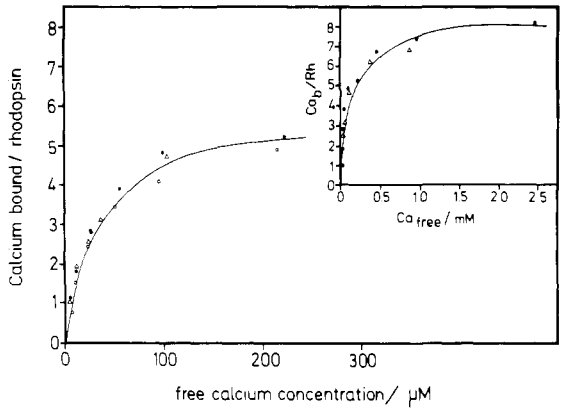

FIGURE 2: Ca^{2+} binding and proton release in Ca^{2+}-depleted intact and leaky ROS. Ca^{2+} binding [(△) intact ROS] is compared with Ca^{2+}-induced proton release [(●) intact ROS; (○) leaky ROS]. Proton release is converted to Ca^{2+} binding by assuming a stoichiometry of one Ca^{2+} bound for two protons released. For the proton release experiments the conditions were as in Figure 1 and the stoichiometry (moles of proton per mole of rhodopsin) was determined by back-titration with KOH. For the Ca^{2+} binding experiments bromcresol purple was omitted from the suspension medium, while 10 mM Mes (adjusted to pH 6.4 with arginine) was added. The rhodopsin concentration in this experiment was 20 μM and Ca^{2+} binding was determined as described under Materials and Methods. Temperature: 22 °C.

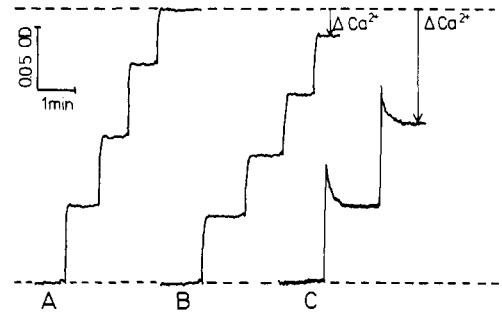

FIGURE 3: Ca^{2+} binding in Ca^{2+}-depleted leaky ROS with and without A23187. Ca^{2+}-depleted leaky ROS were suspended in 600 mM sucrose, 20 mM Hepes, 8.8 mM arginine (pH 7.5), 50 μM arsenazo III, 1 μM gramicidin D, and 14.3 μM rhodopsin. Dual-wavelength time scans are shown (wavelengths 650 and 750 nm with a slit width of 10 nm). In panel A, Ca^{2+} was added in four steps of 2.5 μM under continuous stirring to the above suspension omitting the ROS. This caused stepwise increases in the absorbance difference $A_{650} - A_{750}$. In panel B the same procedure was followed but now including the ROS. In panel C, Ca^{2+} was added in two steps of 5 μM to the ROS in the presence of 1 μM A23187. Temperature: 22 °C.

it with Ca^{2+} binding as inferred from the proton-release data, assuming a stoichiometry of one Ca^{2+} bound for two protons released (Figure 2, closed circles). A close agreement is observed between this inferred Ca^{2+} binding and measured Ca^{2+} binding. The above findings indicate the presence of binding sites in ROS that exchange Ca^{2+} and protons.

Consistent with the presence of Ca–H exchange binding sites is the pH dependence of Ca^{2+} uptake in intact ROS in the presence of A23187. The Ca^{2+} concentration at which half of the maximal binding was observed increased from 10 μM (pH 8.5) to 35 μM (pH 7.5) to about 60 μM (pH 6.4), whereas the maximal binding capacity (at 1 mM free Ca^{2+}) decreased from 12.5 mol of Ca^{2+}/mol of rhodopsin (pH 8.5) to 10 mol of Ca^{2+}/mol of rhodopsin (pH 7.5) to 7 mol of Ca^{2+}/mol of rhodopsin (pH 6.4). Therefore, both the apparent dissociation constant and the maximum binding capacity depended on the pH in the suspension medium. These results suggest competition between Ca^{2+} and protons for common binding sites.

Localization of Ca^{2+} Binding and Proton Release. We determined the site of Ca–H exchange in ROS in two ways. First, we measured the compartmentation of Ca^{2+}-induced proton release by comparing the amount of protons ejected with and without the ionophores. In all cases 250 μM Ca^{2+} was added and the number of protons released was determined. Ca^{2+}-induced proton release was maximal in the presence of A23187 and gramicidin D. This maximal release was of comparable magnitude in intact (Figure 2, closed circles) and in leaky (Figure 2, open circles) ROS. Only a fraction of this maximal proton release was observed when the ionophores were not present. This fraction amounted to 15–20% in intact ROS and probably represents sites located on the outer leaflet of the plasma membrane (including the open disks in the basal part of the outer segment). In leaky ROS this fraction amounted to 20–35%, and the 5–15% difference from the fraction observed in intact ROS probably represents those sites exposed to the cytoplasm. The latter are likely to include sites located on soluble proteins and peripheral membrane proteins. In intact ROS these proteins can be separated from the membrane fraction by lysis at low ionic strength followed by sedimentation of the membranes (Kühn, 1980). When Ca^{2+} was added to this soluble protein fraction, about 16% of the maximal proton release was observed. These results suggest that most of the protons are released from intrinsic membrane sites inaccessible to the cytoplasm of ROS unless A23187 is added.

The second method we used to determine the site of Ca–H exchange in ROS was to compare binding of Ca^{2+} in leaky ROS with and without A23187 (Figure 3). To a 50 μM arsenazo III solution without ROS was added Ca^{2+} in four steps of 2.5 μM Ca^{2+} at intervals of about 1 min. The increase in absorption recorded in pattern A indicates an increase in free Ca^{2+}. Pattern B shows the absorption recording of the same solution with the addition of leaky ROS. In this case the additions of Ca^{2+} caused smaller increases in absorption due to binding of part of the added Ca^{2+} to sites immediately accessible. This binding occurred instantaneously on the time scale of our experiment. The time course of the initial rise in absorption was determined by the time required for complete mixing of the added Ca^{2+} (1–2 s). Pattern C shows the absorption changes after two additions of 5 μM Ca^{2+} to the leaky ROS suspension with the addition of A23187 (note that this Ca^{2+} addition is twice the concentration of the two previous experiments). The initial rise in absorption was similar in pattern B and C. In the latter it was followed by a time-resolved decrease in absorption and reached a constant plateau after about 1 min. This decrease in absorption represents uptake of Ca^{2+} by the disks via the ionophore. The time course of Ca^{2+} uptake in the presence of A23187 (Figure 3C) was similar to that of the Ca^{2+}-induced proton release shown in Figure 1. Pattern C represents about 0.3 mol of Ca^{2+}/mol of rhodopsin taken up by the leaky ROS at a free Ca^{2+} concentration of 1–2 μM. The ROS comprised about 0.5% of the total volume of the suspension, but they contained 41% of the total Ca^{2+} present in the cuvette. A comparison of patterns B and C suggests that the capacity to bind Ca^{2+} in leaky ROS without A23187 was about 20% of that with A23187.

Proton-Induced Ca^{2+} Release from ROS Disks. The reverse process, the effect of protons on the Ca^{2+} content of disks in Ca^{2+}-enriched ROS, also was investigated.

Figure 4 shows an experiment that is complementary to that shown in Figure 1. Protons were added to a suspension of leaky ROS, which contained 1.9 mol of Ca^{2+}/mol of rhodopsin in the presence of A23187. At the indicated arrows, 5 mM Mes was added and the successive decreases in pH were ac-

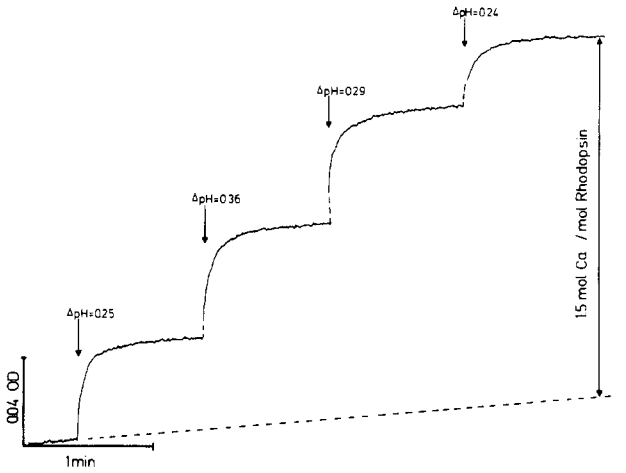

FIGURE 4: Ca^{2+} release from Ca^{2+}-enriched leaky ROS by steps of proton changes. Shown is a dual-wavelength time scan under the same instrumental and medium conditions as described in the legend of Figure 3. The suspension contained 10.6 μM rhodopsin and 2 μM A23187. At the arrows 5 mM Mes was added under continuous stirring and Ca^{2+} release was indicated by an increase in the absorbance difference $A_{650} - A_{750}$. The traces were corrected for the effect of the Mes additions on the arsenazo III spectrum with solutions containing the identical Ca^{2+} concentrations. The Mes additions caused the indicated decreases in the pH of the suspension medium. The amount of Ca^{2+} released was obtained by back-titration with EDTA. Temperature: 22 °C.

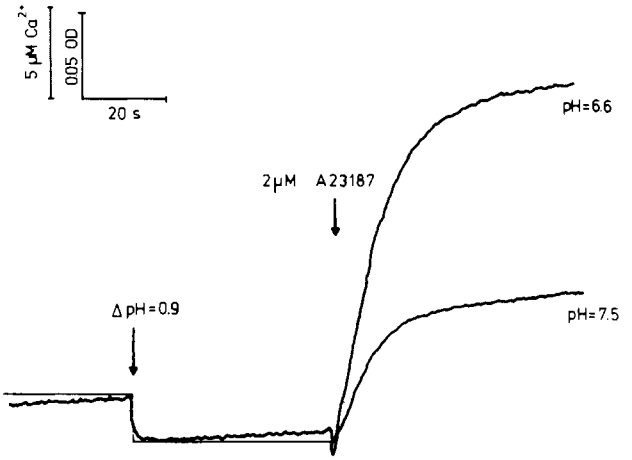

FIGURE 5: Proton-induced Ca^{2+} release in Ca^{2+}-enriched leaky ROS. Shown is a dual-wavelength time scan under the same instrumental conditions as described in the legend of Figure 4. The noisy trace represents a suspension of Ca^{2+}-enriched leaky ROS (final rhodopsin concentration 10.6 μM) in 600 mM sucrose, 20 mM Hepes, 8.8 mM arginine (pH 7.5), and 40 μM arsenazo III. At the first arrow 50 mM Hepes was added under continuous stirring, which caused a decrease of the pH in the suspension medium by 0.9 unit. The smooth thin trace follows the same procedure, but without ROS present. At the second arrow 2 μM A23187 was added (the small dip is an artifact caused by the introduction of the pipet tip into the suspension to ensure the delivery of the minute volume of ethanolic A23187 solution). The trace labeled "pH 7.5" was obtained from a separate cuvette to which no Hepes was added. The trace labeled "pH 6.6" was obtained when A23187 was added after the pH change. Temperature: 22 °C.

companied by large Ca^{2+}-indicating increases in absorption. The pH was lowered by 1.14 units after four steps and the Ca^{2+} release totaled 1.5 mol of Ca^{2+}/mol of rhodopsin. The recordings were corrected for the effect of the buffer additions on the indicator itself (see also Figure 5).

The above experiment was repeated on Ca^{2+}-enriched leaky ROS without A23187, but no proton-induced Ca^{2+} release could be detected (Figure 5). The drift in the noisy trace was caused by slow leakage of Ca^{2+} from the disks, which still contained 2.5 mol of Ca^{2+}/mol of rhodopsin. At the first arrow 50 mM Hepes was added, to change the pH in the suspension medium from 7.5 to 6.6. This addition caused within the mixing time a decrease in absorption, followed by a slow drift similar to that observed prior to the pH jump. An absorption decrease of similar magnitude was observed when 50 mM Hepes was added to the arsenazo-containing suspension medium without ROS (thin smooth line). The absorption change is caused by the pH dependence of the arsenazo III spectrum, by the change in ionic strength, and by the dilution. Results similar to those shown in Figure 5 were obtained when intact ROS were used, when KCl replaced part of the sucrose in the suspension medium (up to 100 mM KCl), or when protonophores such as FCCP or gramicidin were added (the protonphores themselves did not cause any Ca^{2+} release). In none of these cases did lowering of the pH by as much as 0.9 pH unit cause any noticeable Ca^{2+} release from disks. However, addition of Na^+ to the suspension medium resulted in a rapid and large release of Ca^{2+} from disks by Na^+–Ca^{2+} exchange, as described earlier (Schnetkamp, 1980). At the second arrow (Figure 5) A23187 was added to the ROS suspension. This addition caused the release of 0.7 mol of Ca^{2+}/mol of rhodopsin at pH 7.5, whereas at pH 6.6 the release of 1.9 mol of Ca^{2+}/mol of rhodopsin was observed (note that the trace labeled "pH 7.5" was obtained from a separate cuvette to which no Hepes was added).

Discussion

Our results show that isolated ROS from bovine retinae contain binding sites that exchange Ca^{2+} and protons. At physiological pH the maximal binding capacity is about 10 mol of Ca^{2+}/mol of rhodopsin. Ion exchange at these binding sites can operate in both directions when the ionophore A23187 is present. Addition to Ca^{2+} to ROS caused the release of protons that was accompanied by the uptake of Ca^{2+} (Figures 1 and 2), while addition of protons caused the release of bound Ca^{2+} (Figure 4). The use of the Ca–H exchange ionophore A23187 allowed us to determine the localization of the Ca–H binding sites in ROS. The majority of these sites (65–80%) were inaccessible in ROS with a leaky plasma membrane when A23187 was absent. The accessible fraction probably included the buffering groups of soluble and peripheral membrane proteins in the cytoplasm, which contributed 16% of the maximally observed Ca–H exchange. We conclude that the majority of the Ca–H binding sites are sites intrinsic to the membrane and are located in the intradiskal space. In most of our experiments the ionophore A23187 has been applied and Ca–H exchange might be merely a consequence of its operation. However, more than 98% of the observed Ca–H exchange has involved bound Ca^{2+} and protons (Kaupp & Schnetkamp, 1982; Schnetkamp, 1979), and therefore the results must reflect intrinsic properties of membrane binding sites. For example, addition of A23187 to liposomes made of phosphatidylcholine did not result in any of the Ca–H exchange observed in ROS.

The nature of the Ca–H binding sites inside disks is unclear. These sites could be either phospholipids or the portion of rhodopsin protruding into the intradiskal space. A likely candidate for Ca^{2+} binding is phosphatidylserine. Disk membranes contain 9–11 mol of phosphatidylserine/mol of rhodopsin, but most of it is thought to be located on the cytoplasmic surface of the disk membranes (Drenthe et al., 1980; Miljanich et al., 1981). It has been reported that although binding of Mn^{2+} and Co^{2+} to phosphatidylserine membranes causes a release of protons, binding of Ca^{2+} does not (Puskin & Coene, 1980; McLaughlin, 1982). Recently a structure of the rhodopsin molecule in the disk membrane was proposed

on the basis of the amino acid sequence (Ovchinikov et al., 1982). In this model, positively charged residues dominate the part of the rhodopsin protruding into the cytoplasm, whereas the part extending into the intradiskal space has an excess of negatively charged residues (nine carboxyl groups against three basic residues). We have previously argued that the peculiar electrostatic conditions prevailing inside disks (Kaupp & Schnetkamp, 1982) could cause considerable changes in the dissociation constants of phosphatidylserine and/or the acidic residues of rhodopsin for both calcium ions and protons. These changes might account for the observed Ca–H exchange in disks.

It has been suggested that protons act as an intermediate in rod photoreceptors between the light-induced hydrolysis of cGMP and the release of Ca^{2+} (Mueller & Pugh, 1983). This proposal was prompted by the observation that lowering the extracellular pH caused a reduction of the light-sensitive dark current in normal Ringer's solution but not in low-Ca^{2+} Ringer's solution. The interpretation of this finding was that the action of protons was to release Ca^{2+} from intracellular stores. The resulting increase in the free intracellular Ca^{2+} concentration is thought to block the dark current. We have investigated two elements of this hypothesis. First, do ROS contain sufficient Ca–H exchange sites as compared with Ca^{2+}-independent proton buffer sites? Second, do protons added to the cytoplasmic compartment have access to these Ca–H exchange sites? Considering the first question, when we added 5×10^7 protons per outer segment, we observed a release of 4×10^6 calcium ions and a lowering of the pH by 0.1 unit (Figures 1 and 4). Bovine ROS were taken to be cylinders of 1-μm diameter and 20-μm length containing 3 mM rhodopsin. Thus, 13 protons were required to release 1 calcium ion. The actual value is lower since about half of the buffer capacity could be accounted for by the residual external buffer Hepes. In vitro 10^5–10^6 molecules of cGMP are hydrolyzed per bleached rhodopsin per second (Liebman & Pugh, 1982). This results in the production of an equal number of protons, which would be sufficient to release 10^4–10^5 calcium ions. A release of 10^3–10^4 calcium ions is observed in the extracellular space upon absorption of a single photon by a rod cell (Gold & Korenbrot, 1980; Yoshikami et al., 1980). The above calculation was based on Ca–H exchange observed in the presence of A23187.

Regarding the accessibility of the Ca–H exchange sites to the cytoplasmic compartment, we have obtained no evidence for an endogenous Ca–H exchange (transport) system in the disk membrane. Protons added to the cytoplasmic surface of the disks did not cause any Ca^{2+} release without the addition of A23187. Ca^{2+} can be transported by a Na–Ca exchanger (10^6–10^7 Ca^{2+} per outer segment per second) in our ROS preparations (Schnetkamp, 1980). Evidence for a Ca–H exchange transporter has been reported in sonicated disk membrane vesicles, although its manifestation required long sonication times (Racker et al., 1980). In a recent abstract George & Bitensky (1984) report a cGMP- and ATP-dependent uptake of Ca^{2+} in broken ROS from frog retinas. The authors suggest that this Ca^{2+} uptake may be mediated by Ca–H exchange, but in their experiments the uptake of Ca^{2+} was accompanied by a rise of pH of the external solution. Our results do not support the model proposed by Mueller & Pugh (1983) in which protons produced by the light-induced hydrolysis of cGMP release Ca^{2+} from disks, unless one assumes that in our preparations an endogenous Ca–H exchange transporter in the disk membrane was lost.

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Registry No. Ca, 7440-70-2; hydrogen ion, 12408-02-5.

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