ACTION OF LEUKOTRIENE B_4 ON THE CORNEAL EPITHELIAL CELLS OF THE BULLFROG, Rana catesbiana

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The cornea of the bullfrog secretes chloride, an action responsible for maintaining corneal hydration and transparency (Zadunaisky, Amer. J. Physiol. 211:506-512, 1966; Zadunaisky and Lande, Amer. J. Physiol. 221:1837-1844). The model for epithelial chloride secretion includes the transport of chloride basolaterally into the cell coupled to the passive conductance of chloride apically via chloride channels. Agents that elevate corneal cAMP levels result in stimulating chloride transport (Zadunaisky, et al., Exp. Eye Res. 15:577-584, 1973). Leukotriene B_4 (LTB_4) is an endogenous eicosanoid that inhibits the corneal short-circuit current and increases tissue resistance (Schaeffer and Zadunaisky, Invest. Ophthalmol. and Vis. Sci., accepted for publication). We explored the effect of LTB_4 on corneal epithelial cells to elucidate the site of action and cellular events that culminate in the reduction of chloride secretion.

Bullfrog corneas were dissected from the eye by cutting 1 mm from the limbus and then mounted in an Ussing-style perfusion chamber. Each side of the corneal tissue was perfused with frog Ringers gassed with 95% O_2/5% CO_2, pH=7.3 except during the LTB_4 perfusion when the Ringers was equilibrated with 95% air/5% CO_2, to diminish LTB_4 oxidation. Corneal transepithelial potential (TEP) was measured using Ringer-agar bridges to calomel electrodes coupled to a Physiologic Instruments Voltage-current clamp. Tissue resistance (R_t) was monitored periodically with a current pulse of 5 μA. Single-barreled microelectrodes were filled with 0.5 M KCl (tip resistance = 10-40 MΩ).

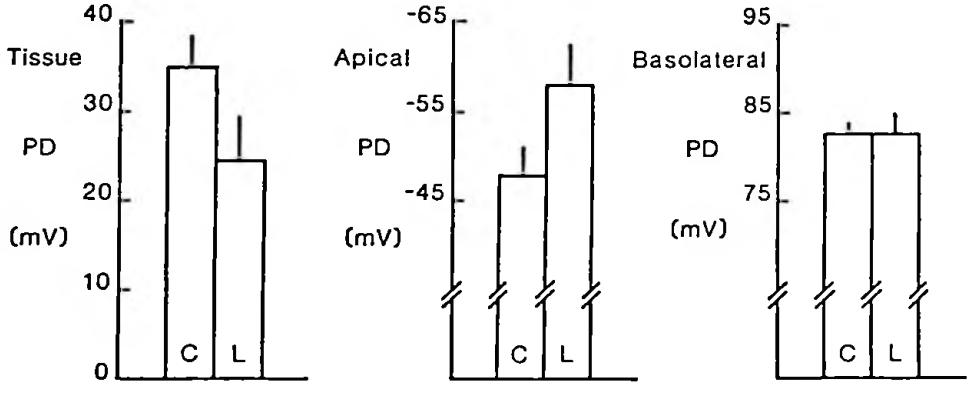

Figure 1. Voltage changes observed with the perfusion of 10^{-7} M LTB_4 serosally. The histogram bars represent the mean, with the vertical lines representing the standard error of the mean (n=6). The letters, C and L, represent the control and 10^{-7} M LTB_4 conditions, respectively.

The addition of LTB_4 (10^{-7} M) serosally caused the TEP to drop 29% and the apical cell voltage to hyperpolarize 21% (figure 1). The decrease in the TEP corresponded to a significant 44% reduction of the calculated corneal current from 30.0 ± 4.7 to 18.4 ± 4.2 uA/cm^2 (n=6). Basolateral cell voltage remained constant at 82.6 mV. The inhibition of the epithelial TEP was based solely on the apical membrane voltage change, a difference of 10.2 mV.

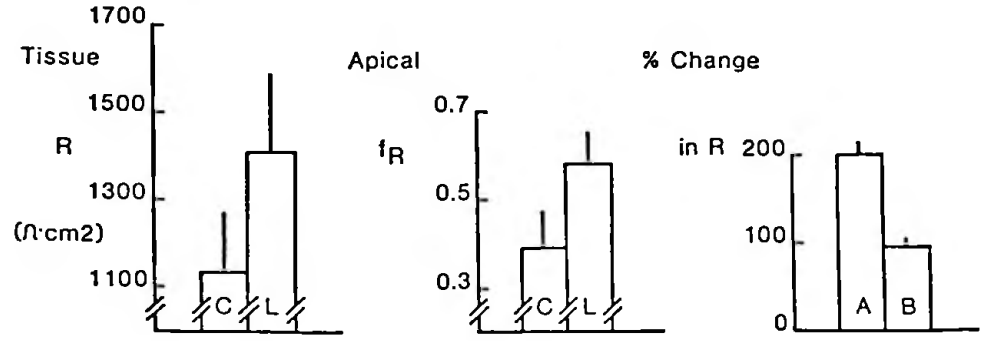

Figure 2. Resistance parameters change with the serosal perfusion of 10^{-7} M LTB_4. The bars are the mean values (n=6) and the vertical lines are the mean standard error. Conditions are represented by C (control) and L (serosal 10^{-7} M LTB_4). Apical resistance change is denoted by A and B represents the basolateral resistance change.

Tissue resistance increases 24% as a result of LTB_4 treatment (figure 2). The perfusion of LTB_4 increases in apical fractional resistance (f_R) 49% from 0.39 ± 0.08 to 0.58 ± 0.07 (n=6). The increase in R_t coupled with the change in apical f_R represents a 100% increase in the apical resistance with only a 15% decrease occurring in the basolateral resistance. Thus, the total resistance increase is primarily from the apical side.

The primary effect of LTB_4 is a decrease in apical chloride conductance that causes an apical cellular hyperpolarization. In the corneal epithelial cells, LTB_4 is acting to inactivate apical chloride channels, thereby increasing apical resistance.

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