0014-4754/94/050438-04$1.50 + 0.20

Research Articles

Regulation of the retinal interphotoreceptor matrix Na by the retinal pigment epithelium during the light response

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Received 4 October 1993; accepted 17 January 1994

Abstract. We examined the rabbit retinal pigment epithelium (RPE) for Na transport properties which would allow it to buffer undesirable changes in Na concentrations in the interphotoreceptor matrix (IPM) during light and dark cycles. The RPE is selectivity permeable to sodium. Open and short circuit transport studies with RPE indicate a circulating (choroid to retina and back) Na current which does not compromise the electrical integrity of the blood brain barrier but together with the Na permselectivity is of sufficient magnitude to buffer both upwards and downwards movements of IPM [Na] during light or dark responses.
Key words. Retinal pigment epithelium; sodium; light response; retina.

The retinal light response induces a series of adventitious changes in the interphotoreceptor matrix (IPM) which are modulated by the actions of the surrounding cells. Changes to IPM [K^+] during the light response are buffered by voltage sensitive [K^+] channels in the Muller (glial) cells[1,2] and there is a smaller contribution to potassium buffering from the horizontal cells[2] and the retinal pigment epithelium[3–5] (RPE) lining the outer surface of the IPM. Light induced IPM acidosis is partly buffered by Na-H exchange across the Muller cells but is mainly buffered by the naturally occurring bicarbonate/CO_2 buffer in the IPM fluid[6]. IPM [Na^+] also changes during the light response: if a typical mammalian dark adapted rod retina (30 pA dark current/photoreceptor: 30 photoreceptors/RPE cell: 6×10^3 RPE cells/mm^2: IPM radial depth 70 μm) experiences a bleaching flash of light (effectively closing all the cGMP gated Na^+ re-entry channels in the rod outer segments[7]) there is an initial rate of increase of IPM [Na^+] of about 0.8 mM/sec. After a brief period, at least two mechanisms will come into effect, driven by the photoreceptors themselves, which will modulate the rate of build up of IPM [Na^+]. They are a) a decline in the activity of the Na^+/K^+ pump activity in rod photoreceptors during maintained illumination[8], and b) light adaptation itself, invoking the Na-Ca, K exchange mechanism across the rod outer segment membrane[9]. These responses would not be sufficiently rapid to cope with IPM hyperpolarisation induced by the light response and its associated osmotic pressures. Driven by an unalleviated (unbuffered) IPM [Na^+] build up, the osmotic pressure increase would generate an IPM hydrostatic pressure rise in a dark adapted bleaching light exposure equivalent to a rise of 16 cm H_2O/sec and within a second or so light generated IPM hydrostatic pressure rises would neutralise intra-ocular pressure and act to detach the neural retinal from the RPE. There is a need for a mechanism to buffer IPM [Na^+] during the light response in order to obviate damaging pressure rises.

While it is a normal function of glial cells, such as the Muller cells in the retina are, to buffer extracellular [K^+] in neural tissue via electrogenic K^+ channels, there is no report of Na^+ channels in Muller cells, although Na coupled glutamate channels exist[10] which act to buffer the retinal neurotransmitter glutamate but are of insufficient magnitude to buffer IPM [Na^+] changes of the magnitude required here. We therefore looked to RPE to check for potential IPM [Na^+] buffering capabilities. Previous studies have demonstrated that the RPE has an intra IPM net Na^+ flux[11,12] and as there is considerable uncertainty about the viability of RPE separated from the neural retina upon which part of the study must inevitably be based, we chose to study the rabbit RPE where in vitro data could be compared to in vivo data as a partial justification of the physiological validity of this study.

Materials and methods

In vivo measurements. The electrical potential difference between the vitreous humour and the retro-bulbar space of rabbits was measured whilst the rabbits were anaesthetised with sodium pentobarbitone or urethane. Instrument zero was recorded before insertion of the electrodes and by inserting both electrodes into the retro-bulbar space or into the vitreous humour. The electrodes gave a slow temporal response of several seconds duration, which helped to stabilise fluctuations in the d.c. voltage output measured across the rear of the eye.

In vitro measurements. Rabbits were overdosed intravenously with sodium pentobarbitone and the eyes dis-

sected. The whole eyes were incubated at 25 °C for 45 min in balanced Ringer (121 mM NaCl, 5 mM KCl, 24 mm $NaHCO_3$, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 1 mM $NaHPO_4$, 5.5 mM glucose, 1 mM reduced glutathione equilibrated against a gas phase of 5% CO_2, 20% O_2, 75% N_2) in order to weaken the adhesive forces between the photoreceptors and RPE. The orb was then attached with cyanoacrylate glue to the scleral half of an Ussing chamber, and the anterior segment of the eye was dissected off the preparation with scalpel and Vannas scissors. The neural retina was teased away with fine forceps whilst the superfusing ringer was stirred. The Ussing chambers were sealed and the sclera-choroid-RPE preparation was perfused with Medium 199 at 20 ml. hr^{-1} whilst stirred with intrachamber paddles at 0.7 Hz. The whole assembly was maintained at 37 °C in an oil bath. Hydrostatic pressure across the preparation was set at 10 cm H_2O. Stabilisation periods of 40 min were allowed before electrically short circuiting the spontaneous electrical potential of a few mV with externally applied current from Ag/AgCl electrodes set into the ends of the Ussing chamber. The whole assembly is illustrated in figure 1. Radio-isotopes ^{22}Na, ^{36}Cl, $H^{14}CO_3$, were introduced to the Ringer on one side of the preparation and their rate of appearance in the alternate chamber used to calculate the flux rates of the ions across the preparation. In order to calculate the flux rates across the RPE alone, at the end of each experiment, as described later, the RPE was scraped off the preparation and the flux rates determined for the choroid and sclera. The magnitude of fluxes (determined, by isotope fluxes) was alternated, so that it was possible to determine uni-directional fluxes both in the direction retina to choroid and also choroid to retina. In some preparations, the retina was also mounted in the Ussing chamber in order to compare spontaneous electrical signals in vitro to those found in vivo, across the whole posterior segment of the eye.

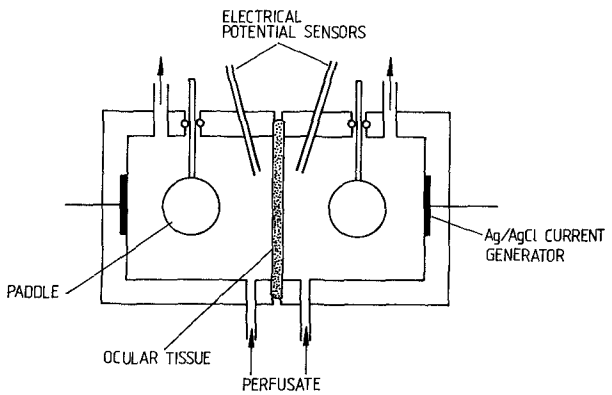

Figure 1. The tissue (usually RPE-choroid-sclera) is mounted between Ussing chambers. Spontaneously generated electrical potentials are detected by sensor tubes and if desired the spontaneous electrical potential can be neutralised by the (series resistance corrected) application of current between the Ag.AgCl current generating electrodes. Ion fluxes are measured by the introduction of suitable radioisotope into one half chamber and detecting its rate of appearance in the other half chamber. Temperature 37 °C.

Results and discussion

In vivo measurements of the electrical potential difference between the vitreous humour and the retro-bulbar space of rabbits anaesthetised with sodium pentobartibone or urethane were similar (3.4 ± 0.4 mV, vitreous humour positive, mean $\pm$ SEM, $n = 14$) to that found across the retinal pigment epithelium membrane in vitro (3.1 ± 0.3 mV, vitreous side positive, mean $\pm$ SEM, $n = 27$). After an initial stabilisation period of a few minutes, the potentials were stable for about 5 h. These series of trans epithelial potential measurements indicated that our isolated RPE preparations behaved similarly in vitro and in vivo. The electrodes used in vivo had a relatively long response time, of the order of seconds, and so it was not possible to detect electrical transients generated by the retinal light response.

Studies of rabbit trans RPE net Na^+ transport under conditions of electrical short circuit, indicated a flux into the retinal IPM of magnitude 3.11 ± 0.27 µmoles/cm^2 h (mean $\pm$ SEM, $n = 27$) and no significant net chloride flux, in contrast to a relatively small reported intra choroidal net chloride flux across amphibian RPE[13,14]. A typical data set of bi-directional Na^+ and Cl^- fluxes is shown in figure 2. In the choroidal direction (when both Na^+ and Cl^+ fluxes were passive), it was clear that the passive Na^+ fluxes were consistently higher than the passive Cl^- fluxes. This apparent RPE permselectivity in favour of Na^+ was monitored in more detail. Ion fluxes of the major ions in the culture medium (Na^+, Cl^-, HCO_3^-) were measured in the direction retina to choroid across preparations of RPE/choroid/sclera and then, after wiping off the RPE cells with gently mechanical rubbing, across preparations of choroid and sclera alone. Electrical resistance of the first preparation was designated R_{E+S} and the electrical

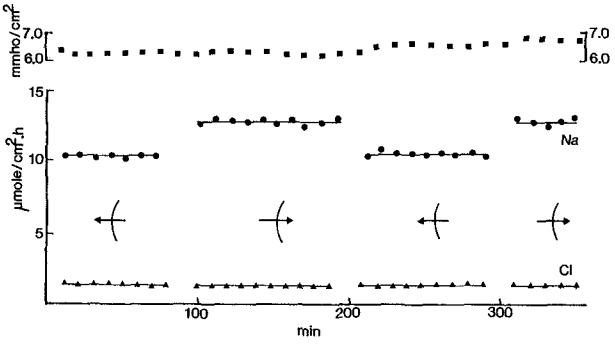

Figure 2. Short circuited stabilised rabbit retinal pigment epithelia have a high net sodium flux (3 µoles/cm^2 h) into the interphotoreceptor matrix (IPM). Passive sodium fluxes (into the choroid) are about eight times higher than passive chloride fluxes. No net chloride transport was noted. Typical electrical resistance of the RPE was 150 Ω cm^2.

resistance when the epithelium was removed was designated R_S. The calculated electrical resistance of the RPE, R_E, was the simple difference of the two resistances.

The ionic permeabilities were calculated from the measured ion fluxes of both the RPE/choroid/sclera preparation (P_{E+S}) and the choroid/sclera preparation (P_S). The ionic permeability of the RPE alone (P_E) was then calculated from the relationship[15]

$$\frac{1}{P_E} = \frac{1}{P_{E+S}} - \frac{1}{P_S}.$$

The ionic conductance g_i, for each ion was then calculated from Hodgkin's equation[16] $g_i = (F^2/R2)P_iC_i$ where F = Faraday's constant, RT has its usual meaning, P_i is the measured permeability of the ion 'i' across the RPE and C_i is the concentration of the ion in the bathing medium. It was then possible to calculate the electrical conductance of each ion (Na^+, Cl^-, or HCO_3^-) across the RPE and compare their summed conductance (the summed conductance of these three ions approximated well to the whole conductance of the RPE) to the measured electrical conductance of the RPE (which is, of course, the reciprocal of the electrical resistance). Over the range of RPE electrical resistances recorded in nine preparations, (110–180 Ω cm²) Na^+ conductance consistently accounted for about 95 ± 4% (mean ± SEM, n = 9) of the total ionic conductance of each RPE preparation (fig. 3). In a non perm-selective monolayer of cells such as rabbit corneal endothelium, Na^+ and Cl^- permeabilities are about equal and Na^+ conductance accounts for about 50% of total trans-endothelial ionic conductance[17]. RPE is about 20 times more permissive of the (passive) passage of Na ions across itself than would be expected from its free diffusion coefficient. As would be expected from this extreme permselectivity, histological examination of a number of our preparations by scanning electron microscopy indicated an intact mosaic of functional RPE cells.

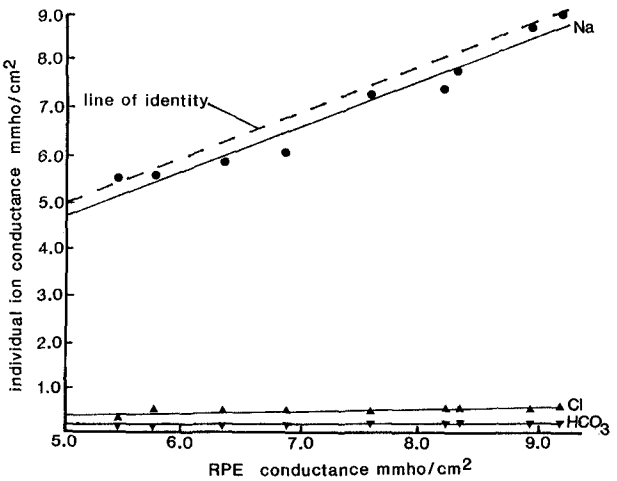

Figure 3. Preparations of RPE choroid and sclera were maintained as in figure 1. Radio-isotopes (^{22}Na, ^{36}Cl, $H^{14}CO_3$) were introduced into the retina side and their rate of efflux through the tissue gave estimates of the ionic permeabilities of the preparation. The chambers were opened, the RPE was removed from the preparation with tissue paper and the rate of efflux through the choroid and sclera preparation determined. Ionic conductances of Na, Cl and HCO_3 across the RPE were calculated as described in the text and compared to the electrical conductance of the RPE. Nearly all (95 ± 4%) trans RPE electrical conductance is carried by Na^+.

We can conclude from the data that rabbit RPE pumps Na^+ into the IPM and that the Na^+ electrochemical gradient across the RPE between the IPM and choroid is dissipated mainly by a paracellular Na^+ current generated by the permselective barrier properties probably located at the zonula occludens at the apical end of the paracellular route between the cells. The model derived from the current study is that RPE generates a circulating Na^+ current. The Na^+ travels from the cytoplasm into the IPM via the apical Na^+-K^+-ATPase[11], back through the paracellular route into the choroid and then returns into the cell via baso-lateral Na-K-Cl co-transporters[18], or Na-HCO_3[19] co-transporters.

If we calculate events arising in the IPM over a single cell of the RPE, then in the dark, the circulating sodium current of all 30 rod photoreceptors is, in total, 900 pA and the circulating RPE current for one cell is 200 pA. After light is absorbed by the photoreceptor the increased IPM [Na^+] will drive the excluded Na^+ across the RPE. Calculations based on the sodium conductance of the RPE (fig. 3) indicate that this light-driven-increase in IPM [Na^+] will be effectively buffered by the associated increased passive Na^+ current through the RPE.

We suggest that RPE acts to protect the IPM from undesirable increases in [Na^+] during increased light intensities by acting as a Na^+ permselective membrane. Its active intra IPM Na^+ pump is further suggested to have the physiological role of restoring the total Na^+ in the photoreceptor/IPM space during recovery in the dark period.

Acknowledgment. This study was supported by the British Retinitis Pigmentosa Society.

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