Biochimica et Biophysica Acta, 375 (1975) 309–316

BBA 76844

AN ELECTROGENIC COMPONENT OF THE POTENTIAL DIFFERENCE IN THE RABBIT LENS

CHRISTOPHER A. PATERSON*, MARGARET C. NEVILLE, RAEBURN M. JENKINS II and JOHN P. CULLEN

From the Division of Ophthalmology and Department of Physiology, University of Colorado Medical Center, 4200 East Ninth Avenue, Denver, Colo., 80220 (U.S.A.)

(Received July 23rd, 1974)

SUMMARY

The normal resting potential of the rabbit lens, −70 mV, is altered to −59 mV by ouabain concentrations up to $5 \cdot 10^{-6}$ M, and to −52 mV at 4 °C. Ouabain acts only at the anterior lens surface. The temperature effect is completely reversible. The Hodgkin–Katz–Goldman equation can be used with the measured lens potentials and Na^+ and K^+ levels in the lens and bathing medium to obtain α, the ratio of the membrane permeabilities to Na^+ and K^+. The α-values obtained were 0.052 at 4 °C and 0.053 in $5 \cdot 10^{-6}$ M ouabain. These data suggest that the change in potential due to cold and ouabain is caused by an inhibition of an electrogenic Na^+ pump in the anterior lens epithelium.

INTRODUCTION

Using microelectrode techniques, an electrical potential difference can be measured between the interior of the crystalline lens of the eye and its environment. Potentials in living tissue result from the steady-state ionic distribution and, in many tissues, the activity of an electrogenic Na^+ pump. In some nerve cells, this electrogenic component may contribute up to 60 % of the total resting membrane potential [1].

Values of the rabbit lens resting potential determined in this laboratory [2] were considerably higher than those previously reported [3, 4] as well as those calculated from the ionic concentrations in the lens and bathing medium. The experiments reported in this paper provide evidence that an electrogenic pump exists in the anterior lens epithelium and that the electrical potential measured at the posterior lens surface is partly dependent upon that pump.

* Reprint requests to Dr C. A. Paterson, Box 2404, 4200 East Ninth Avenue, Denver, Co. 80220.

METHODS AND MATERIALS

New Zealand Dutch strain albino rabbits (2–3 kg) were killed by a blow on the head. The eyes were removed, opened posteriorly, and the lens totally excised by carefully cutting around the zonules.

Instrumentation

Microelectrodes were pulled from 1.2-mm (outer diameter) Pyrex glass tubing and filled with 3 M KCl. These electrodes, with tip diameters in the range 0.7–1.0 μm, resistances of 3–7 mΩ, and tip potentials of less than 2 mV were introduced into the lens using a Prior Instruments micromanipulator. The potential difference between the lens interior and a reference electrode in the bathing medium was measured on a Keithley Model 602 electrometer (10^{14} Ω input impedence) and recorded on a Houston Instruments pen recorder. Silver–silver chloride electrodes provided reversible junctions for both recording and reference electrodes.

Only the posterior lens surface was penetrated with microelectrodes due to the difficulties encountered in penetrating the thick anterior lens capsule.

Temperature experiments

Lenses were placed in a depression cut into a disc of Sylgard (Dow Corning Corp.) at the bottom of the lens bath and batched in TC199 culture medium with bicarbonate buffer, at pH 7.4 [5]. The temperature of the bath, monitored by a thermocouple and meter, was maintained by means of a water jacket (Fig. 1a).

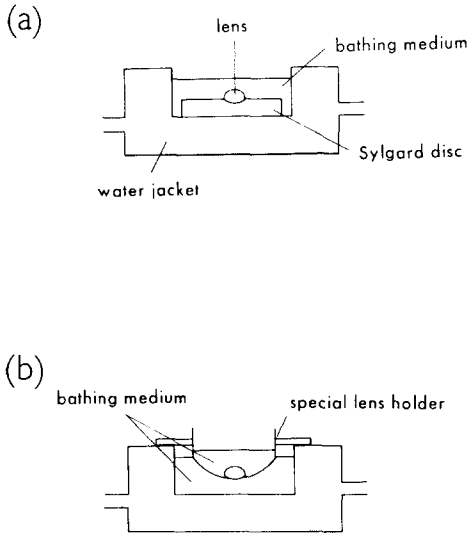

Fig. 1. (a) Diagram of water jacketed bath used to hold lens for potential difference measurements. (b) Same bath as in (a), but with "Sylgard" lens holder removed and special lens holder in position. The diameter of the opening at the base of the holder was made slightly smaller than the diameter of the lenses used. The lens was placed anterior surface down into the opening and the fluid level in the special lens holder filled to about 0.5–1 cm above the level in the bath. The positive hydrostatic pressure in the upper compartment effectively sealed the lens into the opening of the holder, thereby isolating anterior from posterior lens surface. The leak between compartments was less than 0.1 %.

At the end of each experiment, the lens was assayed for Na^+ and K^+ by flame photometry.

Ouabain experiments

Using the lens bath described above, each lens was exposed to one ouabain concentration at 37 °C, the potential measured, and the Na^+ and K^+ contents determined.

In another series of experiments, the posterior surface was effectively isolated from the anterior surface using a special lens holder (Fig. 1b). This allowed measurement of the potential at the posterior lens surface when only one surface was treated with ouabain. [^{14}C]Sucrose was added with the ouabain to check for leaks between the two compartments. The leak between compartments was less than 0.1 % in all experiments. Lenses were analysed for Na^+ and K^+ at the end of each experiment.

Flame photometry

Lenses were dried at 104 °C to constant weight to obtain water content. The dried lenses were digested in hot concentrated HNO_3. Aliquots of suitably diluted lens digest were read against prepared Na^+ and K^+ standards on a Beckman Model B spectrophotometer with flame attachment. Intracellular Na^+ and K^+ concentrations were expressed as mequiv/kg of lens cell water. This was calculated using an extracellular space value of 5 % [6], and assuming the extracellular Na^+ and K^+ concentration to be the same as in the bathing medium, i.e. 150 and 5.5 mM, respectively.

RESULTS

As previously reported from this laboratory [2], the normal resting potential of the rabbit lens, measured at the posterior surface, was in the range −68 to −74 mV. The mean potential of the 46 freshly dissected lenses used in this study was -70.0 ± 2.8 mV (S.E.). This potential was maintained for at least 3 h at 37 °C following the first electrode penetration.

Effect of cooling and rewarming

The change in potential as the bath temperature was reduced from 37 to 4 °C over a period of 1 h is shown in Fig. 2. Between 32 and 27 °C the potential fell rapidly from −70 mV to around −56 mV. In general, potentials arising from ionic distribution across membranes are directly proportional to the absolute temperature [7] (see Eqn 1). This effect provides an adequate explanation for the change in potential difference from −56 to −52 mV as the temperature was reduced from 27 to 4 °C. If, after cooling at 4 °C for 2 h, the lens was then rewarmed in the bath to 37 °C, the potential returned to normal (-69 ± 0.6 mV) within 1 h (Fig. 3). This demonstrates the reversibility of the temperature effect. In all of these experiments the Na^+ and K^+ levels in the lens did not change significantly from control values during the period of the experiment (Table I).

Effect of ouabain

The effect of various concentrations of ouabain on the lens potential is shown in Fig. 4. Concentrations in the range 10^{-7} to $5 \cdot 10^{-6}$ M caused the potential to fall

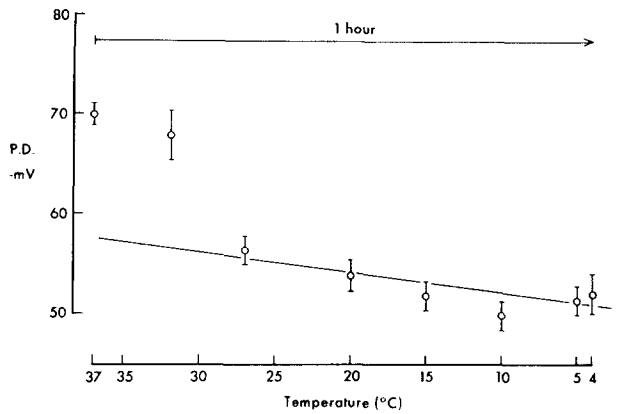

Fig. 2. Effect of cooling on the electrical potential of the rabbit lens. The data were obtained from 8 lenses taken through the entire temperature range. The distance between horizontal bars represents two standard errors of the mean. The solid line represents the theoretical change in the potential as the temperature varies from 4 to 30 °C.

to about −59 mV within 7–10 min. If one takes into account the temperature difference, the potential of −59 mV is equivalent to that of −52 mV measured at 4 °C. Ouabain concentrations greater than $5 \cdot 10^{-6}$ M caused an additional fall in potential to about −50 mV. Lens Na^+ and K^+ levels did not change significantly from control values during the experimental time period.

This finding that both cooling and ouabain concentrations in the range 10^{-7}–$5 \cdot 10^{-6}$ M reduced the rabbit lens potential to equivalent values lead us to postulate the existence of an electrogenic Na^+ pump in this tissue. It is well established that the

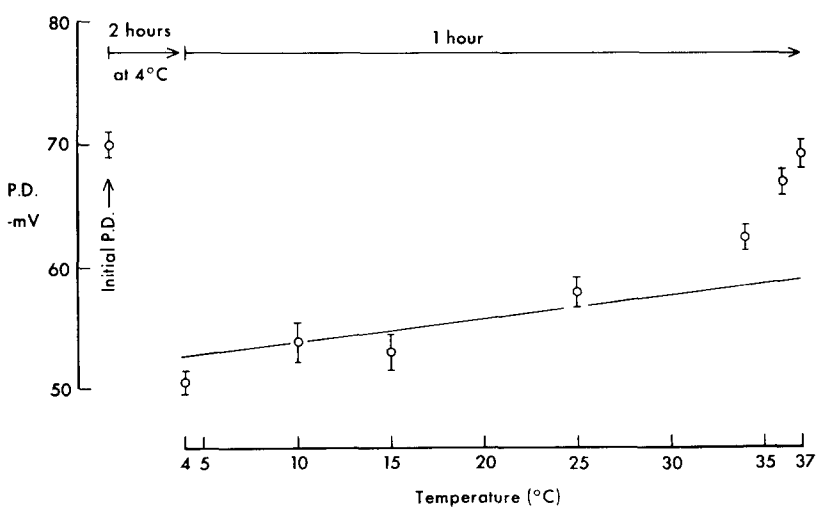

Fig. 3. Effect on lens potential of rewarming to 37 °C following maintenance at 4 °C for 2 h. The data were obtained from 6 lenses. The standard error is shown by the bars, as in Fig. 2. The solid line represents the theoretical change in potential as the temperature varies from 4 to 30 °C.

TABLE I

MEASURED RABBIT LENS POTENTIALS, Na^+ and K^+ DATA AND CALCULATED α VALUES

Figures given are mean ± S.E.

	Measured potential (−mV)	Na^+ (equiv/kg cell water)	K^+ (equiv/kg cell water)	α
Fresh lenses at 37 °C, $n = 46$	70.0±2.8	13.0±2.0★	129.0±3.6★	—
Lenses cooled to 4 °C, $n = 8$	52.5±1.3	15.0±1.0	122.0±3.2	0.052±0.002
Lenses maintained at 4 °C for 2 h, then rewarmed to 37 °C, $n = 6$	69.0±0.6	20.7±2.1	130.0±2.8	—
Lenses exposed to $5 \cdot 10^{-6}$ M ouabain, $n = 5$	59.3±0.6	16.1±1.1	124.0±3.6	0.053±0.004

★ Data from Paterson and Eck[5]; $n = 65$.

Na^+ pump of the lens is confined to the anteriorly located lens epithelium [8]. If our postulate is correct, treatment of the anterior lens with ouabain should reduce the measured potential; application of ouabain to the posterior surface should have no effect.

Using the special lens holder (Fig. 1b), we determined the changes in potential at the posterior surface when ouabain was applied to either anterior or posterior surface alone. The concentration of ouabain which apparently abolished the electrogenic potential was used, i.e. $5 \cdot 10^{-6}$ M. The results are shown in Fig. 5. When ouabain was applied to the anterior surface, the potential measured at the posterior

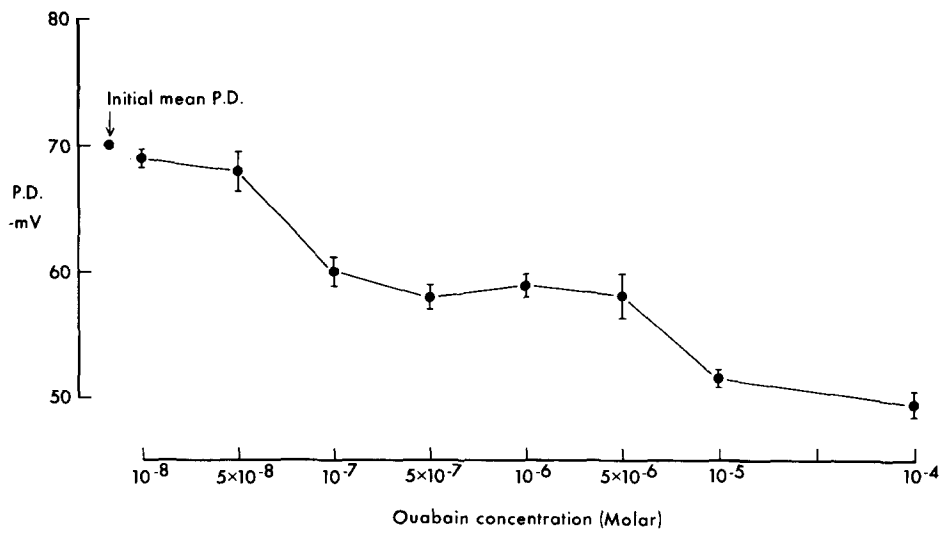

Fig. 4. Effect of specific ouabain concentrations on rabbit lens potential. Each filled circle represents the mean of 5 lenses, with the standard error shown as in Fig. 2. After an initial measurement, ouabain was added to the bath, and potential readings taken until no further change occurred (approx. 15 min). P.D., potential difference.

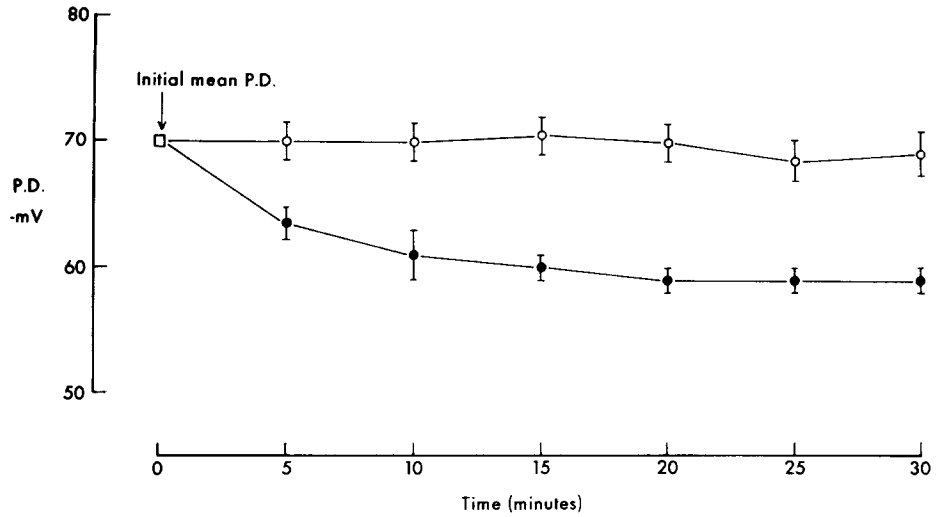

Fig. 5. Response of rabbit lens potential to $5 \cdot 10^{-6}$ M ouabain applied to anterior or posterior lens surface. The data represent the mean and standard error from measurements on 5 lenses. After an initial measurement, $5 \cdot 10^{-6}$ M ouabain was applied to either anterior or posterior surface and the potential measured at given time intervals. The presence of ouabain at the posterior lens surface caused no significant change (open circles). The potential fell characteristically when the anterior surface was exposed to ouabain (filled circles).

surface fell to -58.8 ± 0.2 mV, precisely the same as when the whole lens was exposed to the inhibitor (filled circles). Exposure of the posterior surface only to ouabain did not result in any significant change in potential over a 30-min period (open circles). If ouabain was then applied to the anterior surface, the potential fell characteristically.

DISCUSSION

The reduction of the normal rabbit lens resting potential from about -70 mV to about -59 mV and -52 mV by $5 \cdot 10^{-6}$ M ouabain and cold, respectively, suggested the existence of a component contributed by an electrogenic pump. The characteristics of such pumps have been summarized by Kerkut and York [1]. When a Na^+ pump which is not 1 : 1 coupled to K^+ actively moves Na^+ across the membrane a potential develops. The magnitude of the potential depends upon the resistance of the membrane; a high resistance leads to a large electrogenic component in the total potential. The electrogenic component falls quickly when the pump is inhibited, as for example, by reduced temperature or metabolic poisons. The fact that ouabain is effective only at the anterior surface provides additional evidence that this component of the potential results from an electrogenic Na^+ pump.

If one makes the assumption that the lens cation pump is abolished by $5 \cdot 10^{-6}$ M ouabain or reduced temperature, the remaining potential difference under these conditions should be due solely to the steady-state ion distribution and the permeability of the lens membranes to Na^+ and K^+, as described by the Hodgkin–Katz–Goldman equation [7]. The following version of that equation assumes chloride equilibrium in the lens, an assumption supported by the rabbit lens chloride analyses

of Paterson and Eck [9], a calculation by Duncan [10], and our unpublished observation that substitution of chloride by sulfate in the bathing medium results in only a transient change in the lens resting potential:

$$E = \frac{RT}{F} \ln \frac{[K]_o + \alpha[Na]_o}{[K]_i + \alpha[Na]_i} \quad (1)$$

where E is the membrane potential, R is the gas constant, T is absolute temperature, F is the Faraday constant, and α is the ratio of the membrane permeability to Na^+ and K^+. The bracketed quantities with subscripts o and i refer to external and internal ion concentrations, respectively. In general, at the ionic strength in biological systems, the errors introduced by using concentrations rather than activities in this equation are insignificant.

Using the Na^+ and K^+ concentrations of the lens and bathing medium from flame photometry data with the measured potential difference allows a calculation of α. The value obtained for lenses at 4 °C was 0.052 ± 0.002, and that for lenses exposed to $5 \cdot 10^{-6}$ M ouabain was 0.053 ± 0.004 (Table I). The fact that both cold and ouabain result in similar values for α implies that both treatments reduce the potential through inhibition of the Na^+ pump, rather than by affecting membrane permeability. The estimated values for α are close to that of 0.05, calculated by Duncan [11] for toad lenses. However, Brindley [3] calulated an α-value of 0.03 for rabbit lens on the basis of a -66 mV potential. From our results, Brindley's potential contains an electrogenic component which he did not take into account.

The fact that an electrogenic component of the potential generated at the anterior surface can be measured at the posterior lens surface implies (i) there is a high resistance to the passive movement of ions across the posterior lens surface, and (ii) there must be a low resistance pathway from the anterior to the posterior lens cells. Evidence for low resistance pathways from cell to cell in the lens has been provided by Rae [12], who demonstrated dye diffusion across "low resistance" tight junctions. In addition, the anatomical arrangement of the long lens fibers, which reach from anterior to posterior, might provide a suitable pathway.

The presence of a low resistance pathway through the lens, with an apparent high resistance across the lens surface may explain the extraordinary sensitivity of the lens potential to trauma. Even though the pump is not directly impaired, minor trauma may greatly reduce the resistance across the lens surface. Under this condition, the electrogenic component of the potential difference may no longer be measurable at the posterior surface. The low value of -42 mV reported by Kinsey and McGrady [4] might have resulted from damage extensive enough to alter the lens permeability.

The two-part effect of ouabain is also of some interest. It might be hypothesized that concentrations of ouabain up to $5 \cdot 10^{-6}$ M affect only the electrogenic activity of the lens, presumably by specifically inhibiting (Na^+–K^+)-ATPase. Increasing concentrations of ouabain alter α, leading to a change in potential as predicted by the Hodgkin–Katz–Goldman equation. Evidence that ouabain at 10^{-5} M concentration may affect lens cell permeability has been presented previously [13, 14].

ACKNOWLEDGEMENT

This study was supported by P.H.S. Research grant No. EY-00506-05 from the National Eye Institute.

REFERENCES

1 Kerkut, G. A. and York, B. (1971) The Electrogenic Sodium Pump, Scientechnica, Bristol
2 Paterson, C. A. and Rae, J. L. (1974) Exp. Eye Res., in the press
3 Brindley, G. S. (1956) Br. J. Ophthalmol. 40, 385–391
4 Kinsey, V. E. and McGrady, A. V. (1971) Invest. Ophthalmol. 10, 282–287
5 Paterson, C. A. and Eck, B. A. (1973) Exp. Eye Res. 15, 767–778
6 Paterson, C. A. (1970) Am. J. Physiol. 218, 797–802
7 Katz, B. (1966) in Nerve, Muscle and Synapse, p. 62, McGraw-Hill, New York
8 Paterson, C. A. (1972) Doc. Ophthalmol. 31, 1–28
9 Paterson, C. A. and Eck, B. A. (1971) Exp. Eye Res. 11, 207–213
10 Duncan, G. (1973) The Human Lens–in Relation to Cataract, Ciba Foundation Symposium 19, p. 99, Amsterdam
11 Duncan, G. (1969) Exp. Eye Res. 8, 315–325
12 Rae, J. L. (1974) Invest. Ophthalmol. 13, 147–150
13 Paterson, C. A. (1970) Exp. Eye Res. 10, 331–338
14 Kinsey, V. E. (1973) Exp. Eye Res. 15, 699–710