EVIDENCE FOR A P-GLYCOPROTEIN-LIKE TRANSPORTER IN THE APICAL PLASMA MEMBRANES OF SHARK (SQUALUS ACANTHIAS) RECTAL GLAND EPITHELIAL CELLS

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Rhodamine 123 is a fluorescent cationic lipophilic dye which is selectively concentrated in respiring mitochondria due to the high proton-dependent, inside negative potential across their inner membranes (Chen, Ann. Rev. Cell Biol. 4:155, 1988). In a previous study, FCCP/DNP-sensitive rhodamine 123 staining of mitochondria in native and cultured shark rectal gland cells was described (Smith, Bull MDIBL 29:14, 1990). When rhodamine was selectively applied to either the apical or basolateral side of cultured SRG cells grown on permeable supports in Millipore Millicells, staining was observed only when rhodamine was present in the basolateral bath. Differential plasma membrane permeability was proposed to explain this unexpected finding.

Recent studies show that rhodamine is transported from a variety of cell types by the multidrug transporter, P-glycoprotein (Neyfakh, Exp. Cell Res. 174:168, 1988). P-glycoprotein catalyzes the efflux of a variety of hydrophobic xenobiotics from cells and is localized specifically in the apical plasma membranes of several epithelia (Endicott and Ling, Ann. Rev. Biochem. 58:137, 1989). The endogenous substrates for P-glycoprotein are unknown. These data suggest an alternative explanation for polarized rhodamine staining of SRG cells (Fig. 1). According to this model, when rhodamine is added to the basolateral bath, it diffuses into cells and is rapidly accumulated by mitochondria. In contrast, apically applied rhodamine enters cells but P-glycoprotein transporters present in the apical plasma membrane rapidly catalyze its efflux. Because mitochondria in cultured SRG cells are excluded from the apical cytoplasm (Valentich and Garretson, J. Cell Biol. 105:140a, 1987), rhodamine entering cells apically does not encounter mitochondria before it is pumped from the cytoplasm by P-glycoprotein.

A prediction of this model is that inhibiting P-glycoprotein activity should result in rhodamine staining when the dye is added to the apical bath. To test this hypothesis, SRG monolayer cultures in Millicells (Valentich & Forrest, Bull. MDIBL 26:91, 1986) were treated with 10^{-5}M reserpine for 30 min before adding 10ug/ml rhodamine to the apical bath. Reserpine is a P-glycoprotein inhibitor and enhances rhodamine staining of multidrug-resistant fibroblasts, presumably by inhibiting dye efflux (Neyfakh ibid.). After incubating for 10 min with rhodamine, cultures were rinsed thoroughly with shark Ringer and examined by epifluorescence microscopy. In confirmation of the results of Smith (ibid.), control cultures not treated with reserpine failed to exhibit staining when rhodamine was applied apically. However, in reserpine-treated cultures cells displayed low, but distinct, mitochondrial staining in response to apical rhodamine. Electrophysiological studies showed that treating SRG cultures with reserpine caused no change in transepithelial resistance, indicating that monolayer integrity remained intact. These data are consistent with the model shown in Fig. 1.

Direct functional and biochemical assays for P-glycoprotein are necessary to unequivocally demonstrate the existence and resolve the function of this ubiquitous transport moiety in SRG cells. Molecular genetic studies show

extensive structural similarities between P-glycoprotein and the cystic fibrosis transmembrane regulator protein, CFTR (Juranka et al., FASEB J. 3:2583, 1989). Mutations in the latter molecule are responsible for the anomalous regulation of epithelial chloride secretion which underlies the pathogenesis of cystic fibrosis (Riordan et al., Science 245:1066, 1989). Recently, Grzelczak et al. (Pediatr. Pulmonol. Suppl. 5, p. 195, 1990) have described the cloning and expression of a gene present in SRG cells which encodes a protein sharing 70% amino acid identity with human CFTR. The functional relationships between P-glycoprotein and CFTR and their role in regulating epithelial chloride secretion are critical questions for which the SRG may provide a powerful investigative tool.

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Figure 1

Interpretation of Rhodamine 123 Staining Results