EVIDENCE THAT CFTR DOES NOT TRANSPORT MRP SUBSTRATES

Timothy J. Jensen, Xiu-bao Chang, John R. Riordan
Mayo Graduate School of Medicine, Mayo Clinic, Scottsdale, AZ 85259

Preliminary observations had suggested that CFTR may share some functional capabilities with the related multidrug resistance proteins (Linsdell and Hanrahan, Am J Physiol 275:C323-C326, 1998; Linsdell and Hanrahan, Br J Pharmacol 126:1471-1477, 1999). This possibility seemed reasonable since CFTR and the MRPs belong to the same subfamily (ABCC) within the large superfamily of ABC proteins (Klein, I, et al., Biochim Biophys Acta 1461:237-262, 1999). However in the past several years a great deal of effort had been devoted to demonstrating that, despite their sequence and presumed structural similarity, the proteins are distinct in their primary functions. That is MRPs actively export anionic hydrophilic conjugates of hydrophobic compounds out of cells whereas CFTR forms a channel for the passive conductance of halides. Nevertheless, the finding that glutathione conjugates that are transported by MRP1 and 2 when present at the cytoplasmic side of CFTR block Cl^- conductance suggested that CFTR might also have a transport site for these compounds. Our goal in this project was to determine if CFTR could transport MRP substrates.

Our basic approach was to apply the same method that is commonly used to assay the transport of organic anions by MRPs (Chang, X.-B., et al., J Biol Chem 272:30962-30968, 1997) using everted membrane vesicles from cells expressing these proteins. We had previously expressed both CFTR (Chang, X.-B., et al., J Biol Chem 268:11304-11311, 1993) and MRP1 (Hou, Y.-X., et al., J Biol Chem 275:20280-20287, 2000) in a mammalian cell line (BHK21). For this project the cDNA for MRP2 was obtained from Dieter Keppler and expressed in the same cells. We also prepared recombinant baculoviruses capable of expressing both MRPs in insect Sf9 cells; we had already done this for studies of CFTR channel function (Kartner, N., et al., Cell 64:681-691, 1991). Membrane vesicles were isolated from both cell types and uptake of ^{3}H-leukotriene C_4 measured as we have described for MRP1 (Hou, Y.-X., et al., J Biol Chem 275:20280-20287, 2000).

Western blots showing the proteins in the Sf9 vesicles are presented in Fig. 1A and in BHK in Fig. 1B. A time course of uptake of ^{3}H-LTC_4 by vesicles from BHK cells is shown in Fig. 2. The clear-cut result is that there is robust ATP-dependent uptake by the MRP1 containing vesicles but not by those containing CFTR even though they were phosphorylated by protein kinase A to fully activate its channel activity. Very similar results were obtained with Sf9 vesicles. Although MRPs do not transport reduced glutathione alone, others have suggested that CFTR may do so and hence it's absence might be responsible for the lower levels of GSH reported in the airway surface fluid of patients (Linsdell and Hanrahan, Br J Pharmacol 126:1471-1477, 1999). Therefore we also assayed for ATP-dependent uptake of ^{3}H-GSH by the same vesicle preparations but none was detected.

These experiments have essentially confirmed the null hypothesis, i.e. CFTR does not transport an MRP organic anion substrate under conditions where MRP does nor does it appear to transport GSH. Therefore it can be concluded that while ABC transporters including the MRPs do contribute to excretion of toxic organic substances from marine organisms, it is highly unlikely that CFTR plays such a role.

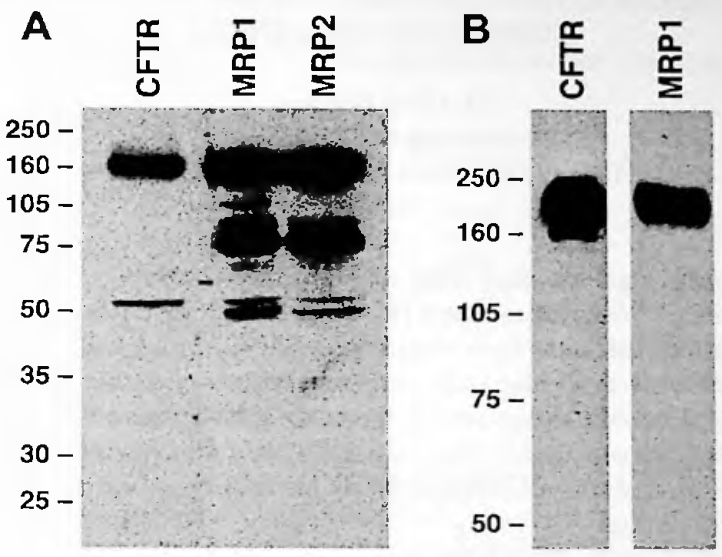

Figure 1. Western blots showing CFTR and MRP1 and MRP2 proteins in membrane vesicles from Sf9 cells infected with the baculoviruses containing the cDNAs for each of these proteins (**A**) and from BHK cells stably expressing CFTR and MRP1 (**B**).

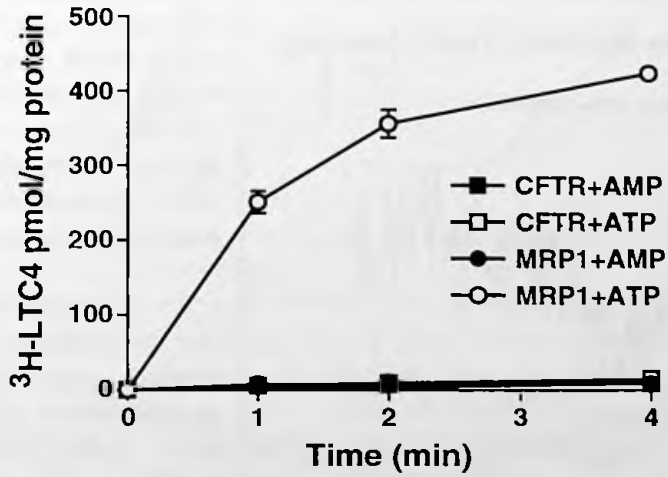

Figure 2. Time course of ^{3}H-leukotriene C_4 uptake by BHK membrane vesicles containing either CFTR or MRP1 in the presence of either 4mM ATP or AMP.