Facilitation of cholinergic neurotransmission via 5-HT4 receptors in the murine gastrointestinal tract

Vicky Pauwelyn, Els Van Deynse and Romain Lefebvre

Department of Pharmacology - Heymans Institute, Ghent University, De Pintelaan 185, B-9000 Ghent, Belgium

1. INTRODUCTION

Recent data in rats with mosapride and in mice with prucalopride, both selective 5-HT4 receptor agonists, showed that these agents activate the cholinergic anti-inflammatory pathway in the rodent small intestine. This was suggested to be due to activation of 5-HT4 receptors on cholinergic myenteric neurons dampening activation of resident macrophages. Till now however, even the presence of 5-HT4 receptors on cholinergic enteric neurons inducing smooth muscle contraction, a location established in the pig and human gastrointestinal (GI) tract, was not firmly established in the murine GI tract.

2. AIM

The aim of this study was therefore to investigate the influence of prucalopride on submaximal cholinergic contractions in different regions of the murine GI tract.

3. METHODS

Circular smooth muscle strips were prepared from the fundus, jejunum and colon (mucosa removed for jejunum and colon) and mounted between 2 stimulation electrodes in an organ bath with oxygenated Krebs solution; isometric tension was registered.

For each tissue, electrical field stimulation (EFS) was performed with different stimulation parameters (voltage, frequency and interstimulus interval) to select those parameters inducing neurogenic cholinergic on-contractions as checked with tetrodotoxin (3 µM) and atropine (1 µM) respectively. EFS at these parameters induced reproducible contractions; the contraction amplitude was halved by decreasing the voltage (V50%) to study the influence of prucalopride (0.003 to 0.03 µM). The 5-HT4 receptor antagonist, GR113808 (0.3 µM), was studied versus 0.03 µM prucalopride.

4. RESULTS

EFS-induced cholinergic on-contractions were obtained in the presence of guanethidine (4 µM) and L-NAME (300 µM) to exclude noradrenergic and nitric influences respectively. Additionally, for colon strips the P2Y1 receptor antagonist MRS 2500 (1 µM) had to be added to avoid influences of the relaxant neurotransmitter ATP. EFS with 10 s trains (500 µs pulse duration; maximal voltage; 4 [fundus] or 8 Hz [jejunum and colon]) at 5 (fundus and colon) or 10 min (jejunum) interval induced reproducible contractions. Upon reduction of the stimulation voltage to V50%, reproducible submaximal contractions were obtained; after adding prucalopride, contractions by EFS at V50% were followed for 50 min.

Prucalopride concentration-dependently increased contractions at V50%. The mean contraction force (%) 50 minutes after administration of prucalopride (0.003, 0.01 and 0.03 µM) was 133, 181*, 204*** in the fundus; 122*, 124**, 138***
in the jejunum; 133, 136, 152** in the colon (*P < 0.05; **P < 0.01; ***P < 0.001 versus control; one-way ANOVA with Bonferroni correction; n = 6-9). The effect of 0.03 µM prucalopride was abolished by the selective 5-HT4 receptor antagonist GR113808 (0.3 µM).

5. CONCLUSION

These data suggest the presence of 5-HT4 receptors on cholinergic neurons innervating circular smooth muscle in murine fundus, jejunum and colon. The effective concentrations of prucalopride are lower than those needed at 5-HT4 receptors in the human and porcine GI tract.