The CFTR inhibitor-172 has minimal effects on shark CFTR as compared to human CFTR

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Recently, a new small molecule CFTR inhibitor, a structural analog of the thiazolidinone family, was identified and synthesized. The new compound, $CFTR_{inh}$–172, reversibly inhibited CFTR mediated short circuit current in a voltage dependent manner when stimulated by a cocktail of activators[2]. $CFTR_{inh}$–172 did not prevent cAMP elevation at concentrations that fully inhibited CFTR, nor did it inhibit non – CFTR chloride channels or similar ABC binding cassette channels[2].

We sought to examine the inhibitory effects of $CFTR_{inh}$–172 on shark CFTR (sCFTR) and to compare this response to the human CFTR chloride channel (hCFTR). We employed three techniques in our study. *In vitro* inhibition was examined using both isolated shark rectal glands in perfusion studies[1] and by measurement of short circuit current (I_{sc}) in primary culture monolayers of rectal gland tubular cells[4]. Direct comparison of the $CFTR_{inh}$ on shark vs human CFTR was examined using two electrode voltage clamp (TEVC) conductance studies in *Xenopus laevis* oocytes injected with hCFTR and sCFTR cRNA[3].

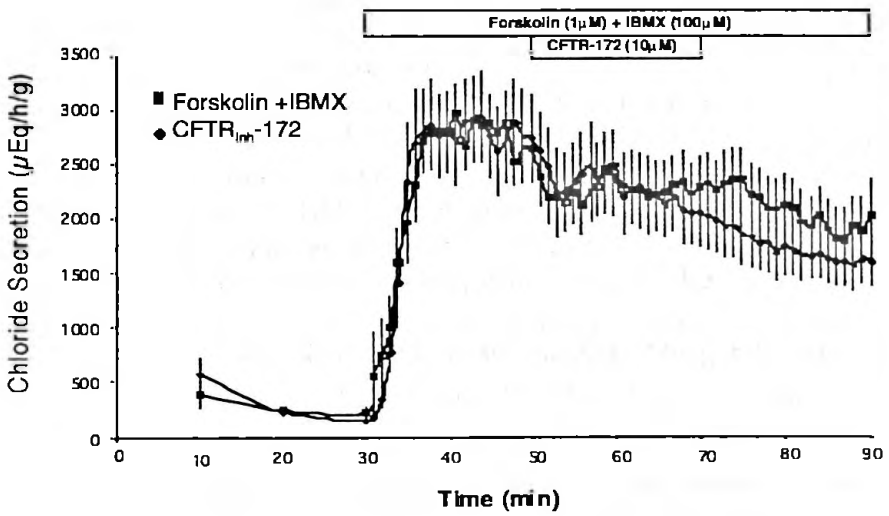

Figure 1. Chloride secretion in perfused shark rectal glands with 1 μM forskolin and 100 μM IBMX added to the perfusate. CFTRinh–172 (10 μM) was added from 50-70 min in experimental studies (n=5) and effects were compared to controls (n=18). Values are mean ± SEM.

Perfusion studies demonstrated that $CFTR_{inh}$–172 had no effect on chloride secretion in the shark rectal gland (Figure 1). The glands were perfused with shark Ringer's solution for thirty minutes to reach basal levels of chloride secretion (~250 μEq/h/g). Forskolin and IBMX were added to the solution at respective concentrations, 1 μM and 100 μM, and chloride secretion was measured in one minute intervals. After twenty minutes of stimulated secretion, 10 μM $CFTR_{inh}$–172 was added to the

perfusion solution for thirty additional minutes (50-80 min) and then removed. $CFTR_{inh}$–172 (10 μM) had no effect on chloride secretion in stimulated rectal glands.

The effects of $CFTR_{inh}$–172 on chloride secretion in rectal gland monolayer cells were consistent with the perfusion studies. Addition of 1, 5 and 10 μM $CFTR_{inh}$–172 to 1 μM forskolin and 100 μM IBMX stimulated cells showed no inhibition in four experiments (results not shown).

To compare inhibitory effects of human vs. shark CFTR, TEVC electrophysiological studies were employed in *Xenopus* oocytes. cRNA of shark and human CFTR were prepared and 5 ng of human and 15 ng of shark were injected into oocytes. These oocytes were perfused with frog Ringer's solution until baseline conductances were observed (~2-10 μS.) Then, 10 μM forskolin and 100 μM IBMX were added to the perfusate for thirty to forty minutes to create a stimulatory plateau. $CFTR_{inh}$–172 at 10 μM was then added and removed after thirty to forty minutes to examine reversibility.

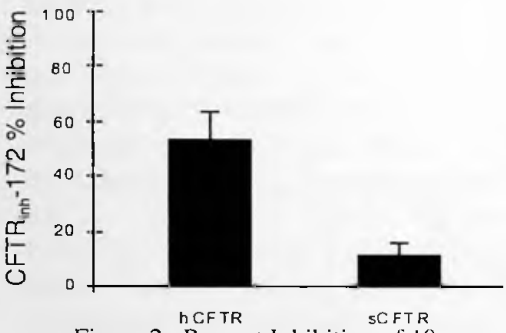

Figure 2. Percent Inhibition of 10 μM $CFTR_{inh}$ –172 on 10 μM forskolin and 100 μM IBMX activated human and shark CFTR injected oocytes (n=10 oocytes each).

In oocytes expressing either human or shark CFTR, forskolin + IBMX resulted in a steady-state increase in chloride conductance to 120-170 μS. $CFTR_{inh}$–172 (10 μM) inhibited this steady-state conductance in sCFTR-injected oocytes by only 11.8 ± 2.7 % (Figure 2). In contrast, hCFTR was much more sensitive to inhibition with $CFTR_{inh}$–172, with 57.1 ± 3.8 % inhibition observed.

In summary, we have demonstrated that $CFTR_{inh}$–172, at a concentration of 10 μM, does not inhibit sCFTR *in vitro* in shark rectal gland perfusion studies and has only modest effect on shark CFTR in expression studies. We have previously shown that human and shark CFTR have different inhibitory responses to the thio-reacting agents mercury and zinc. The present observations indicate a further difference between these two CFTR isoforms. These studies suggest that the binding site(s) for $CFTR_{inh}$–172 may differ in human and shark isoforms.

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