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DIPARTIMENTO DI SCIENZE MEDICHE VETERINARIE

Dottorato di ricerca in Scienze Veterinarie

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Localization of cannabinoid receptors in the equine dorsal root ganglia and ileum

Introduction. The activation of cannabinoid receptors by endogenous, plant-derived or synthetic cannabinoids may exert beneficial effects on inflammatory and neuropathic pain perception thanks to the analgesic, anti-inflammatory, anti-spasmodic and anti-anxiety properties. Their scientific evidence has prompted several companies to produce medical marjiuana and cannabinoid receptor agonists to be used in equine medicine to treat inflammatory disease and pain. The aim of this project was to localize the cellular distribution of nine canonical (CB1R and CB2R) and putative (GPR3, GPR55, PPARα, PPARγ, TRPV1, TRPA1 and 5-HT1a) cannabinoid receptors in the equine cervical dorsal root ganglia (DRG) and ileum.

M&M. Cervical DRG (C6-C8) and ileum were collected from three horses (1.5 years of age) at the public slaughterhouse. The tissues were fixed and processed to obtain cryosections for immunohistochemistry. The primary antibody used in the study are expressed in *Table 1*.

Results. In the DRG, all the receptors were expressed by neurons, satellite glial cells (SGCs), or both cellular types. The neurons showed immunoreactivity (IR) for CB1R, CB2R, GPR3, GPR55 PPARα, TRPV1, TRPA1, and 5-HT1aR. Neuronal processes showed CB1R- and TRPA1-IR. The SGCs showed immunoreactivity for CB2R, GPR55, PPARα, TRPA1, and 5-HT1aR. The PPARγ-IR was expressed by the nuclei of the sensory neurons and SGCs. In the ileum, all the receptors expressed immunolabeling, with the exception of GPR3. In the mucosa, the epithelium was immunoreactive for CB1R, CB2R, GPR55, PPARγ and 5-HT1aR; the lamina propria cells (immunocells) were positive for CB2R, GPR55, 5-HT1aR. The SMP neurons showed immunoreactivity for CB1R, PPARγ, TRPA1 and TRPV1, while SGCs expressed CB1R, PPARα, PPARγ, TRPV1. The MP neurons showed immunoreactivity for CB1R, PPARγ and TRPA1, while SGCs expressed CB1R, PPARα and TRPV1. The muscular layer expressed immunoreactivity for GPR55 and PPARα. *Figure 1* shows examples of the localization of the receptors in the equine DRG and intestine.

Primary AB	CB1R	CB2R	GPR3	GPR55	PPARα	PPARγ	5-HT1aR	TRPA1	TRPV1
Host	Rabbit	Rabbit	Rabbit	Rabbit	Rabbit	Rabbit	Rabbit	Rabbit	Rabbit
Code	Ab23703	Ab45942	Ab106589	NB110-55498	NB600-636	Ab45036	Ab85615	Ab58844	ACC-030
Source	Abcam	Abcam	Abcam	Novus Biol.	Novus Biol.	Abcam	Abcam	Abcam	Alomone

Table 1 - Primary antibodies used in the study.

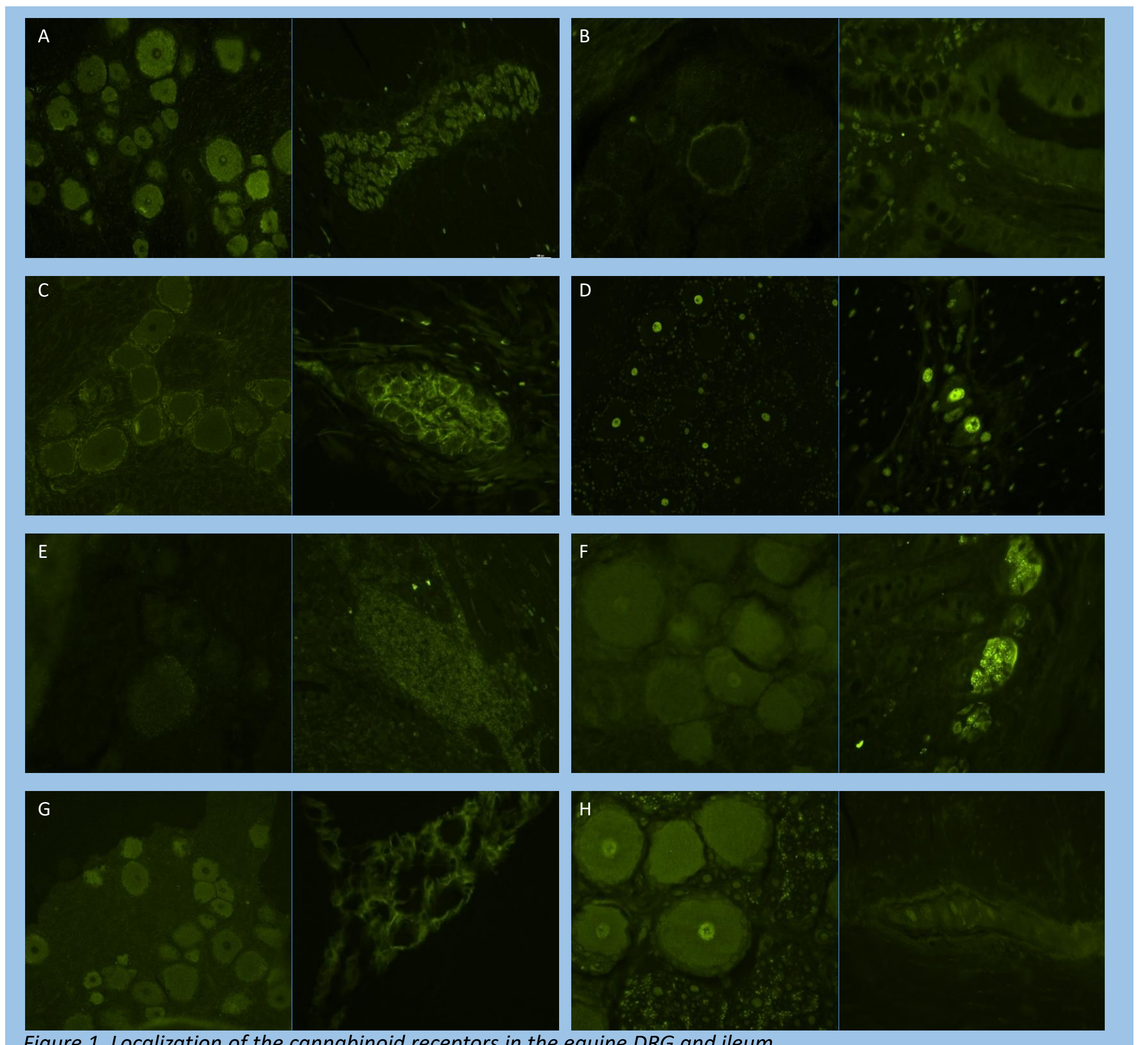

Figure 1. Localization of the cannabinoid receptors in the equine DRG and ileum.
A: CB1R was expressed by DRG (left) and MP (right) neurons; B: CB2R in SGCs (left) and ileal mucosa (right) (epithelium and lamina propria cells); C: PPARα was expressed by SGCs both in DRG (left) and SMP (right); D: PPARγ was expressed in DRG (left) and MP (right) neurons; E: GPR3 was expressed by neurons in the DRG (left); GPR55 was expressed by immunocytes in the ileal Peyer patches (right); F: 5-HT1aR was expressed by DRG neurons (left) and Paneth cells in the intestinal mucosa (right); G: TRPV1 was expressed by DRG neurons (left) and MP glial cells of the ileum (right); H: TRPA1 was expressed by DRG neurons and SGCs (left) and ileal myenteric neurons (right).

Distribution and co-expression patterns of specific cell markers of enteroendocrine cells in pig gastric epithelium

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Introduction. The pig is an accepted model species for human digestive physiology. In this study we have investigated the distribution and the morphology of each of the classes of gastric endocrine cells (EEC) (gastrin, ghrelin, somatostatin-SST, 5-hydroxytryptamine-5HT, histidine decarboxylase-HDC and PYY) in pig stomach, to highlight any differences/parallelism with human stomach.

M&M. Three grower pigs (30-35 kg females) from the *University of Melbourne School of Agriculture and Food* were used for this study. Samples from gastric fundus (F), corpus (C) and antrum (A) were collected and prepared for immunohistochemistry.

Results. As in human, gastrin cells were almost exclusively in the antrum, ghrelin cells were most abundant in the oxyntic mucosa and PYY cells were rare. In the pig, 70% of ECL cells in the antrum and 95% in the fundus contained 5-HT, higher proportions than in human *(Figure 1, 2 and 3)*. Unlike the EEC cells of the small intestine, most gastric EEC did not contain colocalised hormones (*Figure 4*); this is similar to human and other species. We conclude that the pig stomach has substantial similarity to human.

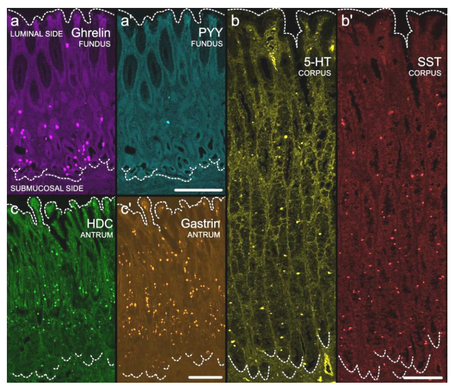

Figure 3. EEC morphologies and relationships. Examples of cells immunoreactive for ghrelin (a and b), SST (c and d), gastrin (e), HDC (f), 5-HT (g), and PYY (h). Arrows indicate small basal processes in a, c, d, and g. Scale bars: 20 µm.

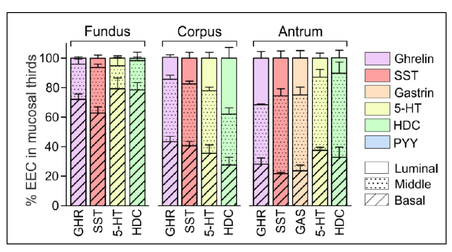

Figure 2. Distribution of EEC across the width of the mucosa in pig gastric F,C, and A.

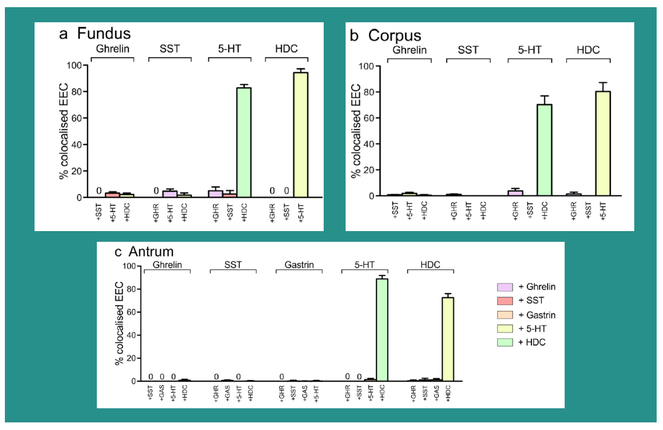

Figure 4. Overlaps between hormones in pig gastric F (a), C (b), and A (c).

Water immersion vs air insufflation in canine duodenal endoscopy: is the future underwater?

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Introduction. In human medicine, during colonscopy, the use of air (air insufflation, AI) to distend the intestinal lumen can induce pain. More and more frequently it is used warm-to touch water, instead of air, to distend the lumen (water immersion, WI). This method decreases the spasm of the musculature of the bowel, reducing the pain, and significantly increases the visualization of mucosal texture and the adenoma detection rate (ADR).

In this study we compare AI and WI during duodenoscopy in anesthetized dogs, in order to evaluate any differences in procedural nociception and in the quality of mucosal visualization (*Figure 1*).

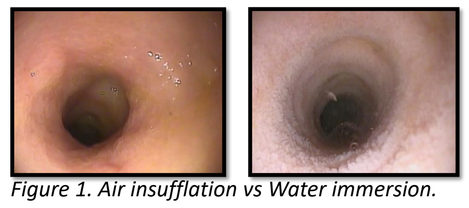

Figure 1. Air insufflation vs Water immersion.

M&M. Twenty-five dogs, subjected to gastroduodenoscopy under general anesthesia, were included in the study. The heart rate and the arterial blood pressure were measured throughout the procedure. To evaluate the quality of the images, the same mucosal image of the duodenum was recorded with both AI and WI, and subjected to a texture analysis (*skeletonization- Figure 2* and *entropy* evaluation) and to a subjective blind evaluation by three expert endoscopists.

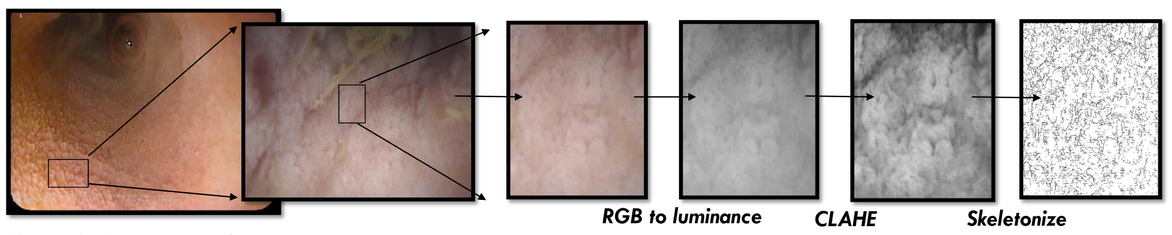

Figure 2. Application of the skeletonization method.

Results. No systematic significant differences were detected for the cardiovascular parameters (*Figure 3*), maybe influenced by the drugs used for the anesthesia , which well control nociception.

The texture analysis did not give significant results, except for the subjective evaluation by the endoscopists, who identified the WI images as qualitatively better *(Figure 4).*

Based on the evaluation of the endoscopists, the WI allows to get better quality images, with a detailed visualization of the intestinal villi, while this is still not confirmed by objective texture analysis.

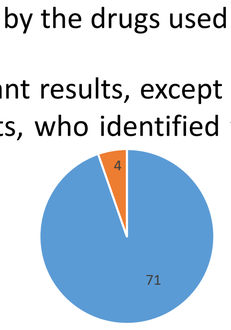

Figure 4. Results of the subjective evaluation. Orange: AI, blue: WI.
***K** (fixed-marginal K) **=0,74** (0,61-0,80).*

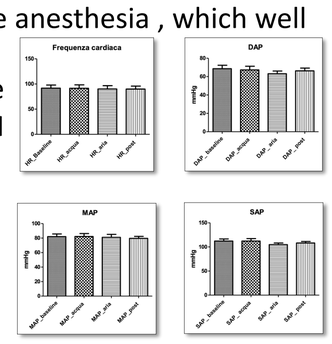

Figure 3. Results of the evaluation of the cardiovascular parameters.