DOTTORATO DI RICERCA – XXXII CICLO

Dottoranda: **Agnese Stanzani**
Tutor: Prof. Paolo Clavenzani
Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Italia

DISTRIBUTION OF TASTE SIGNALING MOLECULES IN ENTEROENDOCRINE CELLS IN RAT PILORUS IN AN ARTIFICIAL TORPOR STATE

Background: The GI mucosa serves as "sensory organ" and expresses a variety of sensory receptors, mostly located on enteroendocrine cells (ECs) and brush cells, which respond to luminal content activating intrinsic and extrinsic neuronal network. Moreover, ECs are the largest endocrine organ of the human body, which produces a variety of hormones and/or biogenic amin.[1]
These chemoreceptor include a family of G-protein coupled receptor (GPCR): taste 1 receptors (T1Rs) that detect sweets and umami and taste 2 receptors (T2Rs) that detect bitter tastes. Upon stimulation taste receptors interact with specific G-protein a-subunits (a–transducin and a–gustducin) leading to Ca^{2+} increase and transmitters release.[2]
We observed that in mice a long term high fat diets induces a selective upregulation in the stomach of the T2Rs receptor subtype T2R138[3]. We focused our attention to the fasting that occurs physiologically in some animals during the ibernation. In collaboration with Dr M. Luppi (DIBINEM – Università degli studi di Bologna) we have decided to evaluated if there are any changes in the TRs pathway (expression) in an animal artificial torpor model to better understand the changes that occurs in the GI tract in this condition.

Aims & Methods: The present study, is aimed at evaluating the number of T2R38, grelin (GRE) and colecystokinin (CCK) (-IR) cells in piloric sections of rats in three different conditions: control (CTRL), hypothermic-nadir (minumum central temperature achieved) (HYP) and rewarm 36h (sacrified after 36 hour from the temperature recovery of 35.5°C) (R36H). The torpor has been obtained with the Hypothermia protocol proposed by Cerri et al. 2013. We isolated the pylorus of each animals, and serial section (10um thick) were processed for single and double immunofluorescence. We ramdomly selected 10 field per sample in order to determine the average number of IR cells in 3 mm^2 of mucosa. The values are expressed as mean ± standard deviation (SD) and were analyzed with Student's *t*-test.

Preliminary Results: We didn't observed any significant variations in the mean number of T2R38, GRE and CCK-IR cells (data not shown), although we observed a lower number of CCK-IR cells in nadir samples and rewarm (**Fig.** 1). We observed a redistribution, in particular for CCK in control and rewarm 36h CCK is mainly located in the basal mucosa (in contact with basal lamina), instead in nadir it is distributed throughout the entire mucosa (**Fig.** 2).

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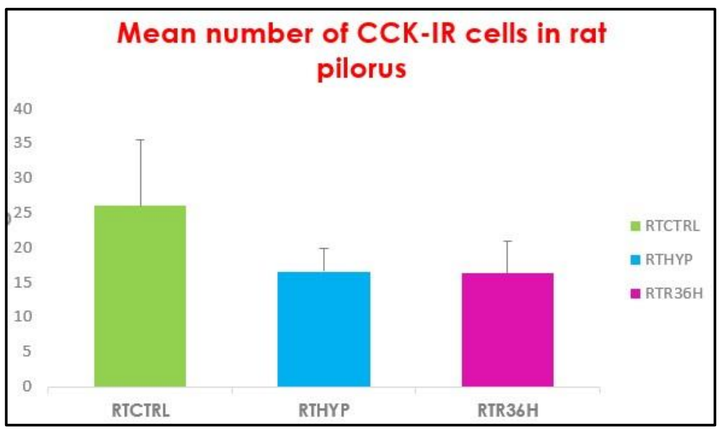

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Fig. 1: Mean number of CCK-IR cells in rat pilorus in the three different condition. Control (**RTCTRL**), Hypothermic-nadir (**RTHYP**) and Recovery 36h (**RT36H**). The values are expressed as mean + standard deviation.

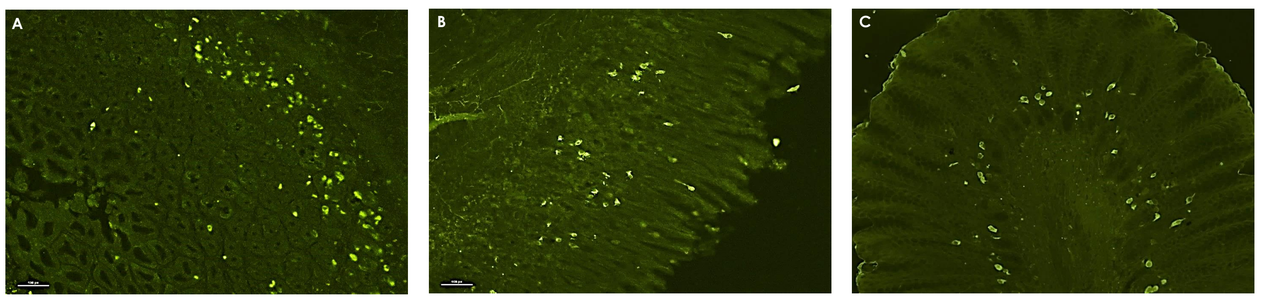

Fig. 2: Distribution of CCK-IR cells in rat pylorus in the three different condition. (**A**) RTCTRL, (**B**) RTHYP and (**C**) RTR36h. In RTCTRL and RTR36H CCK-IR cells were mostly located in the basal membrane, while in the RTHYP, CCK-IR cells were distributed along the pyloric glands.

[1] Sternini C. et al., Curr Opin Endocrinol Diabetes Obes. 15. 2008; [2] Caicedo A. et al., J Neurosci. 23. 2003;

LOCALIZATION OF CANNABINOID RECEPTORS IN THE CAT GASTROINTESTINAL TRACT

Background: Cannabinoid receptors regulate (reduce) gastrointestinal tract (GIT) motility and secretion, sensation, emesis, satiety, and inflammation [1][2][3][4][5][6][7][8][9]. Several evidences indicate that substances acting on GIT cannabinoid receptors may be beneficial for gut discomfort and pain [10][11].

Aims and Methods: The present *ex vivo* study was aimed to investigate immunohistochemically the distribution of the canonical cannabinoid receptors CB_1 and CB_2, and the putative cannabinoid receptors G protein-coupled receptors 55 (GPR55), nuclear peroxisome proliferator-activated receptors alpha (PPARα) and gamma (PPARγ), transient receptor potential ankyrin 1 (TRPA1), and serotonin receptor 5-HT1a (5-HT1a) on the cat gastrointestinal tract. Gastrointestinal tissues were collected *ex-vivo* from five European cats, that did not have history of gastrointestinal disorders and did not show gross alteration of gastrointestinal wall. GIT samples (pylorus, descending duodenum and distal colon) were harvested within 1 hour from death and were longitudinally opened along the gastric small curvature (pylorus) and mesenteric border (intestine).The samples are then processed for immunohistochemistry.

Results: CB_1 receptor immunoreactivity (CB_1-IR) was observed in gastric epithelial cells, intestinal enteroendocrine cells (EEC) and globet cells (**Fig.**1), and in lamina propria mast cells (MCs)(data not shown). CB_1-IR was also weakly displayed by myenteric plexus (MP) neurons (data not shown). CB_2 receptor-IR (CB_2-IR) was expressed by EEC, intestinal epithelial cells, and lamina propria macrophages (**Fig.** 2). GPR55 receptor-IR (GPR55-IR) was expressed by EEC, macrophages, and lamina propria and Peyer's patches immunocytes. In addition, GPR55-IR was also expressed by MP neurons (**Fig.** 3). PPARa receptor-IR was expressed by putative gastric parietal cells and intestinal immunocytes. PPARa receptor-IR was also expressed by smooth muscle cells of the *tunica muscularis* and enteroglial cells. PPARγ receptor-IR was weakly expressed by some nucleus of MP neurons. TRPA1 receptor-IR was expressed by gastrointestinal MP and submucosal plexus neurons and globet cells. 5-HT1a receptor-IR was expressed by gastrointestinal epithelial cells and gastric smooth muscle cells (**Fig.** 4).

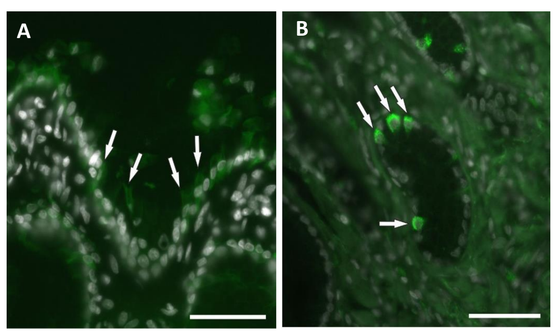

Fig. 1: Distribution of CB_1-IR cells in:
- **A**, gastric mucosa;
- **B**, intestinal enteroendocrine cells;
- **C**, intestinal globet cells;
- **D**, intestinal myenteric plexus

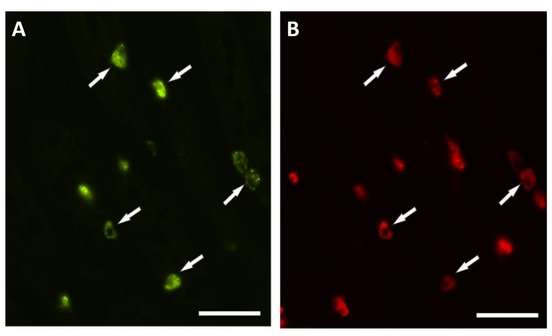

Fig. 2: Distribution of CB_2-IR cells in:
- **A** and **B**, intestinal enteroendocrine cells Cromogranin A positive;
- **C**, luminal surface of enterocytes;
- **D**, intestinal globet cells

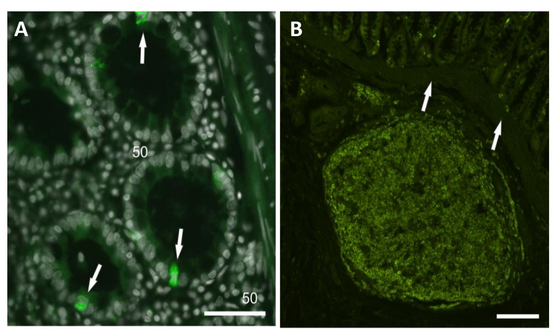

Fig. 3: Distribution of GPR55-IR cells in:
- **A**, large intestine enteroendrocrine cells;
- **B**, Immunocytes of lamina propria and above of Peyer's;
- **C**, gastric myenteric plexus;
- **D**, lamina propria IgA-IR plasma cells

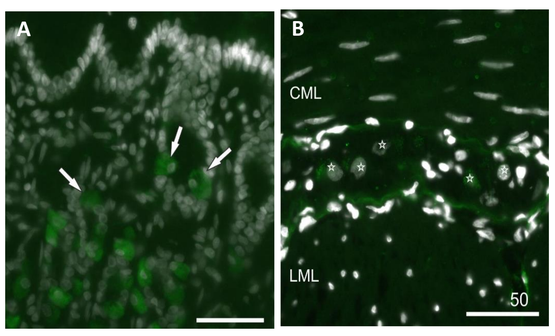

Fig. 4: Distribution of PPARa (**A**), PPARγ (**B**), TRPA1 (**C**) and 5-HT1a (**D**)-IR cells in:
- **A**, parietal cells of the pyloric glands;
- **B**, intestinal myenteric plexus;
- **C**, small intestine globet cells;
- **D**, gastric epithelial cells

Conclusions: Cannabinoid receptors showed a wide distribution in the feline GIT layers. Although not yet supported by functional evidences, the present research might represent an anatomical substrate that might be useful to support, in feline species, the therapeutic use of cannabinoids during gastrointestinal inflammatory diseases.

[1] Hornby and Prouty, 2004; [2] Izzo et al., 2004; [3] Duncan et al., 2005; [4] Storr and Sharkey, 2007; [5] Duncan et al., 2008; [6] Wright et al., 2008; [7] Sharkey and Wiley, 2016; [8] Lee et al., 2016; [9] Di Patrizio et al., 2016 [10] Di Carlo and Izzo, 2003; [11] Hornby and Prouty, 2004)