In focus in HCB

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In this June issue of the journal, we will highlight three original articles describing (1) the morphological and immunofluorescence characterization of 5-HT-containing endocrine cells in the gastrointestinal tract, including their interaction with nerve fibers; (2) the impact of airborne organic dust exposure on mitochondrial function, morphology, and biogenesis in a cultured monocytic cell line; and (3) analysis of the uptake of different-sized fluorescently tagged thio-organosilica nanoparticles into the mouse female genital tract following intravaginal administration. We hope you enjoy these brief “snippets”, as well as the entire issue, and we wish you all a pleasant beginning to the summer.

The GI empire of 5-HT

Ever since its discovery by Feyrter (1938), the Helle-Zellen-System, nowadays known as the diffuse neuroendocrine system, has attracted much interest among histologists, histochemists, and pathologists. In the gastrointestinal (GI) tract, the most abundant type of endocrine cell is the 5-hydroxytryptamine (5-HT)-containing enterochromaffin cell (EEC) (Sjölund et al. 1983). It is now established that intestinal 5-HT fulfills diverse functions (Gribble and Reimann 2019; Jones et al. 2020). Furthermore, variations are found not only in the number of 5-HT-containing EECs in the different parts of the GI tract, but also in their morphology and peptide hormone content (Fothergill and Furness 2018; Fothergill et al. 2019; Reynaud et al. 2016). In continuation

of their authoritative studies, the Furness team (Koo et al. 2021) has investigated the morphology and peptide hormone content of 5-HT-containing EECs and their relationship with other endocrine cell types and nerve fibers throughout the GI tract of male mice. In their study, they used 60 µm-thick cryostat sections for immunohistochemistry and captured Z-stack images with a super-resolution confocal microscope. Three-dimensional rendering of triple-labeled sections was applied to study the spatial relationship between 5-HT EEC and other endocrine cell types and nerve fibers. The authors report the existence of six morphologically different 5-HT-containing EECs along the GI tract (Fig. 1).

Fig. 1 Co-distribution of 5-HT (green) and histidine decarboxylase (red) in cells in the gastric antrum is common, as indicated by the yellowish color in the overlay shown. The interrupted lines indicate the outer, middle, and inner parts of the mucosa. From Koo et al. (2021)

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They further observed a changing morphology during their migration from the crypt base to the luminal epithelium. Although colocalization of 5-HT and histidine decarboxylase was common in the antrum, no overlap with gastrin or somatostatin was observed. In the colon, 5-HT cells were distinct from oxyntomodulin and peptide YY cells, although they were sometimes in close contact. Even with the use of a super-resolution confocal microscope, no specific relationships between 5-HT cells and nerve fibers could be detected. The main conclusion drawn by the authors was that five major features, i.e. region, morphology, hormone content, receptor repertoire, and cell lineage, can be used to define 5-HT cells.

The mighty mitochondrion

Hearken back to secondary school biology class, where during discussion of cellular organelles we were taught that mitochondria are “the powerhouse of the cell”. This sentiment of course was certainly true, and over the decades the role of mitochondria in myriad diseases and pathological conditions has become a bona fide canon of cell biology. Indeed, mitochondrial dysfunction has been implicated in a variety of lung diseases, including asthma, viral infections, allergies, and other inflammatory conditions (Bruno and Anathy 2021). This link between mitochondria and lung diseases is likely the result of cellular oxidative damage occurring via release of reactive oxygen species from mitochondria during a stress response. Such cellular stress activity can be induced in the lung by inhalation of environmental toxins or pollutants. For many years, Charavarymath and colleagues have been studying the mechanisms underlying pulmonary effects of exposure to airborne organic dusts (Bhat et al. 2019; Charavarymath et al. 2005, 2008; Charavarymath and Singh 2006). They have now focussed their attention on mitochondria (Bhat et al. 2021), and provide an extensive and detailed analysis of mitochondrial structure and function in cells treated with organic dust extract (ODE). Using cultured human monocytic cells (THP1) as an *in vitro* model representing human lung macrophages, they performed a wide variety of mitochondrial ultrastructural and physiological analyses, including cell viability and the MTT assay, mitochondrial DNA isolation, quantitative RT-PCR, Western blot analysis, mitochondrial activity via the MitoSOX assay, calcium influx measurements, nitric oxide secretion via the Griess assay, multi-cytokine identification, subcellular fractionation, and transmission electron microscopy followed by morphological image analysis. They also tested the potential protective effects of the mitochondrial-specific antioxidant reagents ethyl pyruvate and mito-apocynin on ODE-induced mitochondrial biogenesis (Fig. 2).

Fig. 2 Organic dust extract (ODE) exposure induces selective targeting of mitochondria for autophagy (mitophagy) as indicated by increased Parkin protein amounts in cultured human monocytic cells. Co-exposure to ethyl pyruvate (EP) or mito-apocynin (MA) significantly reduced the increase in ODE exposure-induced Parkin protein. From Bhat et al. (2021)

To summarize some of the extensive data presented, the authors found that exposure of THP1 monocytic cells to ODE resulted in the following: (1) morphological alterations in mitochondrial structure; (2) leakage of mitochondrial DNA into the cytoplasm; (3) elevated cellular mitophagy (mitochondrial autophagy) as evidenced by increases in PINK1, Parkin, and cytoplasmic cytochrome c levels; and (4) treatment of cells with pyruvate and mito-apocynin could only partially protect cells from the enumerated ODE-induced mitochondrial alterations. Therefore, these data demonstrate the effects of *in vitro* ODE exposure on mitochondrial function and biogenesis, with future studies aimed at similar investigations using primary pulmonary alveolar macrophages as well as mouse models.

Thiol-organosilica nanoparticles as a potential drug delivery system for the female genital tract

The complex anatomy of the female reproductive tract presents a potential barrier for local delivery of pharmacological agents. This issue is further complicated by the morphological changes presented by vaginal tissue throughout the estrous cycle (Byers et al. 2012). Awaad and Nakamura (2021) have investigated the use of two different-sized fluorescently tagged thiol-organosilica nanoparticle preparations (tOS-NPs) for use as drug delivery systems through local vaginal administration. They restricted their study to animals in the diestrus phase of the estrous cycle to allow direct comparisons between specimens, and because during this stage the vaginal mucosa displays decreased cell numbers, an absence of stratum corneum, and epithelial proliferation. tOS-NPs were prepared in 100 and 1000 nm size distributions, and then conjugated to either rhodamine B or fluorescein isothiocyanate (FITC). Animals were then administered intravaginally either size of the fluorescently tagged

Fig. 3 Thiol-organosilica nanoparticles (1000 nm diameter, red) can be observed in the vaginal epithelium (VE) and vaginal stroma (VS) 6 h after vaginal administration. The number of F4/80-positive macrophages (green), which may contain nanoparticles, is significantly increased. VL vaginal lumen. From Awaad and Nakamura (2021)

tOS-NPs, or as a combination of the two different-sized particles. The distribution of the fluorescence signal was then determined in different areas of the genital tract, including the vagina, endometrium, and ovaries. Moreover, the potential ingestion of the particles by resident macrophages was analyzed by immunostaining tissue sections with a FITC-tagged macrophage-specific F4/80 antibody (Fig. 3).

Their results showed that (1) smaller particles (100 nm) showed more tissue accumulation than larger particles (1000 nm), especially in the endometrium; (2) the larger particles elicited a macrophage response in the vagina; (3) particles of both sizes were found to be co-distributed with macrophages in the vagina, endometrium, and ovary; and (4) both sizes of particles were also found in the spleen, indicating that they likely entered the blood vessels through the vaginal lumen. In summary, their results suggest that optimization of nanoparticle size and stage of the estrous cycle are critical factors to consider when considering drug delivery systems for the female genital system.

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