Pro- and anti-inflammatory roles of 5-HT and its receptors in the gut

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Serotonin (5-HT) is an important signaling molecule in the gut, where it is synthesized and released from enterochromaffin (EC) cells in the mucosa, and from a subset of enteric neurons in the myenteric plexus. In addition to its actions on intrinsic and extrinsic reflexes of the gut, 5-HT can act as both a pro- and anti-inflammatory molecule in the intestinal mucosa.

The Gershon and Khan research teams have demonstrated that experimental colitis is augmented in mice lacking the serotonin transporter, which increases serotonin availability when it is released from EC cells[1], and that mice are protected from colitis when mucosal serotonin synthesis is diminished by inhibition of tryptophan hydroxylase, and in *Tph1*-knockout mice [2-4]. Dendritic cells, which express 5-HT_7 receptor are likely mediators of the pro-inflammatory response [5,6].

Serotonin can also exert an anti-inflammatory effect in the intestinal mucosa via activation of epithelial 5-HT_4 receptors[7]. Activation of epithelial 5-HT_4 receptors reduces the development of colitis, and accelerates healing of established colitis. Mechanisms include enhanced epithelial proliferation, accelerated wound healing, and increased resistance to oxidative-stress-induced apoptosis. Daily administration of a 5-HT_4 antagonist to normal mice causes mild colitis indicating that this receptor is important for epithelial integrity.

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Micro-coordination of pacemaker potentials may characterize functional states of gut motility.

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Functional movement of the gut, such as peristalsis, segmentation, etc. requires sophisticated coordination of micro-regions. Textbooks of medicine and physiology describe the 'law of the intestine' that indicates that the intestine is considered to be under the control of the intrinsic nervous system. Nevertheless, in vivo observations of the gut, for example, during surgical operations and cine MRI, often display peristaltic motions without local pressure. A multitude of excitable cellular systems, including network-forming pacemaker cells, are likely to cooperate in coordinated movements of the gut.

In this study, we thus employed a dialysis membrane-enforced micro-electrode array (MEA) technique measure spontaneous electrical activity in the ileum showing typical pacemaker potentials, and visually analyzed the spatio-temporal coordination of micro-regions. The micro-coordination of spontaneous field potential oscillations (pacemaker potentials) was tentatively classified into three patterns: 'bumpy', 'expanding' and 'migrating'. In addition, features of micro-coordination fluctuated, and occasionally underwent large changes even in the same sample during a long period of measurements. For example, some samples of 'migrating' patterns showed a reversal of propagating direction. Furthermore, in preliminary experiments, we observed a shift of the micro-coordination pattern after application of 5-HT.

With further refinement of this classification in future studies, the pattern of micro-electric activity potentially could be used as a sensitive indicator of the functional state of the interstitial cell network and GI tract in physiological and pathological conditions. Elucidation of mechanisms underlying the micro-coordination of pacemaker activity may provide researchers with a new therapeutic strategy for various functions related to gut motility.

Neuronal anti-inflammatory signaling via 5-HT_4 receptor stimulation in small intestine

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Background: Gastrointestinal prokinetic agent, serotonin-4 receptor (5-HT_4R) agonists activate myenteric plexus neurons to release acetylcholine (ACh), which in turn induces anti-inflammatory action, but detailed pathway remains to be elucidated.

Aim: Aim of this study is to clarify the anti-inflammatory mechanism of intramural plexus stimulation via 5-HT_4R on leukocytes infiltration in animal model for postoperative ileus.

Methods: Postoperative ileus was induced by intestinal manipulation in wild type C57BL6/J (WT), 5-HT_4R knock-out (S4R KO), α7nAChR knock-out (α7R KO) and M2 muscarinic AChR (M2AChR) knock-out (M2R KO) mice.

Results: Intramural plexus stimulation via 5-HT_4R attenuated leukocytes infiltration in WT. The intramural plexus stimulation-induced anti-inflammatory action was completely suppressed in S4R KO mice. The intramural plexus stimulation-induced anti-inflammatory action against macrophages infiltration but not neutrophils infiltration was attenuated in α7R KO mice. Selective α7nAChR agonists, PNU-282987 and AR-R17779 also inhibited only macrophages infiltration. The intramural plexus-mediated inhibition of neutrophils infiltration was diminished by atropine, M2AChR antagonist, methoctramine and in M2R KO mice.

Conclusion: 5-HT_4R stimulation inhibits leukocytes infiltration in POI possibly through intramural plexus activation. Released ACh inhibited macrophages and neutrophils infiltrations by activation of α7nAChR presumably on macrophages, and M2AChR, respectively. Thus, macrophages and neutrophils recruitment into the inflamed site is inhibited by different types of AChRs in small intestine.

Signal transmission via purinergic receptors to sensory nerves from mechanical stimulation of bone or joints

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The mechanical stimulation (MS) plays critical roles for the healthy bone and joints. Previous studies have shown that bone formation is controlled via the sympathetic nerve by central nervous system, and the existence of sensory nerve is thought to be essential for the normal bone homeostasis. The role of sensory nerves, however, remains unknown. The aim of the 1st study was to reveal the mechanotransduction mechanism for the bone homeostasis. The possible mechanisms of interactions between osteoblasts (OB) and dorsal root ganglionic (DRG) sensory neurons were investigated in co-culture system. MS of OB by a glass micropipette induced increases of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in neurites of DRG neurons; afferent signal transmission. Pre-treatments with antagonists, suramin or pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate, abolished the increase of $[Ca^{2+}]_i$ in DRG neurons. The afferent signal transmission is found to be elicited by opening stretch-activated calcium channel and followed by release of ATP. These results suggested the possibility that MS of bone tissue elicited afferent response of sensory neurons mediated purinergic transmission by P2X receptor[1]. In addition, mechano-sensitivity of OB is regulated by $P2Y_2$ receptor mediated via autocrine ATP stimulation, which may affect interactions between OB and neurons. The 2nd studies using fibroblast-like synoviocytes (FLS) with DRG neurons in co-culture system were performed to reveal interactions between joints and sensory neurons. The results suggested the possibility that MS of synovium elicited Ca^{2+}-dependent signal transmissions from FLS to sensory neurons.

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