ORGANIC ANION UPTAKE BY TRABECULAR MESHWORK (TBM) CELLS FROM HUMAN EYE

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Certain fluid compartments of the body, e.g., aqueous humor and cerebrospinal fluid, possess their own "internal kidneys," responsible for the removal of potentially toxic normal metabolites, drugs and drug metabolites (Pritchard and Miller, Physiol. Rev. 73:765, 1993). Many of these substances are transported as charged compounds and the tissues responsible possess separate plasma membrane transport systems for organic anions and organic cations. In the present report, we investigated the organic anion transport properties of cultured trabecular meshwork cells (TBM) from human eye using a fluorescent organic anion, fluorescein (FL), video microscopy and digital image analysis.

Transfected TBM cells from human eye (Alcon Laboratories) were grown at 37° C on 4x4 cm glass cover slips in Dulbecco's Modified Eagle's Medium (DMEM, low glucose) supplemented with 10% fetal bovine serum, glutamine and Pen/Strep. For experiments, cover slips were mounted in a chamber containing DMEM with 1 μM FL and viewed by means of an inverted microscope equipped with epi-fluorescence optics and a video camera and connected to a Macintosh computer (Miller et al., Am. J. Physiol. 264:R882, 1994).

TBM cells incubated in medium with FL rapidly concentrated the dye, reaching steady-state levels within 10 min (Fig. 1A). At steady state, diffuse fluorescence filled both the cytoplasm and nucleus. Also, areas of intense punctate fluorescence were seen throughout the cytoplasm. These were most concentrated in the perinuclear region, which is the thickest part of the cell. FL uptake was abolished when metabolism was inhibited by KCN. Addition of prostaglandin PGE2 (0.5-5 μM) to the medium caused a concentration dependent decrease in FL accumulation (Fig. 1B). At the highest concentration used, PGE2 reduced FL accumulation by 59%. The organic anion, probenecid, at 1 mM, reduced FL uptake by 52%. p-Aminohippurate did not inhibit FL uptake, rather it caused a small increase (30-40%, not shown).

To date organic anion uptake mechanisms have only been found in those cells functioning to absorb or secrete metabolites and xenobiotics, e.g., renal and hepatic epithelial cells. The present preliminary findings indicate that human TBM cells possess a transport system for organic anions that is specific, uphill and dependent on cellular metabolism. This system may play a role in removing drugs and physiologically important metabolites from aqueous humor. Supported by NIH grant EY 01340.

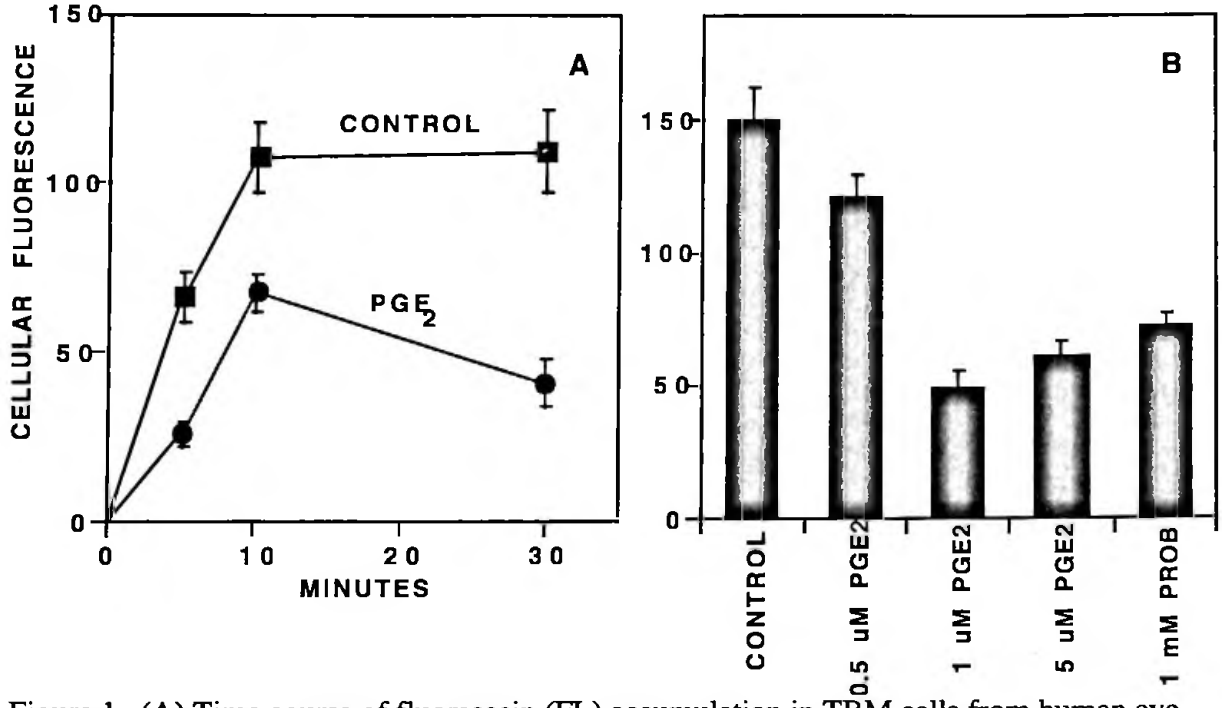

Figure 1. (A) Time course of fluorescein (FL) accumulation in TBM cells from human eye. The PGE_2 concentration was 5 μM. (B) Effects of PGE_2 and probenecid on the 30 min uptake of 1 μM FL. Values are mean pixel intensities ± SE for 10-19 (A) or 28-29 (B) cells. Statistical comparisons: (A) PGE_2 reduced fluorescence intensity at all times, $P<0.01$; (B) 1-5 μM PGE2 and probenecid reduced fluorescence intensity, $P<0.01$.