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BIOCHEMICAL ASPECTS OF THE VISUAL PROCESS

XXXVI. CALCIUM ACCUMULATION IN CATTLE ROD OUTER SEGMENTS: EVIDENCE FOR A CALCIUM-SODIUM EXCHANGE CARRIER IN THE ROD SAC MEMBRANE

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Summary

Accumulation of calcium has been studied in bovine rod outer segments (rods), isolated by sucrose density gradient centrifugation. Calcium-depleted rods are obtained by having ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*'-tetra-acetic acid (EGTA) present during isolation.

Rods thus isolated have a leaky plasma membrane, as shown by the effects of ionophore A23187 and by their light-induced phosphorylation behaviour. The accumulation of ^{45}Ca, determined by incubation followed by a single fast washing-filtration procedure, thus represents translocation across the rod sac membrane.

Accumulation in non-depleted rods is independent of the external calcium level and of ATP, suggesting exchange of ^{40}Ca by ^{45}Ca. In depleted rods in the presence of ATP there is net uptake, sigmoidally increasing with the external calcium concentration to the level attained in non-depleted rods. This net uptake is abolished by omission of ATP, its replacement by β,γ-methylene ATP and lowering the temperature to 0° C, suggesting involvement of enzymatic hydrolysis of ATP.

Replacement of KCl by NaCl in the medium causes marked inhibition of ^{45}Ca uptake, both net uptake and exchange. Oligomycin, ruthenium red, lanthanum and ouabain do not inhibit accumulation.

Efflux of ^{45}Ca from pre-loaded rods is slow in a KCl medium ($t_{1/2} \sim 30$ min at 25° C), but is greatly accelerated by addition of NaCl or Ca^{2+} ($t_{1/2} \sim 10$ s at 25° C).

It is concluded that the rod sac membrane contains a carrier system, which is sensitive towards Ca^{2+} and Na^+ and which requires ATP for net uptake of Ca^{2+} but not for exchange transport of Ca^{2+} with Ca^{2+} or Na^+.

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*'-tetraacetic acid

Introduction

It has been suggested that Ca^{2+} acts as a transmitter in rod outer segments (rods) between the rod sac membrane and the rod plasma membrane [1]. The rod sacs would store Ca^{2+}. Part of this would be released during photolysis of rhodopsin, thus increasing the cytoplasmic calcium concentration. The released Ca^{2+} would diffuse to the plasma membrane, where it would close sodium channels, thus reducing the sodium permeability of this membrane.

In our laboratory Hendriks et al. [2] have found that frog rods, after careful and fast isolation in the presence of ATP, have a very high calcium content. They have also reported a shift in calcium distribution in these rods upon illumination after osmotic lysis. In cattle rods, which require a much more extensive and drastic isolation procedure, they find a rather variable and lower calcium content.

The high calcium content of rods, the probably saccular location and the fast release of Ca^{2+} from the rod sacs upon photolysis of rhodopsin would require the existence in the rod sac membrane of an efficient system for binding or translocation of calcium.

Binding of calcium to rod sac membranes has been studied in our laboratory by means of the equilibrium dialysis method [3]. Cattle rods have been used in these and all further experiments. The binding studies indicate that at the presumed low cytoplasmic calcium concentration (approx. 10^{-6} M, see ref. 4) far too little calcium would be bound to the cytoplasmic side of the rod sac membrane to explain the high calcium content and the light-induced release of calcium.

Thus a calcium translocation mechanism must be considered. Therefore, accumulation and efflux of calcium in cattle rods has been studied by means of ^{45}Ca. Through the use of calcium-depleted as well as non-depleted rods it has been possible to distinguish between net calcium uptake and exchange of ^{45}Ca with endogenous ^{40}Ca. These experiments have demonstrated the presence of a calcium translocation system in the rod sac membrane.

Methods and Materials

Isolation of cattle rod outer segments. All procedures, except for the calcium determinations, are carried out in dim red light or darkness.

Cattle rods are normally isolated according to de Grip et al. [5], involving mild homogenization in Tris · HCl buffer (160 mM, pH 7.4), followed by sucrose density gradient centrifugation. After gradient centrifugation the rod layer is collected, diluted with three volumes of the ice-cold isolation medium, centrifuged (1000 × *g*, 15 min, 4° C) and resuspended in the desired medium. The preparation is stored in ice and used within 2 h. Calcium-depleted rods are prepared by addition of 1 mM EGTA, an efficient calcium chelator, to the isolation medium.

Calcium determination. A 0.5 ml aliquot of rod suspension is incubated for 15 min at 18–20° C with 4 μM A23187 (gift of E. Lilly and Co., Indianapolis, Ind., U.S.A.) and 5 mM EDTA. The ionophore A23187 is added as an ethanolic

solution (final concentration ethanol: <0.4%) with agitation. The suspension is centrifuged and calcium is determined in an aliquot of the clear supernatant. The calcium content of the sediment remaining after ionophore treatment is less than 1% of the original content (Drenthe et al., to be published).

Calcium is determined with a Pye Unicam SP1950 double-beam atomic absorption spectrophotometer. All samples contain 0.5% $LaCl_3$ to overcome anionic interference. Calcium standard solutions are prepared from anhydrous $CaCO_3$, which is dissolved in HCl containing 0.5% $LaCl_3$.

Rhodopsin determination. Rhodopsin is determined in a rod preparation by determining the 500 nm absorbance before and after complete bleaching in the presence of 1% Triton X-100 and 50 mM hydroxylamine. The molar concentration is calculated by using a molar absorbance of 40 600 at 500 nm for rhodopsin [6].

Determination of ^{45}Ca accumulation. 1 ml of cattle rod suspension (15—30 nmol rhodopsin) is incubated at 25° C in a medium containing 100 mM KCl, 20 mM Tris · HCl (pH 7.4), 2 mM $MgCl_2$, about 2 μCi ^{45}Ca (The Radiochemical Centre, Amersham, U.K., 1 Ci/50 mg Ca^{2+}), varying concentrations of $^{40}CaCl_2$ and other additions. Aliquots of 200 μl are transferred to 2.5 ml ice-cold washing medium on a borosilicate glass fiber filter (high flow rate, no adsorption of calcium), immediately followed by addition of another 2.5 ml ice-cold washing medium and suction through the filter. The entire washing procedure takes about 5 s. The washing medium has the same composition as the incubation medium, except for omission of ^{40}Ca and ^{45}Ca and addition of sufficient EGTA (0.25 mM) to chelate all calcium present. Blanks consist of ^{45}Ca-free suspension and radioactive isotope solution mixed in the washing medium and immediately subjected to the same washing and filtration procedure. These blanks give the same values as tissue-free blanks. The filters are placed in counting vials filled with 10 ml Aquasol (New England Nuclear, Boston, Mass., U.S.A.). Total radioactivity is determined by pipetting an aliquot of the labeled suspension directly into a counting vial, to which an unused filter is added.

The single fast washing-filtration procedure yields a rapid and complete separation between ^{45}Ca in the medium and ^{45}Ca accumulated inside the rods due to complete and instantaneous chelation by EGTA of all accessible ^{45}Ca.

Phosphorylation procedure. 1 ml outer segment suspension (20—30 nmol rhodopsin) is preincubated at 25° C. Phosphorylation is started by addition of ATP (final concentration 1 mM) and 2 μCi [γ-^{32}P] ATP (The Radiochemical Centre, Amersham, U.K., 3 Ci/mmol). The sampling, washing and counting procedure is the same as used in the determination of ^{45}Ca accumulation except for the following modifications. The washing medium consists of ice-cold 0.12 M sodium phosphate buffer (pH 7.4) and two additional portions of washing medium are used. The filter is not allowed to run dry.

Results

Endogenous calcium content of isolated cattle rod outer segments

The calcium contents of non-depleted and depleted rods are 2.0 and 0.43 mol Ca^{2+}/mol rhodopsin, respectively (Table I). These endogenous calcium

TABLE I

ENDOGENOUS CALCIUM CONTENT OF VARIOUS CATTLE ROD OUTER SEGMENT PREPARATIONS

	Non-depleted	Depleted
Calcium content (mol Ca^{2+}/mol rhodopsin)	2.0	0.43
S.E.	0.4	0.09
Range	0.7—4.0	0.18—0.66
Number of preparations	8	5

contents must be taken into account in interpreting the results of ^{45}Ca accumulation experiments.

The rather variable results indicate that the rods easily lose calcium during the lengthy (2—2.5 h) isolation procedure. This point is further illustrated by the dramatic decrease of the calcium content when EGTA is present during isolation (depleted rods). Major contamination by extracellular calcium seems unlikely, since non-depleted rods in vitro do not show a net uptake of extracellular calcium (see Fig. 2 and its discussion).

$^{45}Calcium$ accumulation as a function of time

Fig. 1 shows that ^{45}Ca accumulation in the presence of excess EGTA does not significantly exceed the isotope blank level. Accumulation in the absence of EGTA extrapolates at zero time to the isotope blank level, indicating the validity of the blank corrections. The rapid approach to a maximal level, many times higher than the blank level, indicates that ^{45}Ca is indeed accumulated inside rod outer segments.

Steady-state filling, i.e. the condition where influx of ^{45}Ca equals its efflux,

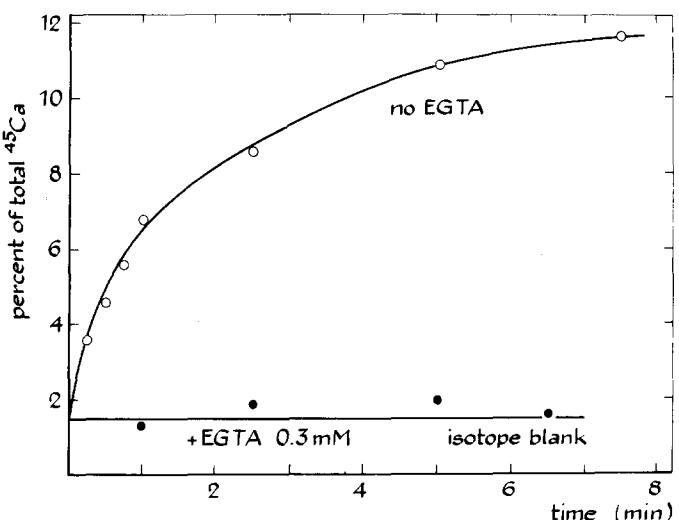

Fig. 1. ^{45}Ca accumulation in non-depleted cattle rod outer segments. Open symbols, no EGTA; closed symbols, 0.3 mM EGTA. The horizontal line represents the isotope blank level. Medium: 100 mM KCl, 2 mM $MgCl_2$, 19 μM $CaCl_2$, ImM Tris · ATP, 20 mM Tris · HCl (pH 7.4).

is reached after 10—30 min incubation at 25° C. This is shown for non-depleted rods in Fig. 1, but it is also true for depleted rods. In all further experiments, where only the steady-state filling level is determined, a standard incubation time of 45 min at 25° C has therefore been used. Steady-state filling levels are calculated from triplicate determinations of the tracer content. In calculating the amount of accumulated calcium, the isotope is assumed to be equally distributed over endogenous and exogenous calcium in the steady-state. This assumption is supported by the horizontal curve in Fig. 2 for the calcium content of non-depleted rods, calculated from the accumulated ^{45}Ca.

Effect of medium composition on accumulation

In a number of experiments with depleted rods the major osmotic component in the accumulation medium has been varied (Table II). The only significant deviation is a large decrease in accumulation in the presence of 100 mM Na^+. Experiments at varying Na^+ concentrations indicate that 100 mM Na^+ has approximately maximal effect and that half-maximal effect is obtained somewhere in the range of 10—30 mM Na^+. We shall return to this observation in the section describing efflux experiments. Omission of ATP from the media gives lower, but relatively similar accumulation levels (not shown). In view of these results, the experiments reported in the next four sections have been carried out in the basic medium with 100 mM KCl present as the major osmotic component.

Effect of external calcium concentration

Fig. 2 combines the results from four experiments, in which depleted and

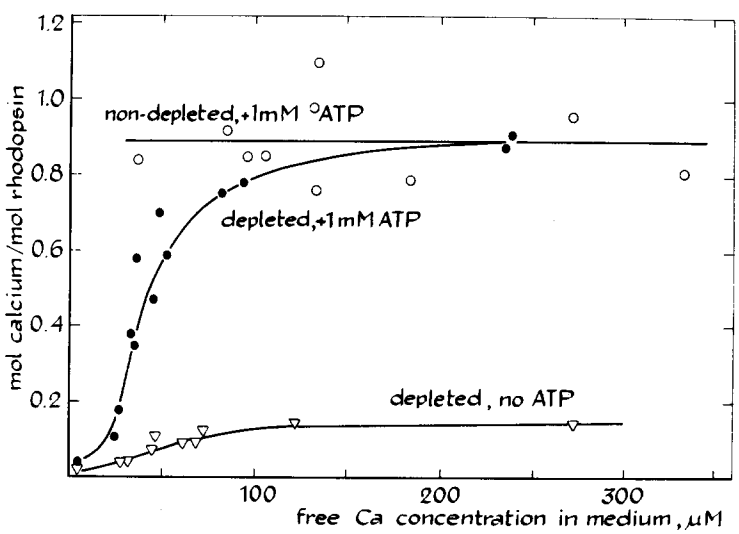

Fig. 2. Effect of external calcium on ^{45}Ca accumulation by depleted and non-depleted rods. Medium: 100 mM KCl, 2 mM $MgCl_2$, 20 mM Tris · HCl (pH 7.4). Free external calcium concentrations are calculated by subtracting from the total amount of calcium present, endogenous and exogenous, the amount of calcium incorporated in the rods. The results of four experiments are combined. ○, non-depleted rods, 1 mM ATP present in medium; ●, depleted rods, 1 mM ATP present in medium; △, depleted rods, no ATP present. Steady-state levels after 45 min incubation at 25° C are presented.

TABLE II

EFFECT OF MEDIUM COMPOSITION ON ^{45}Ca ACCUMULATION OF DEPLETED RODS

Basic medium contains: $MgCl_2$, 2 mM; Tris · HCl, 20 mM; ATP, 1 mM; $CaCl_2$, 50 μM (pH 7.4). Results refer to steady-state levels after 45 min incubation at 25° C and of the accumulation in the medium containing 100 mM KCl, with S.E. and number of experiments. Each experiment is carried out with a single depleted rod preparation.

Addition to basic medium	Accumulation	*n*
100 mM KCl	≡100%	7
100 mM NaCl	30 ± 6	7
100 mM Tris · HCl (pH = 7.4)	73 ± 9	2
100 mM sucrose	90 ± 2	3
200 mM sucrose	117 ± 22	4

non-depleted rods with and without ATP are compared. Non-depleted rods in the presence of ATP accumulate calcium to a level, which is independent of the external calcium concentration over a wide range (25—350 μM). The same effect is observed when ATP is omitted from the medium, except that the ^{45}Ca level remains about 35% lower (see Table III). This suggests that there is in these cases no net accumulation, but only an exchange between exogenous ^{45}Ca and endogenous ^{40}Ca.

Depleted rods show only in the presence of ATP at increasing calcium concentration a net calcium accumulation to a level equal to that of the original calcium content of the non-depleted rods. This suggests that the same calcium compartment is being filled with ^{45}Ca in the two cases. These experiments indicate that only after prior calcium depletion ^{45}Ca uptake experiments will yield true net uptake of calcium.

Effect of ATP on accumulation

ATP stimulates calcium accumulation significantly, but much more so in depleted than in non-depleted rods. The uptake in depleted rods is increased 429% and in non-depleted rods from the same batches of eyes by only 54% (Table III). Replacement of ATP by β,γ-methylene ATP (which is not hydrolyzed by ATPases and kinases) abolishes the stimulation of accumulation.

TABLE III

EFFECT OF ATP AND β,γ-METHYLENE ATP ON ^{45}Ca ACCUMULATION

Medium composition: 100 mM KCl, 2 mM $MgCl_2$, 50 μM $CaCl_2$, 20 mM Tris · HCl (pH 7.4). Steady-state levels after 45 min incubation at 25° C are presented as percent of the levels achieved in the absence of ATP. Results are averages with S.E. and number of experiments for depleted and non-depleted rods prepared from the same batch of eyes.

	No ATP	ATP (1 mM)		β,γ-Methylene ATP (1 mM)	
	%	%	*n*	%	*n*
Depleted rods	≡100	529 ± 50	4	69 ± 2	2
Non-depleted rods	≡100	154 ± 3	3	90 ± 2	2

This suggests that the stimulating effect of ATP involves its enzymatic hydrolysis.

Effects of temperature and ionophore on accumulation

In view of the stimulating effect of ATP on net calcium accumulation in depleted rods we have investigated the effects of the temperature. Table IV shows that low temperature only slightly lowers the accumulation in the absence of ATP, but completely abolishes the stimulation of ^{45}Ca uptake by ATP. This means that ^{45}Ca-^{40}Ca exchange shows little temperature dependence, whereas net uptake of calcium is greatly temperature dependent.

The presence of A23187 in the medium completely inhibits accumulation of calcium (Table IV, line 3). Conversely, addition of A23187 to a rod suspension after accumulation rapidly abolishes the calcium gradient (Fig. 3). This effect is virtually completely reversed by addition of 50 μM lanthanum, probably due to its complexation of the ionophore. These effects of A23187, which are consistently found in a large number (n = 18) of experiments, strongly suggest that accumulation involves translocation into the rod sacs, resulting in a concentration gradient across the rod sac membrane. Calculation shows that at low external calcium levels rod/medium ratios far above 1 are reached, e.g. a ratio of 25 at 50 μM external Ca^{2+}.

Effect of various substances on net accumulation

Table V shows the effects of some substances, which might influence ATP-dependent calcium accumulation in depleted rods. None of these substances has a clear effect, although ruthenium red (100 μM) may cause up to 60% inhibition in experiments with a high stimulation (3—5-fold) of accumulation by ATP.

Structural integrity of isolated rods

The calcium level in non-depleted rods and net calcium accumulation in depleted rods are very sensitive to mechanical disruption and ageing. Additional centrifugation for 15 min at 3000 × g causes 60 and 40% reduction, respectively, of calcium level and accumulation capacity, ageing by storage for 24 h at 4° C and freeze-thawing reduce both parameters by 70%, and lyophilization reduce them by virtually 100%. Electron microscopic inspection reveals that ageing and freeze-thawing result in loss of stacked-sac structure and swelling of

TABLE IV

EFFECT OF TEMPERATURE AND IONOPHORE A23187 ON ^{45}Ca ACCUMULATION OF DEPLETED RODS

Medium composition as in Table III. Results for one representative experiment from a total of four experiments. Steady-state levels after 45 min incubation at 25° C are presented as percent of the control level at 25° C.

Conditions	25° C	0° C
1. control (45 min at indicated temperature)	≡100%	53%
2. as 1, +1 mM ATP	403	64
3. as 2, +10 μM A23187	36	—

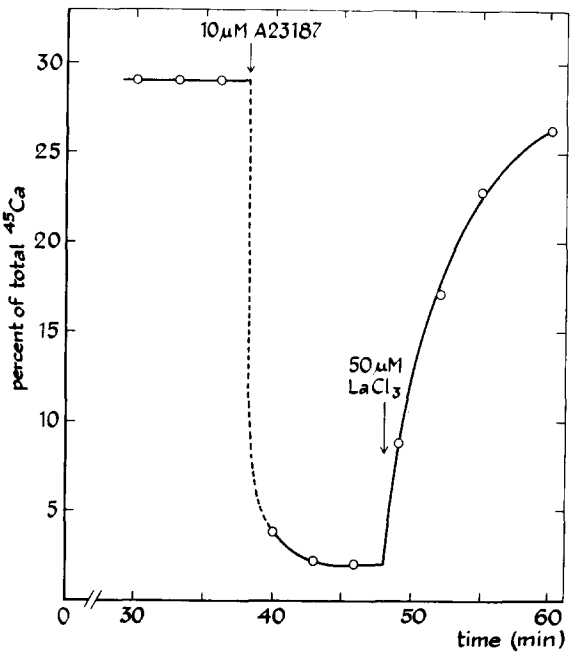

Fig. 3. Effect of ionophore A23187 on ^{45}Ca level of pre-loaded rods. Medium: 100 mM KCl, 2 mM $MgCl_2$, 1 mM ATP, 10 μM Ca^{2+}, 20 mM Tris · HCl (pH 7.4). Arrows indicate time of addition of A23187 and $LaCl_3$ to the indicated final concentrations.

the sacs, but does not permit a conclusion as to the intactness of the plasma membrane in these preparations.

We have used light-dependent phosphorylation of rhodopsin [7,8], which is nearly entirely located in the rod sac membrane, to establish whether externally applied solutes, in this case [γ-^{32}P] ATP, are immediately available to the rod sacs. Fig. 4 shows that the isolated rods are phosphorylated at exactly the same rate with or without prior freeze-thawing, indicating that the plasma membrane in the isolated rods does not function as a permeability barrier for small solutes.

Efflux of ^{45}Ca from pre-loaded rods

The calcium uptake experiments have been complemented by studies of ^{45}Ca

TABLE V

EFFECT OF VARIOUS SUBSTANCES ON ^{45}Ca ACCUMULATION OF DEPLETED RODS

Medium composition as in Table III. Steady-state levels after 45 min incubation at 25° C are presented as percent of the level achieved without added substance. Results are averages with S.E. for the indicated number of experiments (*n*).

Substance added	0 mM ATP	*n*	1 mM ATP	*n*
None	≡100%		≡100%	
Ouabain, 100 μM	80 ± 22	2	88 ± 7	4
Ruthenium red, 100 μM	100 ± 3	2	60 ± 21	3
Oligomycin, 5 μg/ml	92 ± 1	4	82 ± 6	4
La^{3+}, 50 μM	108 ± 3	3	122 ± 7	4

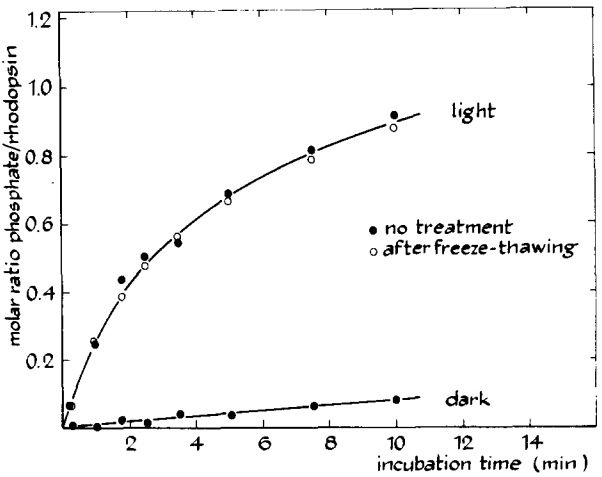

Fig. 4. Light-dependent phosphorylation of non-depleted rods. Rods, without (•) or after (○) prior freeze-thawing, are incubated at 25° C in a medium containing 100 mM KCl, 20 mM Tris · HCl (pH 7.4) and 2 mM $MgCl_2$. Illumination with white light is started 30 s before addition of 1 mM Tris · ATP and 2 μCi [γ-^{32}P] ATP. Incorporation values for a control experiment in darkness are shown, and are deducted from the incorporation values in the light. Maximal incorporation is close to one phosphate per rhodopsin, and the incorporation curve approaches first-order kinetics, as would be expected when the ATP concentration and the kinase activity remain constant during the full incubation period.

efflux from rods pre-loaded in the absence of ATP. By omitting all calcium from the efflux medium and adding EGTA, net efflux of calcium can be measured in the absence of exchange fluxes, such as always occur in uptake experiments where even depleted rods still contain appreciable amounts of endogenous calcium (see Table I).

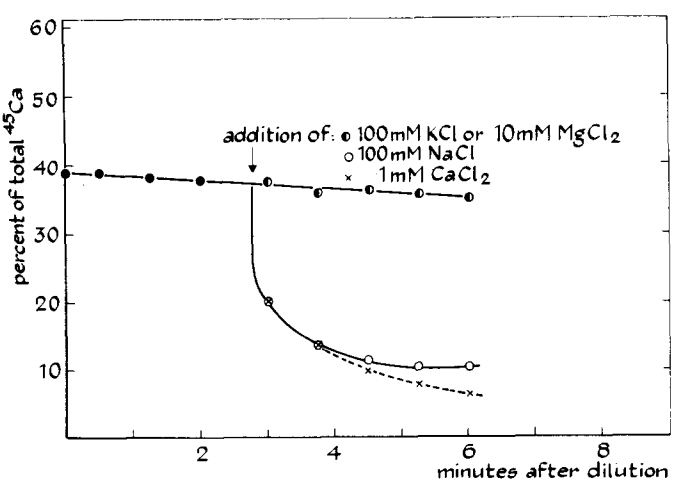

Fig. 5. Calcium efflux from non-depleted rods, equilibrated with ^{45}Ca. Equilibration medium: 100 mM KCl, 2 mM $MgCl_2$, 20 mM Tris · HCl (pH 7.4), 2 μCi ^{45}Ca, 25° C. Efflux is started by 10-fold dilution (at 25° C) in the same medium without Ca^{2+} and 250 μM EGTA (•). The point on the ordinate represents a determination of tracer content before dilution. After 2.75 min a concentrated salt solution is added, which results in an additional final concentration of 100 mM KCl or 10 mM $MgCl_2$ (•), 100 mM NaCl (○) or 1 mM $CaCl_2$ (X).

The efflux experiment is started by the addition of nine volumes of a medium, lacking all calcium and containing 0.25 mM EGTA, to a suspension of rods preloaded with ^{45}Ca (Fig. 5). Efflux in a medium with 100 mM KCl (no Na^+) is slow ($t_{1/2} \sim 30$ min), which is also the case for efflux in media containing sucrose (100 or 200 mM) or Tris (120 mM) or lacking Mg^{2+}. This slow efflux indicates a low passive calcium permeability of the rod sac membrane.

However, when 100 mM NaCl is added to the medium there is a fast and large release of ^{45}Ca with a half-time of $t_{1/2} \sim 10$ s, whereas addition of 100 mM KCl or 10 mM $MgCl_2$ do not noticeably affect the efflux rate. This finding and the reduced ^{45}Ca accumulation in the presence of 100 mM NaCl (Table II) suggest the operation of a Ca^{2+}-Na^+ exchange carrier system. Addition of ATP to the efflux medium affects neither the slow nor the fast calcium efflux.

Addition of 1 mM ^{40}Ca to the efflux medium also greatly accelerates ^{45}Ca efflux (Fig. 5). The rapid phase of the efflux curve after addition of 1 mM $^{40}Ca^{2+}$ virtually coincides with that of 100 mM Na^+, suggesting that the same Ca^{2+}-Na^+ exchange carrier may operate here in ^{45}Ca-^{40}Ca exchange.

Discussion

Accumulation of ^{45}Ca by rods has previously been reported by several authors [9—14]. Although they use different terms, binding, uptake and accumulation, it may be assumed that in all these cases accumulation of ^{45}Ca inside rod outer segments or rod outer segment fragments has been measured, since all authors use sampling procedures based on dilution with isotope-free media. Unfortunately, rather little information about the accumulation system has been gained from these studies. Neufeld et al. [10] and Mason et al. [11] have used sonicated rod material and Weller et al. [14] have applied a severe hypo-osmotic shock to their rod preparation, so that in these cases the original intact rod sac structure has presumably been lost. Hemminki [12] treats his earlier results as representing a mere binding process, neglecting the accumulation through translocation, which according to our results occurs even in the absence of ATP. His later attempt to distinguish between binding and active transport through the use of ATP and the ionophore A23187 [13] suffers from the fact that he makes the questionable assumption that the isolated bovine rods have an intact plasma membrane. Moreover, in none of these studies has the endogenous calcium content been taken into account or removed, which at the low external calcium concentrations generally used may lead to false net uptake curves.

For the accumulation experiments in this paper we have used cattle rods isolated by means of sucrose density gradient centrifugation. The average calcium content, immediately after centrifugation, is 2.0 mol Ca^{2+}/mol rhodopsin for non-depleted rods, and 0.4 mol Ca^{2+}/mol rhodopsin in EGTA-depleted rods.

Parallel losses in calcium content and accumulation capacity are caused by centrifugation and resuspension, ageing, freeze-thawing and lyophilization. Conversely, depleted rods are capable of net uptake of calcium until the calcium content of non-depleted rods is reached. These observations suggest the presence of a single well-defined, structure-dependent calcium storage and accumulation capacity.

The rapid ^{45}Ca efflux from preloaded rods upon addition of A23187, Na^+ or $^{40}Ca^{2+}$ suggests that the rods behave like a two-compartment system. The phosphorylation experiment shows that the plasma membrane does not constitute a rate-limiting barrier to small solutes. Hence the two compartments appear to be medium and intrasaccular space, and accumulation of calcium must involve translocation across the rod sac membrane. The achievement of rod/medium ratios far above 1 and the rapid abolition of such a ratio by A23187 indicate that entering calcium can be translocated against a concentration gradient.

In our accumulation and efflux experiments three modes of calcium translocation across the rod sac membrane can be distinguished. The first mode is the net uptake of calcium in depleted rods, which is clearly ATP dependent. The steady-state level is sigmoidally related to the external calcium and maximally reaches the originally present calcium level. Enzymatic hydrolysis of ATP appears to be involved, since β,γ-methylene ATP cannot replace ATP and cooling to 0° C abolishes the stimulating effect of ATP. The second mode of translocation is a Ca^{2+}-Ca^{2+} exchange process, which is observed in ^{45}Ca accumulation by non-depleted rods as well as in Ca^{2+}-stimulated ^{45}Ca efflux from preloaded rods. This process appears to be independent of ATP and has a low temperature coefficient. ^{45}Ca accumulation by Ca^{2+}-Ca^{2+} exchange proceeds to a calcium level, which is virtually independent of the external calcium concentration in a range of 25—350 μM. The third mode of translocation is a Ca^{2+}-Na^+ exchange process, which is indicated by the fact that external Na^+ inhibits calcium accumulation and stimulates calcium efflux. The effects of external Ca^{2+} and Na^+ appear to be selective, since K^+ and Mg^{2+} do not stimulate Ca^{2+} efflux.

These three modes of translocation seem to be mediated by a single system, since (1) net uptake by depleted rods in the presence of ATP leads to about the same calcium level (determined with ^{45}Ca) as reached by exchange uptake in non-depleted rods, (2) net ^{45}Ca uptake and ^{45}Ca-^{40}Ca exchange uptake are similarly reduced by external Na^+, (3) conversely, external Na^+ accelerates ^{45}Ca efflux, and (4) the ^{45}Ca efflux rates after addition of external Na^+ or Ca^{2+} are about equal.

This translocation system differs from the calcium pumps in sarcoplasmic reticulum [15—17], mitochondria [15,18,19] and erythrocytes [15,20,21], as indicated by the ineffectiveness of lanthanum, oligomycin and ruthenium red and by the effects of external sodium on the steady-state filling levels of the rods. In accordance with this, we have so far not been able to detect a (Ca^{2+} + Mg^{2+})-activated ATPase activity in rod outer segment preparations [22]. How the system in the rod sac membrane utilizes the energy derived from hydrolysis of ATP in the case of net uptake is unclear. Coupling to a (Na^+ + K^+)-ATPase pump system, i.e. a calcium accumulation system driven by a sodium gradient, also seems unlikely, since ouabain does not affect calcium accumulation in rods.

The translocation system, in so far as it seems to be capable of both Ca^{2+}-Na^+ and Ca^{2+}-Ca^{2+} exchange, seems to resemble the calcium extrusion systems operating in plasma membranes of nerve, muscle and other cells [15]. The latter systems are also selectively activated by Ca^{2+} and Na^+ and appear to utilize ATP under certain conditions [23,24], but apparently not through a (Ca^{2+} + Mg^{2+})-activated ATPase system as occurring in sarcoplasmic reticulum.

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