Control of the signalling pathway of 5-HT$_4$ receptors in cholinergic neurons of human colon by phosphodiesterases

Introduction: 5-HT$_4$ receptors are present on cholinergic neurons innervating gastrointestinal smooth muscle cells in mouse, dog, pig and man. Activation of those receptors with a selective 5-HT$_4$ receptor agonist facilitates the ongoing acetylcholine release resulting in increased smooth muscle contractions. In porcine stomach and colon the signalling pathway of the adenylyl cyclase linked 5-HT$_4$ receptor in cholinergic neurons is controlled by phosphodiesterase 4 (PDE4). The aim of this study was to investigate whether PDEs also control the 5-HT$_4$ receptor in human colon.

Methods: The study was approved by the Ethics Committee of Ghent University Hospital. After removing the mucosa, circular smooth muscle strips from human colon were prepared and after loading with [^{3}H]-choline chloride, tritium outflow as a measure for acetylcholine release was induced by electrical field stimulation (EFS). Two trains (2 min, 4 Hz, 1 ms, 15 V) were applied at 60 min interval (S_1 and S_2). Compounds were added before S_2: prucalopride (selective 5-HT$_4$ receptor agonist; 0.01, 0.03 and 0.3 µM); 3-isobutyl-1-methylxanthine (IBMX; non-selective PDE inhibitor; 10 µM) and rolipram (selective PDE4 inhibitor; 1 µM). The ratio of acetylcholine release by S_2 compared to that by S_1 was calculated.

Results and discussion: Prucalopride (0.3 µM) significantly increased the electrically induced acetylcholine release (fig. 1A). Prucalopride (0.03 µM) still had a significant facilitating effect (fig. 1B) but not prucalopride (0.01 µM; fig. 1C). IBMX (10 µM) per se did not affect EFS-induced acetylcholine release. It tended to increase the effect of prucalopride (0.03 µM) (fig. 1B) but this did not reach significance; in the presence of IBMX, prucalopride (0.01 µM) significantly increased acetylcholine release (fig. 1C).Preliminary experiments with rolipram indicated that rolipram (1 µM) does not influence EFS-induced acetylcholine release, but enhances the influence of prucalopride (0.01 µM): S_2/S_1 0.71 ± 0.05 for controls, n = 6; 0.73 ± 0.03 for rolipram, n = 5; 0.77 ± 0.05 for prucalopride, n = 5; 0.94 ± 0.03 for prucalopride in the presence of rolipram, n = 5.

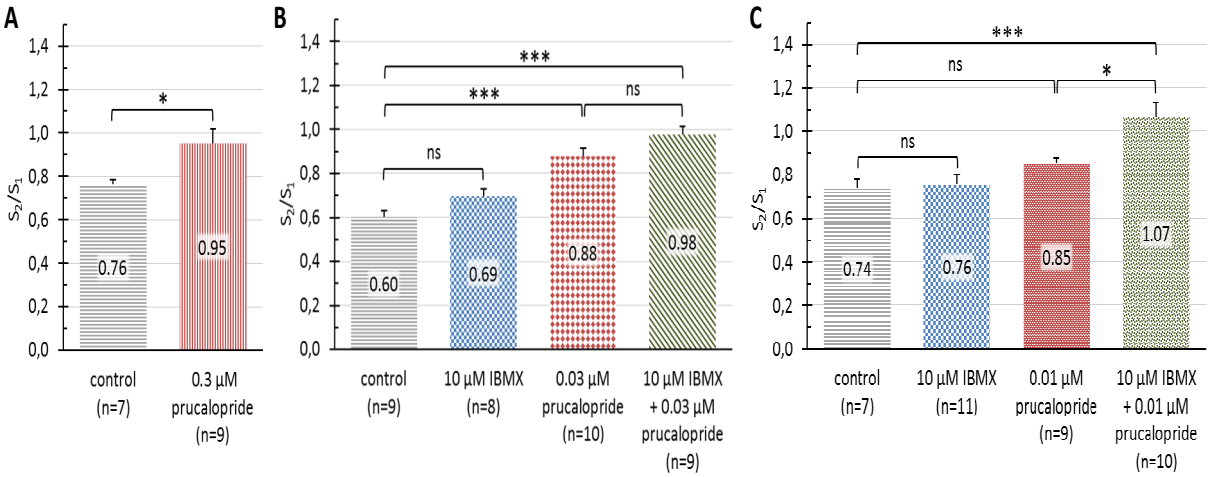

Figure 1 - Influence of: 0.3 µM prucalopride (A), 10 µM IBMX and 0.03 µM prucalopride (B) and 10 µM IBMX and 0.01 µM prucalopride (C). MEANS ± SEM are shown. Statistical analysis: t-test (A) or one-way ANOVA with Bonferroni corrected t-test (B and C) with ns not significant, * p < 0.05 and *** p < 0.001.

Conclusion: These data demonstrate that just as in pigs, the 5-HT$_4$ receptor pathway in cholinergic neurons in human colon is controlled by one or more phosphodiesterases. The preliminary results with

rolipram suggest a role for PDE4, but further investigation with selective PDE inhibitors is required to identify the PDE subtype(s) involved.