Hyperosmolarity-induced hyperpolarization of the membrane potential of the retinal pigment epithelium

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Abstract. The hyperosmolarity-induced changes of the apical (V_{ap}) and basal (V_{ba}) membrane potentials of the retinal pigment epithelium (RPE) were studied in an in-vitro RPE-choroid preparation of the frog. Both V_{ap} and V_{ba} were simultaneously hyperpolarized by hyperosmolarity at either the apical or basal side of the RPE. Hyperosmolarity at the apical side hyperpolarized V_{ap} greater than V_{ba}, and increased the trans-epithelial potential (TEP) across the RPE. Hyperosmolarity at the basal side hyperpolarized V_{ba}, simultaneously hyperpolarized V_{ap} by a smaller amount, and reduced the TEP. The hyperosmolarity response (a decrease of the ocular standing potential induced by an intravenous hypertonicity) is due mainly to a hyperpolarization of V_{ba}.

Introduction

The hyperosmolarity response, a decrease of the ocular standing potential induced by an intravenous hypertonicity, originates mainly in the retinal pigment epithelium (RPE), and is useful for diagnosis of pigment epitheliopathy in diseases of the eye such as diabetic retinopathy, retinal detachment, retinitis pigmentosa, and genetic macular dystrophies (Yonemura et al., 1979; Madachi, 1982a–c; Kawasaki et al., 1984; Madachi-Yamamoto et al., 1984; Yonemura et al., 1984). Hyperosmolarity has been previously shown to reduce the transepithelial potential (TEP) across the isolated RPE-choroid in the human and the frog when applied to the basal side of the RPE (Mukoh et al., 1982; Kawasaki et al., 1983). The hyperosmolarity response, therefore, depends mainly on the effect of hyperosmolarity on the basal side of the RPE.

In the present study, effects of hyperosmolarity on membrane potentials of the RPE cells were investigated to determine whether a hyperosmolarity-induced decrease of the TEP was generated at the apical or basal membrane or across the paracellular shunt.
Materials and methods

The isolated RPE-choroid preparations of the bull-frog (Rana catesbiana) were used. The RPE-choroid preparation was dissected by the method previously described (Mukoh et al., 1982; Kawasaki et al., 1983), and then mounted in a chamber similar to that described by Miller and Steinberg (1977). The area of the tissue exposed to the perfusate was 0.07 cm².

The two sides of the tissue, apical and basal, were immersed in 3.0-ml baths and continuously perfused at 10 ml/min. The bathing solution was a modified Ringer's with the following composition (in mM): 94.0 NaCl, 2.0 KCl, 1.0 MgCl₂, 1.8 CaCl₂, 15.0 NaHCO₃, 10.0 glucose; it was perfused after bubbling with 100% oxygen. The pH, the osmolarity, and the temperature of the solution were 8.05 ± 0.05, 2.2 × 10² mOsmol, and 20 ± 1 °C respectively.

A pair of silver—silver chloride electrodes was placed symmetrically on each side of the tissue to record the TEP. The TEP was led to a DC amplifier (Nihon Kohden, RDU-5, −3 dB at 200 Hz), and recorded on a pen recorder (Riken, SP-G6P). The glass-microelectrode was used for an intracellular recording from RPE cells. It was filled with 3 M KCl, bevelled to an impedance of 30–80 MΩ, and then inserted into the RPE cell from the apical surface. The apical membrane potential (Vₐₚ) was measured differentially between the intracellular microelectrode and the silver—silver chloride electrode placed in the apical bath by means of a high-impedance preamplifier (Nihon Kohden, MEZ-7101). The basal membrane potential (Vₐₚ) was measured between the microelectrode and the reference electrode in the basal bath by means of a high-impedance preamplifier (Nihon Kohden, MEZ-8201). Both Vₐₚ and Vₐₚ were further amplified (Nihon Kohden, AVH-10, −3 dB at 100 Hz), displayed on both a pen recorder (Riken, SP-G6P) and a memory oscilloscope (Nihon Kohden, VC-10), and stored on magnetic tape (Sony, NFR-3000).

The osmolarity of the bathing solution was increased 100 mOsmol above the control level by the addition of fructose.

Results

In all 13 tissues tested, the apical surface of the RPE was electrically positive with respect to the choroidal surface, and the TEP was between 4.00 mV and 9.30 mV (6.32 ± 1.93 mV). Membrane potentials of the RPE were measured from 30 to 180 min after the cells were impaled with a microelectrode. Vₐₚ of 24 cells ranged from −80.0 to −97.0 mV (−88.1 ± 4.8 mV). Effects of hyperosmolarity at the apical or basal side on the TEP and membrane potentials are illustrated in Figures 1–4. These potentials were recorded on the same tissue and the same cell. When the osmolarity of the solution in the apical bath was raised by adding fructose, both Vₐₚ and Vₐₚ transiently