

Dottorato di Ricerca – XXXII Ciclo

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Localization of cannabinoid receptors CB1, CB2, GPR55, and PPARa in the canine gastrointestinal tract

The endocannabinoid system (ECS) is composed of cannabinoid receptors, their endogenous ligands and the enzymes involved in endocannabinoid turnover. A growing body of evidence indicates that activation of cannabinoid receptors by endogenous, plant-derived, or synthetic cannabinoids may exert beneficial effects on gastrointestinal inflammation and visceral pain. The present *ex vivo* study aimed to investigate immunohistochemically the distribution of cannabinoid receptors CB1, CB2, G protein-coupled receptor 55 (GPR55), and peroxisome proliferation activated receptor alpha (PPARα) in the canine gastrointestinal tract. CB1 receptor immunoreactivity was observed in the lamina propria and epithelial cells and in myenteric plexus neurons. CB2 receptor immunoreactivity was expressed by lamina propria mast cells and immunocytes, blood vessels and smooth muscle cells. Faint CB2 receptor immunoreactivity was also observed in neurons and glial cells of the submucosal plexus. GPR55 receptor immunoreactivity was expressed by lamina propria mast cells, and glial cells of the myenteric plexus. Cannabinoid receptors showed a wide distribution in the gastrointestinal tract of the dog. Since cannabinoid receptors have a protective role during intestinal inflammatory processes, the present research provides an anatomical basis supporting the therapeutic use of cannabinoid receptor agonists in relieving motility disorders and visceral hypersensitivity in canine acute or chronic enteropathies.



Cryosections of canine colon: CB1 receptor immunoreactivity in the mucosa. Open arrows indicate enterochromaffin cells co-expressing moderate CB1- and bright 5-HT-immunoreactivity.



Cryosections of canine intestinal tract: CB2 receptor immunoreactivity in the mucosa. Arrows indicate bright CB2-immunolabelled endothelial cells of blood capillaries running along the major axis of a



Cryosections of canine gastrointestinal tract: GPR55 receptor immunoreactivity in the mucosa. Small white arrows indicate lamina propria cells showing bright immunoreactivity. The small open arrows



Cryosections of canine gastrointestinal tract: PPAR α receptor in the mucosa (a,b) and muscle layers (c). Open arrows and white arrows indicate, respectively, blood vessels and fascicles of smooth muscle in a

The white arrow indicate a 5-HT immunoreactive cell of the lamina propria.



Cryosections of canine intestinal tract: CB1 receptor immunoreactivity in the myenteric plexus. The arrow indicates a neuron showing weak and punctate CB1 receptor immunoreactivity.

duodenal villus. White and open arrows indicate, respectively, the nuclei of smooth muscle and endothelial cells expressing strong CB2 immunoreactivity.



Cryosections of canine gastrointestinal tract: CB2 receptor immunoreactivity in the muscle layers. Stars indicate the nuclei of myenteric plexus neurons, which were CB2-negative. On the contrary, the smooth muscle cells of the longitudinal (LML) and circular muscle layers (CML) showed intense CB2 immunoreactivity.

indicate GPR55-immunolabelled enterochromaffin cells. Large open arrows indicate epithelial cells of the inner portion of the mucosa of the colon, which showed diffuse GPR55 immunoreactivity.



Cryosections of canine gastrointestinal tract: GPR55 receptor immunoreactivity in the muscle layers. Arrows indicate bright GPR55-immunolabelling in the external portions of the small intestine LML and in the whole LML of the colon.

villus of ileal mucosa showing bright PPARα. In figure c, arrows indicate bright PPARα-immunolabelling in the external portions of the LML.



Cryosections of myenteric plexus (MP) of canine duodenum: PPAR α receptor immunoreactivity. Stars indicate MP HuC/HuD-immunoreactive neurons which were PPAR α -negative. On the contrary, a network of PPAR α -positive cellular processes belonging to enteric glial cells is visible around HuC/HuD neurons.

ENDOSCOPIC BRONCHIAL ANATOMY IN THE DOG

Bronchoscopy is an important diagnostic procedure for the evaluation of many respiratory diseases and the removal of foreign bodies. During bronchoscopy, it is fundamental to know precisely the bronchial topography, in order to recognize abnormal anatomy or pathological changes and to locate foreign bodies or inflammatory processes. Currently, the endoscopic anatomy is based on a paper of 1986¹. Therefore, the aim of this study was to obtain a description of topographic anatomy and morphometric value, to introduce a new standardised nomenclature and draw a correct bronchial map of the dog.

Twelve dogs, different in age, sex and breed, who died spontaneously for reasons other than pulmonary diseases, were included in the study, with owners' consent, and distinguished by weight in 3 groups (<10, 10-25, >25Kg). All the subjects were examined endoscopically in a systematic manner with a HD flexible endoscope (\emptyset 6mm). The endoscopy focused on intracorpore (IC) and extracorpore (EC) exam. The EC was performed after the isolation of the lungs in order to obtain a better examination of cranial lobes, difficult in IC for their orientation. We considered the visualisation and the ability to pass through the lumen of each branch with the endoscope.

After that, on the same lungs, casts of polyurethane foam were made and diameter and length of the



bronchial branches were measured through a digital calliper. Furthermore, to name the structures and to draw the bronchial map, we defined them by looking at their direction and position.

All the casts conformed to the orientation, the branching pattern and the topographic relationship seen during broncoscopy. For each lung lobe it was possible to define a new descriptive nomenclature for the first three series of bronchial division. The morphometric examination allowed to obtain a mean value of diameter and length of bronchi for each group of weight and to confirm the monopodial branching system.

During bronchoscopy it was possible to locate and/or move in the principal, lobar and segmental bronchi, with significant differences between the groups. After comparison with previous studies, we draw a new bronchial map and gave a descriptive nomenclature for the first three series of canine bronchial division. Moreover, we analysed the accessible airways with a 6mm diameter flexible endoscope in the different groups.

Finally, our results provided accurate reference values useful in diagnostic imaging procedures, especially during bronchoscopy.



¹Amis TC & McKiernan BC: Systematic identification of endobronchial anatomy during bronchoscopy in the dog. American Journal of Veterinary Research 1986, 47 (12), 2649-2657.