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Short communication

Block of CFTR-dependent chloride currents by inhibitors of multidrug resistance-associated proteins

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Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) is a membrane protein that belongs to the same family as multidrug resistance-associated proteins whose main function is to expel xenobiotics and physiological organic anions from the cell interior. Despite the overall structural similarity with these membrane proteins, CFTR is not an active transporter but is instead a Cl^- channel. We have tested the ability of known inhibitors of multidrug resistance-associated proteins to affect CFTR Cl^- currents. We have found that sulfinpyrazone, probenecid, and benzbromarone are also inhibitors of CFTR activity, with a mechanism involving blockage of the channel pore.

Keywords: CFTR; Multidrug resistance protein; Chloride channel; Channel blocker

1. Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) is a plasma membrane protein that belongs to the family of ATP-binding cassette (ABC) transporters (Dean et al., 2001; Schmitt and Tampe, 2002). Such membrane proteins share a similar architecture based on various transmembrane helices (from 12 to 17) and two nucleotide binding domains (NBD1 and 2). Interaction and hydrolysis of ATP at two sites in the NBDs induces conformational changes that drive active transport of various types of molecules across the plasma membrane (Dean et al., 2001; Schinkel and Jonker, 2003). CFTR is part of the subfamily C of ABC (ABCC) transporters which includes the multidrug resistance-associated proteins (Kruh and Belinsky, 2003). These proteins work as active transporters of endogenous substrates, like ABCC1 for LTC_4 (Leier et al., 1994; Jedlitschky et al., 1994), and of exogenous substances, called xenobiotics. Such compounds are transported in their native state or as conjugates with glutathione (Ishikawa, 1992), glucunorate, or sulfates (Jedlitschky et al., 1996). In general, ABCC drug transporters have a preference for anionic compounds in contrast to the multidrug resistance protein 1, ABCB1, which is more selective for neutral or slightly basic compounds (Schinkel and Jonker, 2003). The wide spectrum of substances translocated by multidrug resistance proteins is beneficial because it provides protection against potentially toxic exogenous molecules (Leslie et al., 2001; Hipfner et al., 1999). However, many ABCC transporters, as well as ABCB1, are also responsible for the multidrug resistance shown by different types of human tumours (Grant et al., 1994; Kruh et al., 2001; Sawicka et al., 2004).

Among the ABCC subfamily, CFTR is the only protein that does not generate an active transport. In fact, CFTR is a plasma membrane Cl^- channel (Anderson et al., 1991) in which the conformational changes generated by NBD/ATP interactions are not used for active transport but rather for the opening and closing of the pore (Sheppard and Welsh, 1999). However, there are still some intriguing findings that suggest that multidrug resistance-associated proteins and CFTR have some similarities beyond the amino acid sequence homology. For example, it has been reported by some investigators that CFTR is also able to translocate glutathione as done by other ABCC proteins (although by passive diffusion and not by active transport) (Linsdell and Hanrahan, 1998). Furthermore, substrates of

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multidrug resistance-associated proteins inhibit CFTR Cl^- currents by interacting with the CFTR pore from the cytosolic side (Linsdell and Hanrahan, 1999). This suggests a common mechanism of interaction at the level of the transmembrane portion of the proteins.

We have tested the ability of known ABCC inhibitors to affect CFTR Cl^-currents. This is important to further explore the analogies between CFTR and ABCC drug transporters and, possibly, to develop novel CFTR blockers which could be useful for the treatment of secretory diarrhea (Verkman et al., 2006). Our data show that sulfinpyrazone, probenecid, and, particularly, benzbromarone are effective inhibitors of the CFTR channel through a probable block of the pore.

2. Materials and methods

2.1. Cell culture

Fischer rat thyroid (FRT) cells stably expressing human CFTR were cultured on plastic in Coon's modified F12 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. T84 cells were cultured in DMEM/F12 plus 10% fetal bovine

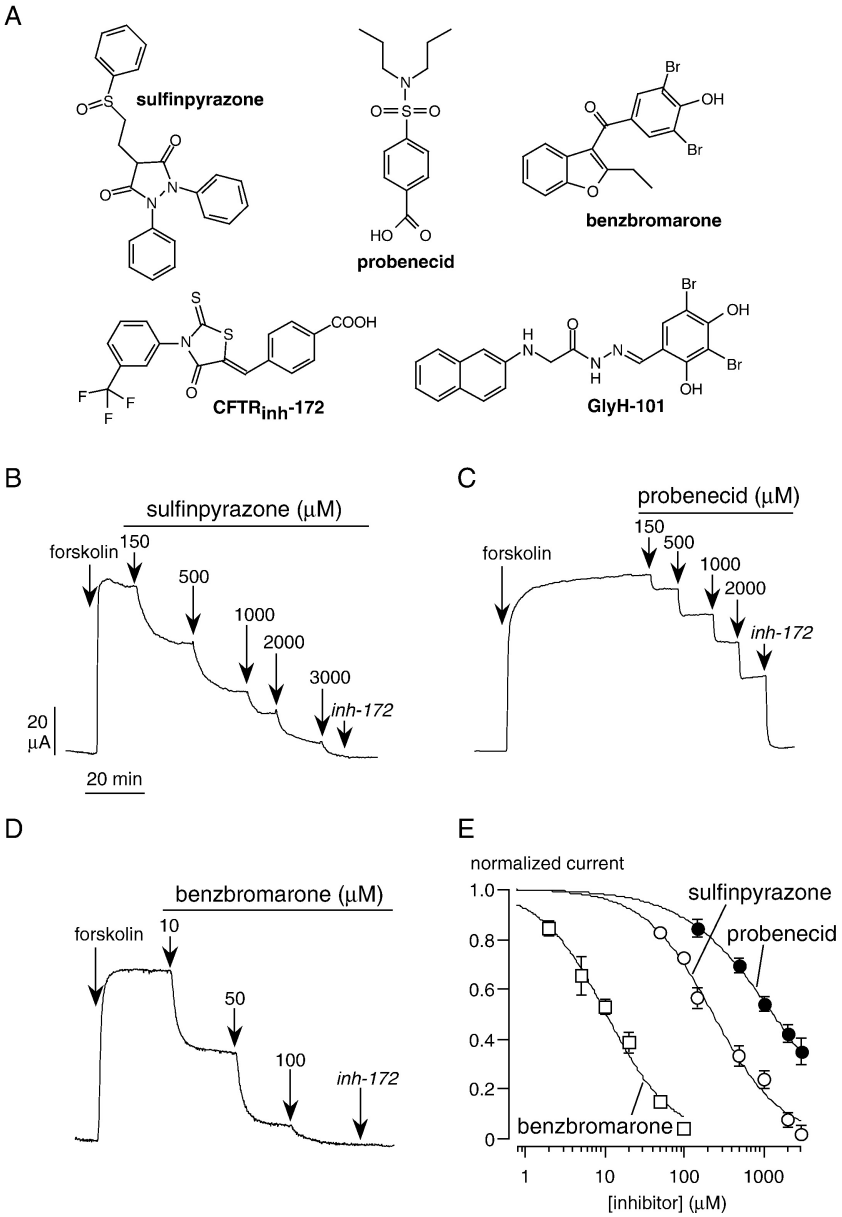

Fig. 1. Block of CFTR Cl^- currents by inhibitors of multidrug resistance-associated proteins. (A) Chemical structure of sulfinpyrazone, probenecid, and benzbromarone compared to known CFTR blockers. (B–D) Representative recordings of transepithelial Cl^- currents in FRT cells expressing human CFTR. After full activation with forskolin (20 μM), CFTR currents were inhibited with increasing concentrations of sulfinpyrazone, probenecid, and benzbromarone. The $CFTR_{inh}$-172 (10 μM) was added at the end of the experiment to block residual CFTR activity. (E) Normalized dose-responses. Each point is the mean ± SEM of 8–10 experiments.