Cell-specific distribution and signal transduction control of 5-HT_4 receptors in the gastrointestinal tract

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Serotonin (5-hydroxytryptamine, 5-HT) is an essential messenger for the coordinated movement of food along the gastrointestinal (GI) tract, by interacting with a wide array of 5-HT receptors. Within the GI wall, 5-HT activates 5-HT receptors located on smooth muscle cells or on intrinsic and extrinsic sensory neurons, contributing to the regulation of peristalsis and communication between the brain and the GI tract. The 5-HT receptors are classified into seven receptor classes; in the gut, a role has been proposed for 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 receptors. The 5-HT_4 receptor is expressed on excitatory cholinergic motor neurons in the GI tract, facilitating acetylcholine release hereby stimulating GI motility. This presynaptic facilitation is thought to be the principal mechanism of the prokinetic action of 5-HT_4 receptor agonists such as prucalopride, explaining their therapeutic use in GI dysmotility related disorders such as chronic constipation, gastroparesis and gastroesophagal reflux disease. The pharmaceutical industry has invested in the search for adequate therapies for patients suffering from GI motility disorders by aiming at mimicking a subset of the actions of 5-HT. Aside the presence of 5-HT_4 receptors on cholinergic neurons in the GI tract, their localization has also been proposed on other cell types within the colon and stomach. In the human colon 5-HT_4 receptor agonists lead to smooth muscle relaxation by stimulating 5-HT_4 receptors on smooth muscle cells and possibly 5-HT_4 receptors located on nitrergic neurons. 5-HT_4 receptor mRNA expression has also been reported in the human and pig gastric and colonic mucosa, and it has been suggested that 5-HT-induced mucosal secretion is mediated by 5-HT_4 receptors. But the cellular 5-HT_4 receptor distribution and its functional implications within the epithelial layer have not yet been investigated. More detailed information on the expression and localization of 5-HT_4 receptors and their signaling pathways would lead to a better understanding of the role of 5-HT_4 receptors in GI motility and secretion.

The aim of this study was to investigate whether the porcine colon descendens could function as a model for the role and location of 5-HT_4 receptors in the control of human colonic motility. Secondly, the influence of phosphodiesterases (PDEs) in the signal transduction pathway upon activation of 5-HT_4 receptors on excitatory cholinergic motor neurons was investigated in pig gastric and colonic circular muscle. Finally, the mucosal expression of 5-HT_4 receptors was studied in the stomach and colon of the pig.

The influence of the selective 5-HT_4 receptor agonist prucalopride on electrically induced acetylcholine (ACh) release from cholinergic nerve endings innervating pig gastric circular muscle and on the contractions induced by this release was investigated; the possible regulation of this effect by PDEs was explored. Prucalopride concentration-dependently increased the amplitude of submaximal cholinergic contractions and of ACh release induced by electrical field stimulation. The effect of prucalopride on electrically induced cholinergic contractions was antagonized by the selective 5-HT_4 receptor antagonist GR113808 and this antagonism was confirmed in the release assay. The non-selective PDE inhibitor IBMX concentration-dependently reduced the amplitude of the cholinergic contractions, but in a concentration that only mildly reduced these contractions, it enhanced the facilitating effect of prucalopride on both cholinergic contractions; IBMX was able to induce and enhance the facilitating effect of prucalopride on electrically induced ACh release. The selective inhibitors vinpocetine (PDE1), EHNA (PDE2) and cilostamide (PDE3) did not influence the effect of prucalopride on acetylcholine release but the PDE4-inhibitor rolipram enhanced the facilitating effect of

prucalopride to the same extent as IBMX. These results demonstrate that 5-HT_4 receptors are present on the cholinergic nerves towards the pig gastric circular muscle, facilitating acetylcholine release and the intracellular transduction pathway of this facilitation is regulated by PDE4. As for the role of PDEs in the control of the cyclic nucleotide content in the porcine gastric circular muscle cells, our results point to a redundant role of PDE3 and PDE4 with slight predominance of PDE3.

The pig colon descendens was then first evaluated as possible model for the different locations of human colonic 5-HT_4 receptors. To evaluate the nervous control in porcine GI motility, the intrinsic excitatory and inhibitory motor neurotransmission was first characterized. In colonic circular smooth muscle strips, electrical field stimulation (EFS) was only able to voltage-dependently induce on-contractions in the combined presence of the NO synthase inhibitor L-NAME and the SK channel blocker apamin. The on-contractions were largely reduced by the neuronal conductance blocker tetrodotoxin and by the muscarinic receptor antagonist atropine, illustrating activation of cholinergic neurons. Prucalopride facilitated submaximal EFS-evoked cholinergic contractions and this effect was prevented by the 5-HT_4 receptor antagonist GR113808, supporting the presence of facilitating 5-HT_4 receptors on the cholinergic nerve endings innervating circular muscle in pig colon descendens. However, the facilitating effect of prucalopride was less pronounced than has been reported for canine and human stomach or colon, and for porcine stomach. This suggests a more limited number of 5-HT_4 receptors on the cholinergic nerve endings and/or less effective signalling of these receptors in pig colon descendens. Relaxations were induced by EFS in strips pre-contracted with tachykinin 1 receptor agonist substance P in the presence of atropine. The responses at lower stimulation voltages were abolished by tetrodotoxin. L-NAME or apamin alone did not influence or only moderately reduced the relaxations, but L-NAME plus apamin abolished the relaxations at lower stimulation voltages, suggesting that NO and ATP act as inhibitory neurotransmitters in a redundant way. Prucalopride did not influence the EFS-induced relaxations at lower stimulation voltage, nor did it per se relax contracted circular muscle strips. No evidence for relaxing 5-HT_4 receptors, either on inhibitory neurons or on the muscle cells was thus obtained in pig colon descendens circular muscle. The pig colon descendens can thus only be used as a model for the 5-HT_4 receptors on cholinergic neurons in the human colon.

The role of PDEs in the control of the facilitating effect of prucalopride on cholinergic contractions and on smooth muscle tone in pig colon descendens was studied. The influence of the non-selective PDE inhibitor IBMX and selective inhibitors vinpocetine (PDE1), EHNA (PDE2), cilostamide (PDE3), rolipram (PDE4) and zaprinast (PDE5) was evaluated. IBMX and cilostamide concentration-dependently reduced the amplitude of the cholinergic contractions, as good as abolishing them at their highest concentrations. EHNA only reduced the contractions significantly at the highest concentration tested. Rolipram showed a biphasic effect, significantly increasing the contractions at 0.1 and 0.3 μM but decreasing them at 30 μM. Vinpocetine did not influence the electrically induced contractions while zaprinast enhanced the responses at 3-30 μM. IBMX, vinpocetine and EHNA did not influence the facilitating effect of prucalopride on electrically induced cholinergic contractions but rolipram tended to enhance it. When rolipram was added after prucalopride, the facilitating effect of prucalopride was significantly enhanced. These results suggest that PDE3 is the main regulator of the cyclic nucleotides regulating circular smooth muscle activity and that the signal transduction of 5-HT_4 receptors on the cholinergic nerves towards the circular muscle layer is regulated by PDE4 in pig colon descendens.

Finally the cellular 5-HT_4 receptor distribution, with attention to the epithelial layer was investigated in porcine stomach and colon by laser microdissection (LMPC) and PCR. 5-HT_4 receptor and GAPDH mRNA expression was detected in mucosa and muscle-myenteric plexus tissue fractions, and in mucosal and muscle-myenteric plexus parts of hematoxylin&eosin (H&E) stained tissue sections of pig colon descendens and gastric fundus. In the mucosal tissue fractions of both GI tissues, the expression of 5-$HT_{4(+h)}$ receptor mRNA, a splice variant containing an additional 14 amino acid segment in the second extracellular loop of the receptor, was significantly higher compared to 5-$HT_{4(-h)}$ receptor expression, and a similar trend was obtained in the mucosal part of H&E stained tissue sections. Large microdissected patches of the epithelial and circular smooth muscle cell layer of pig colon descendens and of the epithelial cell layer of pig gastric fundus, also showed 5-HT_4 receptor and GAPDH mRNA expression. No 5-HT_4 receptor mRNA expression was detected in gastric LMPC-isolated EC cells from IHC stained tissues, while the cells were positive for GAPDH. The expression of 5-HT_4 receptors in individual cell types might be too low to pick them up by LPCM and PCR. Porcine GI mucosa thus predominantly expresses 5-$HT_{4(+h)}$ receptor splice variants suggesting a preferential involvement of this type of 5-HT_4 receptor in mucosal effects of 5-HT.

In conclusion, our findings indicate that 5-HT_4 receptors are present on the cholinergic neurons innervating the muscle layer of porcine stomach and colon. The signal transduction pathway of these receptors is regulated by PDE4 so that concomitant treatment with a PDE4 inhibitor enhances the facilitating effect of the 5-HT_4 receptor agonist prucalopride on Ach release and thus on cholinergic contractions. If further investigation confirms this mechanism in humans, this opens the possibility of prokinetic combination therapy of 5-HT_4 receptor agonists with PDE4 inhibitors. Our results also show the mucosal expression of 5-HT_4 receptors in pig stomach and colon. As the information on 5-HT_4 receptor-mediated effects on GI secretion in mammals is limited, the pig can also be used as a model to further unravel the cellular distribution of 5-HT_4 receptors and the effects of 5-HT_4 receptor activation in the mucosa.