Dottorato di Ricerca – XXXII Ciclo

Dottoranda: Giorgia Galiazzo

Tutor: Prof. Roberto Chiocchetti

Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Italia

Distribution of cannabinoid receptors CB1, CB2, GPR55 and PPARα in gastrointestinal tract of healthy dogs

Background - The Endocannabinoid system (ECS) is composed of cannabinoid receptors, endocannabinoids and the enzymes for their production and degradation[1]. The principal cannabinoid receptors are CB1, expressed mostly in the central nervous system (CNS) and peripheral nerves, and CB2, mainly expressed in immune cells [2]. G protein-coupled receptor 55 (GPR55) and nuclear peroxisome proliferator-activated receptor alpha (PPARα) are considered as novel cannabinoid receptors[3].

Aim - To investigate the distribuition of CB1, CB2, GPR55 and PPARα in different gastrointestinal tracts of healthy dogs.

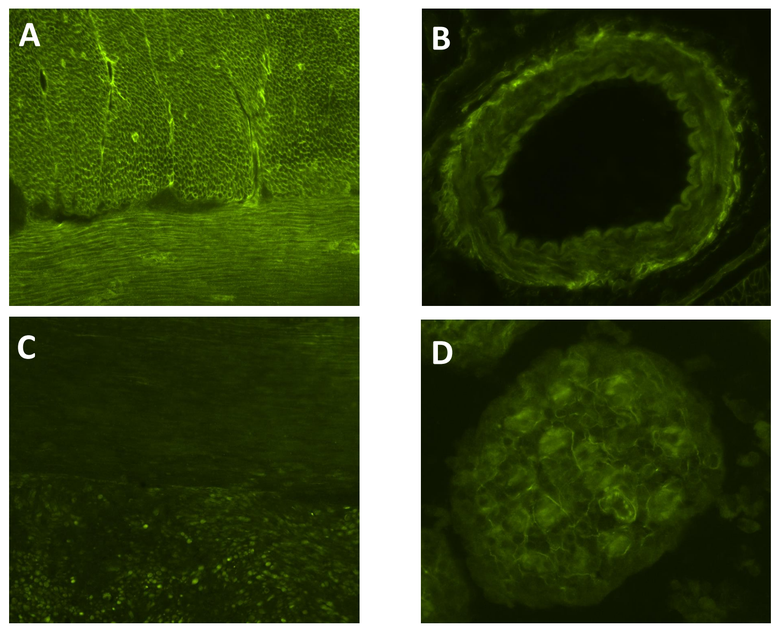
Methods - The tissue were collected ex-vivo from three dogs spontaneously dead or euthanized for human reasons. These dogs did not show gastrointestinal synptoms and did not present macroscopic alterations of gastrointestinal wall. The gastrointestinal tract was collected within 2 hours after each animal's death. Samples from stomach, ileum and colon were processed for immunohistochemistry.

Preliminary results – Table 1 shows the distribution of receptors in stomach, ileum and colon of healthy dogs.

Table 1. Semiquantitative evaluation of density of receptors immunoreactivity (preliminary results).

	PYLORUS				ILEUM				DESCENDING COLON			
	CB1	CB2	GPR55	PPARα	CB1	CB2	GPR55	PPARα	CB1	CB2	GPR55	PPARα
Epithelium	-	-	-	-							+++	
LP	-	-	-	-		++	+++	++			+++	
MM	-	++	++	-		++	+++				+++	
Blood vessels	-	-	-	-		+++		+++				
SMP	-	-	-	-	-	G ++ N -	-		-	G +++, N -	-	
CML	-	++	++	+		+++	+++	+/++		++	-/+	
MP	-	-	-	G +	-	-	-		-		-	
LML	-	++	+	++		+++	+++	++		+	+++	

Immunoreactive cells are graded as: –, not found; +, a very small number; ++, a moderate number; +++, a large number.
Abbreviations: lamina propria (LP); muscolaris mucosae (MM), circular muscle layer (CML); longitudinal muscle layer (LML); myenteric plexus (MP); submucosal plexus (SMP); G, glial cells; N, Neurons.



Cryosections of colon (A-C) and ileum (D) of the dog.
A and B) CB2-immunoreactivity in smooth muscle cells of the *tunica muscularis* (A) and a large submucosal blood vessels (B).
C) GPR55-immunoreactivity in the longitudinal muscle layer of the *tunica muscularis*.
D) PPARα-immunoreactivity in cells (mucosal glial cells?) distributed in the lamina propria of a villus.

[1] Stella N: Cannabinoid signaling in glial cells. *Glia* 2004; 48: 267-77. [2] Di Marzo V and Izzo AA: Endocannabinoid overactivity and intestinal inflammation. *Gut* 2006; 55 (10): 1373-1376.
[3] Izzo AA and Sharkey KA: Cannabinoid and the gut: new developments and emerging concepts. *Pharmacology & therapeutics* 2010; 126: 21-38.

Artificial torpor as a new experimental model for neurodegenerative diseases

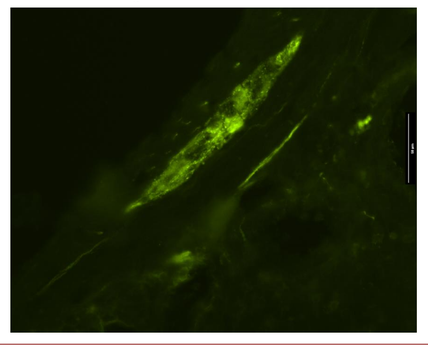
Background - Tau protein is a "microtubule-associated-protein". Its phosphorylated form (pTau) is involved in the pathogenesis of different neurodegenerative processes, such as Alzheimer and Parkinson's disease, called "taupathies"[1]. Injection of GABA agonist muscimol in the brain stem of rat can induce a deep hypothermia, comparable to the hibernation of mammals. During this artificial torpor (AT), there is an overproduction of p-Tau, reversible with the recovery of the euthermia[2].

AT could be an interesting model to evaluate the process of phosphorylation and dephosphorylation of Tau protein, to better understand its role in neurodegenerative diseases.

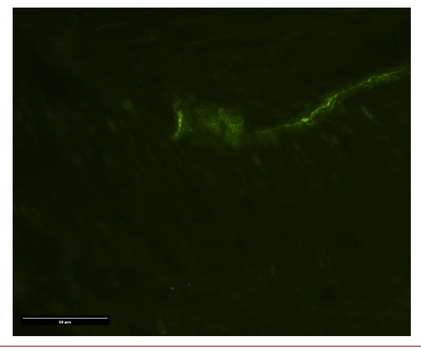
Aim - To introduce AT-rat as an innovative animal model for the comprehension of pTau-dependent cellular process in central (CNS) and autonomic nervous system (ANS), including enteric nervous system (ENS).

Methods - Hypothermia was induced by the injection of muscimol in the brain stem of rats. Animals were euthanized at the minimum of hypothermia, at the recovery from hypothermia (after 6 hours) and as control. Tissue, collected from CNS, ANS and gastrointestinal tract, were processed for immunohistochemistry to evaluate the expression of p-Tau by different neurons. We focus on enteric nervous system.

Preliminary results



Hypothermia, ileum (cryosection). Expression of strong p-Tau-immunoreactivity in the myenteric plexus.



Recovery, ileum (cryosection). Expression of faint p-Tau-immunoreactivity in the myenteric plexus.

[1] Mietelska-Porowska A, Waisk U, Goras M, Filipek A, Niewiadomska G: Tau protein modifications and interactions: their role in function and dysfunction. *International journal of molecular sciences* 2014; 15: 4671-713.
[2] Stieler JT, Bullmann T, Kohl F, Tøien Ø, Brückner MK, Härtig W, Barnes BM, Arendt T: The physiological link between metabolic rate depression and tau phosphorylation in mammalian hibernation. *PLOS ONE 2011;* 6 (1): e14530.

Serotonin (5-HT), 5-HT4 receptor and reuptake of 5-HT in the digestive system of dogs.

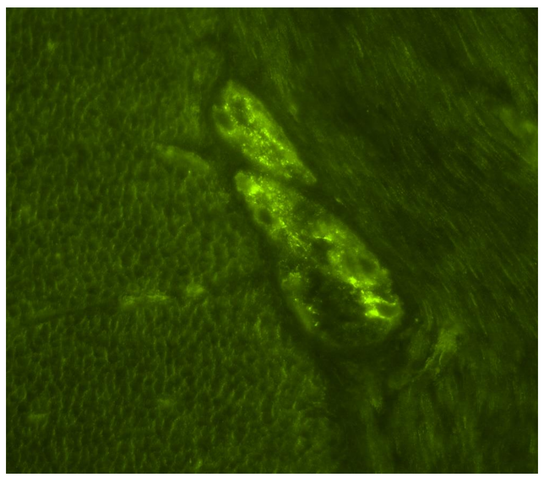
Background – Serotonin (5-HT) is a neurotransmitter produced by intestinal enterochromaffin cells (EC). Linking to its receptors, 5-HT influences intestinal sensitivity, motility and secretion[1]. In human medicine, patients with Crohn's disease or ulcerative colitis show an increased synthesis of 5-HT or a decreased expression of SERT, membrane transporter which inhibits 5-HT functions. Inflammatory Bowel disease (IBD) of dogs shows similarities to human chronic enteropathies. Human serotoninergic system has been deeply investigated; on the other hand, these morphologic studies lack in dogs.

Aim – To identify and quantify cells which express 5-HT, 5-HT4 receptors and SERT in the gastrointestinal tract of healthy and IBD dogs.

Methods – Samples were collected from gastrointestinal tracts of IBD dogs during gastro-duodeno-colonscopy and from control dogs, dead spontaneously or euthanized for human reasons, during necropsy. Tissue were prepared for immunohistochemistry.

Preliminary results in control dogs

Tract	Stomach	Duodenum	Jejunum	Ileum	Colon
% of 5-HT4 positive myenteric plexus neurons	34%	24%	58%	34%	34%



Cryosections of jejunum of a control dog. 5-HT4 receptor-immunoreactivity in myenteric plexus and muscolar layers.

[1] Mawe GM and Hoffman JM: Serotonin signaling in the gut- functions, dysfunctions and therapeutic targets. *Nature Reviews Gastroenterology and Hepatology* 2013; 10 (8): 473-486.