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Serotonin in the gut: pretty when it gets down to the nitty gritty

Serotonin (5-hydroxytryptamine; 5-HT) plays a pivotal role in regulating several aspects of gut function including secretion, motility and sensation. It is a neurotransmitter in the enteric nervous system mediating both fast and slow synaptic events through receptors (R) that may be ligand-gated ion channels (5-HT3R), or G-protein coupled receptors, GPCRs (5-HT4/1PR) expressed at the post-synaptic membrane.[1,2] These receptors are also expressed on the terminals of enteric and extrinsic sensory nerves within the lamina propria, beneath the mucosal epithelium, where they are positioned to detect 5-HT released from enterochromaffin cells (EC cells).[3,4] These cells are a rich source of 5-HT that is concentrated in granular vesicles located at the base of the cell. Enterochromaffin cells have an apical tuft of microvilli projecting into the intestinal lumen. The tuft is proposed to be the sensory apparatus of the cell. An analogy has been drawn between taste buds and EC cells with the hypothesis that these cells are the taste cells of the gut sampling the chemical composition of luminal contents.[5] Stimuli acting on the apical membrane trigger 5-HT release which in turn activates the sub-epithelial sensory nerve terminals. Enterochromaffin cells, therefore, play a pivotal role in coding sensory stimuli and in transmitting this information to enteric nerves involved in local reflex control as well as extrinsic afferent fibres projecting to the brain and spinal cord. Afferents respond in direct proportion to the amount of chemical released, which, in turn, is related directly to the strength of the luminal stimulus.

The action of 5-HT is rapidly terminated by specific re-uptake mechanisms. SERT, the seroronin re-uptake transporter, is expressed in neurons as well as the intestinal epithelium and it efficiently removes 5-HT following its release by the EC cell.[6] The balance between release and re-uptake therefore determines receptor activation and its neural and paracrine action. Release of 5-HT from small intestinal EC cells regulates physiological processes including peristalsis, and is implicated in pathological conditions including the vomiting and diarrhoea after cancer radio- or chemotherapy, in diarrhoeal states caused by bacterial enterotoxins, and possibly in the visceral hypersensitivity in patients with IBS.[7]

Our understanding of 5-HT release from EC cells is based upon levels of 5-HT or its metabolites in the portal blood or the systemic circulation, or following overspill, by 5HT in the lumen. Levels increase following a meal and the increase is exaggerated in some patients with IBS.[8] However, because of the capacity for 5-HT re-uptake, these levels are unlikely to reflect true release and the kinetics of circulating levels of 5HT do not provide sufficient temporal resolution to what is undoubtedly a very dynamic process of synthesis, release, reuptake, and metabolism Attempts to overcome this lack of understanding of the dynamic events following release of 5HT from EC cells by utilizing EC cells in culture have been unsuccessful. Investigators have resorted to isolated crypts and calcium imaging techniques to investigate stimulus-secretion coupling in these cells.[9] Other groups have used a 5-HT secreting pancreatic carcinoma cell line as a model for EC cells in order to investigate their mechanical and chemical sensitivity and the associated signal transduction pathways.[10] However, while isolated cells offer the advantage of absence from interference from other cell types present in the mucosal epithelium, they do not allow the dynamic interaction between EC cells and neural influences that are likely to modulate EC cell responses *in situ*.

Another approach is to utilize electrochemical methods to measure the oxidation of biogenic amine on the surface of an inert carbon fibre electrode. In this issue, Paul Bertrand reports the first description of real time measurement of 5-HT release from gut mucosa.[11] This has been achieved by adapting and refining electrochemical techniques that have been successfully used in the CNS field for a number of years. The measurement of 5-HT release is, however, complicated by the presence of high concentrations of other electroactive species (such as ascorbic and uric acids). These complications, however, can be overcome: first, by coating the electrode with an anionic resin to select for cationic molecules, and second, by selecting oxidation currents that are specific for 5-HT over, e.g. noradrenaline. In this way, the currents detected from the intestinal mucosa are likely to represent the release of 5-HT from a small number of EC cells in proximity to the electrode tip Bertrand estimates the number is about 7. While the current paper is largely a validation of the methodology, the application of this approach in the future will provide insight into the role of EC cells in sensory signal transduction. It will now become

feasible to apply specific mechanical or chemical stimuli, and to measure 5-HT release directly at the site of release and action. Such information is critically important in order to interpret recent findings showing that mucosal 5-HT synthesis and reuptake are significantly reduced in pathological conditions such as ulcerative colitis and IBS.[12] The functional significance of these changes can only be fully appreciated when the consequence for local 5-HT concentration at the level of the sensory nerve terminal are known. The technique described by Bertrand would allow measurement of 5-HT release with a so far unmatched sensitivity and spatiotemporal resolution compared to other approaches using HPLC and may reveal subtle differences between normal and diseased gut.

The potential of the electrochemistry approach is clear, but there are several issues or questions raised by the current paper by Bertrand:

1 How do mechanical and chemical stimulation influence graded 5-HT release? The 5-HT was only detected when the electrode had physical contact with the mucosa, a phenomenon that appears inherent to carbon fibre electrochemistry. However, because EC cells are believed to be mechanosensory, it is possible that such mechanical interaction impacts on the baseline states of the EC cell and the magnitude of this effect is unknown. Certainly, mechanical distortion of the mucosa by distension or stroking is one way of evoking 5-HT release.

2 Is it possible that the detecting electrode is contributing to the release of 5-HT, much like the probing by a von Frey hair, rather than acting solely as a simple detector?

3 In the present study, 5-HT release was triggered by cholinergic agonists. To what extent does this release reflect movement of the tissue? Or does release reflect activation of enteric network as part of a bi-directional control mechanism?

4 It would also be intriguing to know the extent to which 5-HT can diffuse from its site of release, particularly whether it can extend to influence neural processing in submucosal and myenteric ganglia. The latter are supplied by elaborate vagal sensory terminals, the so called intraganglionic laminar endings (IGLEs) which function as tension receptors.[13] 5-HT effects on enteric nerve cells and processes as well as on vagal sensory terminals involve activation of 5-HT3 and 5-HT4 receptors.

The 5-HT3 and 5-HT4 receptor ligands have proved effective in the treatment of visceral hypersensitivity and motility disorders, both prominent factors in inflammatory and functional bowel disorders.[14] The electrochemical approach may help resolve questions regarding mode of action of these and other pharmacological probes.

The electrochemistry technique is not restricted to measurement of 5-HT; in fact, changes in the electrode characteristics as well as the measurement protocol offer the possibility to monitor the release of other important mediators, such as noradrenaline, dopamine or melatonin. Clinical neurogastroenterology will ultimately advance from such studies as they help to identify new targets for drug development.

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