Journal of Animal and Veterinary Advances 11 (22): 4269-4278, 2012
ISSN: 1680-5593

Emerging Immunohistochemical Evidence for Direct Peripheral Control of Endocannabinoids on the Gastrointestinal Tract and Pancreas of Obese (fa/fa) and Lean Zucker Rats (Pathophysiological Implications)

V. Tessitore, G. Bonaventura, F. Carini, D. Cucco, G.F. Spatola and M.L. Uzzo
Department of Biomedicine Experimental and Clinical Neuroscience,
Histology Section and Anatomy Section, University of Palermo, Palermo, Italy

Abstract: This research has the objective to investigate immunohistochemical expression of CB1 receptor and its probable changes in Gastroenteropancreatic system (GEP) of obese and lean Zucker rats and understand the endocannabinoid pathophysiological implications in the obesity. Male obese (fa/fa) and lean Zucker rats 6 weeks old were obtained from Harlan Italy Srl; the rats were sacrificed at 8, 12 and 16 weeks old. Normal rats also were sacrificed. Specimens of stomach, jejunum-ileum and pancreas were fixed in Bouin's mixture and embedded in paraffin; obtained sections were processed with anti-CB1 (Biosource Europe SA) by Streptavidin-Biotin-Complex Method. The findings show that CB1 receptor is expressed not only in enteric neurons as documented by earlier studies up to now but more widely and with stronger intensity in obese animals compared with their lean counterparts by several structures of gastrointestinal tract (epithelium, glands, endocrine cells and immune cells of villi stroma). In obese Zucker rats pancreas unlike the normal rats where the CB1 receptor is essentially expressed by A-cells, the CB1 immunoreactivity even extends with higher intensity to B-cells. It is concluded that GEP System represents a new and wide peripheral target of EC action that have a direct autocrine, endocrine-paracrine and neurocrine control on many functions of GEP. In addition in GEP of obese Zucker rats in comparison with lean ones, the CB1 receptor is overexpressed and consequently the peripheral endocannabinoid system is upregulated and negatively modulated by leptin. It may contribute to increase hyperphagia, body weight and hyperglycaemia.

Key words: Endocannabinoids, food intake, body weight, energy balance, CB1 receptor, GEP System

INTRODUCTION

Experimental evidences, already consolidated, suggest that the Endogenous Cannabinoid System (ECS), made as known of CB1 and CB2 receptors of their respective endogenous ligands, the Endocannabinoids (EC) and of their synthesizing and degrading enzymes is involved in the regulation of several physiological processes of high importance including neuroprotection, immunomodulation, antinociception, inflammation, locomotor function, learning and memory among others (Piomelli, 2003; De Petrocellis *et al*., 2004; Di Marzo *et al*., 2004; Pagotto *et al*., 2007). The discovery of CB1 and CB2 receptors provided, first of all a molecular basis to understand the action mechanism of EC and more, it has stimulated the syntesis of cannabinoid CB1 receptors both agonists and antagonists whose use is an attractive therapeutical strategy for the treatment of those diseases attributed to a disregulation of endocannabinoid system (Fox *et al*., 2004; Zajicek *et al*., 2003; Patton *et al*., 2002; Colombo *et al*., 1998; Fernandez and Allison, 2004; Van Gaal *et al*., 2005; Despres *et al*., 2005; Di Marzo *et al*., 2000).

The wide distribution of CB1 in many encephalic areas (hippocampus, limbic system, amygdala, basal ganglia, cerebellum and hypothalamus) at first created the conviction that it should be considered brain specific that is exclusively expressed by the central neurons (Matsuda *et al*., 1990; Howlett *et al*., 2002). When Munro clones a second receptor (CB2) in the spleen, it was thought that it was escusively expressed by immune peripheral cells (Munro *et al*., 1993). Nevertheless, very recent studies, through different technologies have shown that CB1 can be expressed out of SNC in several peripheral tissues (Osei-Hyiaman *et al*., 2005; Bonaventura *et al*., 2007a, b; Gye *et al*., 2005; Ruiz-Llorente *et al*., 2003; Das *et al*., 1995; Cota *et al*., 2003a; Liu *et al*., 2005; Kulkarni-Narla and Brown, 2000; Pertwee, 1999; Pinto *et al*., 2002).

Corresponding Author: Carini Francesco, Department of Biomedicine Experimental and Clinical Neuroscience (BIONEC), Histology Section and Anatomy Section, Via del Vespro 129, 90127 Palermo, Sicilia, Italy

A very present and really interesting point, both erudite and clinical practical, refers to the key role that the endocannabinoids may have on the energy balance (Kirkham, 2003; Di Marzo and Matias, 2005; Cota and Woods, 2005; Pagotto *et al*., 2007; Komorowski and Stepien, 2007; Cota *et al*., 2003a, b; Wiley *et al*., 2005) whose balance can be altered by a derangement between intake and expenditure of energy, ending in obesity, a disease that got such a popularity to be even represented in the mythology through different sculptures of mother God, cult object (Venus of Willendorf of Catal Hoyuk ecc).

Several recent reports have demonstrated that endocannabinoids modulate appetite, body weight and motivation to consume palatable food, via central orexigenic mechanism through specific activation of CB1 receptor expressed and/or coexpressed by some hypothalamic neurons and of limbic system, producing orexigenic and anorexigenic neurohormones and neurotransmitters such Nociceptin/Orphanin FQ (NOP/OFQ), orexins A-B, leptin, Corticotropin-Releasing-Hormone (CRH), Cocaine-Amphetamine-Regulated-Transcript (CART), Melanin-Concentrating-Hormone (MCH), Vasointestinal Peptide (VIP), Neuropeptide Y (NPY), all neuropeptides known to modulate feeding behavior and energy balance (Elmquist *et al*., 1999; Kalra *et al*., 1999; Cota *et al*., 2006). Other findings suggest that in obese and Hyperphagic Models of animals, the hypothalamic level of endocannabinoids increases and on the contrary it decreases after the administration of leptin (satiety hormone) (Di Marzo *et al*., 2001). The administration of endocannabinoids in animal models stimulates the appetite (Hao *et al*., 2000; Jamshidi and Taylor, 2001) while the intake of a new antagonist of CB1 receptor (rimonabant) reduces in mice and in rat the food intake and body weight (Colombo *et al*., 1998). In conclusion the lack of CB1 receptor in knockout mices causes hypophagia with reduction of fat mass and consequently of body weight (Cota *et al*., 2003a). Nevertheless a growing body of experimental evidence suggests that CB1 receptor could be expressed by some peripheral tissue that have a control role on the nutritional balance (Osei-Hyiaman *et al*., 2005; Cota *et al*., 2003b; Engeli *et al*., 2005; Liu *et al*., 2005; Bonaventura *et al*., 2007a, b; Tessitore *et al*., 2006a). So, it is presumable that the role for the ECS in regulating appetite and metabolism is not restricted to the CNS but it appears to extend to several peripheral organs and tissues (Cota, 2007).

Among these the Gastroenteropancreatic system (GEP) is surely more than any other area that is involved in the control of nutritional balance through intake, absorption, digestion and nutrient in the glicemic homeostasis. Therefore, this distrect can become a vast peripheral target of EC if its structural components will prove capable of expressing the CB1 receptor activated by EC allows the unfolding of their orexigenic effect. Besides the gastrointestinal tract is for its own, a big primary or alternative source of orexigenic (grelin, porphanin orexin) and anorexigenic (leptin) neuropeptides (Tessitore *et al*., 2005, 2006b; Cint *et al*., 2000; Bonaventura *et al*., 2005). So, this is a wide organ able to select and lead a flow of orexigenic and anorexigenic signals that direct to the hypothalamus inform it about the nutritional status of organism in accordance with a correlative axis already confirmed in many other occasion (brain gut axis).

Is to be specified however that the primary role assigned to EC against the gastroenteric apparatus was to exert an inhibitory control on intestinal motility. The >100 years ago in fact, the estract of cannabis were administered in gastroenteric pain and in diarrhea. These empirical therapeutic applications received in recent times confirmations more scientific by the CB1 receptor immunohistochemical identification of neurons in the enteric nervous system (Auerbach plexus submucosal and myenteric plexus) of man, guinea pig, pig and rat (Kulkarni-Narla and Brown, 2000; Pertwee, 1999; Pinto *et al*., 2002; Storr *et al*., 2004; Griffin *et al*., 1997), its activation from the endocannabinoids inhibits the motility and the gastroenteric secretion in rodents and in man with a Neurocrine mechanism that is through the involving of axon terminals, respectively of myenteric and submucosal plexus (Calignano *et al*., 1997; Izzo *et al*., 2001, 1999; Coruzzi *et al*., 1999; Germano *et al*., 2001). Even in more recent time Nabilone (Cesamet), synthetic derivative of delta 9-THC have been used in the treatment of AIDS-related anorexia and cachexia (Plasse *et al*., 1991; Volicer *et al*., 1997; Di Marzo *et al*., 2000). At present, finally has been studied the protective role of EC in a murine model of colitis (Borrelli *et al*., 2009) and in the gut of patients with inflammatory bowel disease (Di Sabatino *et al*., 2011; Schicho and Storr, 2011; Izzo *et al*., 2012).

Regarding the pancreas the dates reported in the literature ascribe that the exocrine and endocrine parenchyma express the CB1 receptor. While there is unanimous agreement to exocrine receptors able to express CB1 and CB2 receptors, relatively to endocrine pancreas the literature supplied no univocal resulted regarding the identification of endocrine cytotipes that this expressed receptors (Tessitore *et al*., 2006b; Juan-Pico *et al*., 2006; Bermudez-Silva *et al*., 2008; Tharp *et al*., 2008; Matias *et al*., 2006; Starowicz *et al*., 2008). This vexed remarks are likely to ascribe to use of

different experimental models (rats, mice and man) studied in different periods of life and to use of different technique (PCR, immunohistochemical).

With reference to what is stated on the EC orexigenic role, researchers have undertaken an immunohistochemical study already started years ago on the expression of CB1 receptor in the no neural structures of gastroenteropancreatic system. The aim of the present study was to give a complete distribution map of CB1-immunoreactive structures that may constitute a basis for morphoistochemical basis for contributing to explain the pathophysiologic implications of EC in obesity and in the associated metabolic comorbidity. Therefore, researchers have used Zucker rats that are genetically obese animal models and have a missense mutation in the leptin receptor gene. All leptin receptor isoforms are affected by this mutation. Consequently the receptors number on the surface membrane of cells and their signal transduction efficency are decreased. Therefore, the rats homozygous for the lepr fa are hyperphagic, hyperinsulinemic and hypercholesterolemic.

MATERIALS AND METHODS

Animals and tissue preparation: The N°10'6 weeks old male obese (fa/fa) and N°10' lean Zucker rats purchased from Harlan Italy Srl, weighting between 250-300 g were used. They were used. In addition, normal male Wistar rats (250, 300 g) were used. All rats were housed in plastic cages and maintained on commercial pelleted diet (Harlan Tekland, Rodent diet No. 8640) in humidity and temperature controlled room with free access to water. All experiments were performed with guidelines for the care and use of laboratory animals of Helsinki Conventionas was demostrated that in obese Zucker rats the citotipe of endocrine pancreas developed an apoptotic damage (8°-12° week) (Shimabukuro *et al*., 1998), researchers have valutated the possible modifications of the pancreatic immunoreactivity sacrificing some Zucker rats of 8, 12 and 16 weeks of life.

Specimens of stomach, jejunum-ileum and pancreas have been drawn, fixed in Bouin mixture, dehydrated in a graded ethanol series and embedded in paraffin blocks for light microscopy and immunohistochemistry.

Immunohistochemistry: Serial sections 8 μ thick were cut on to Leica microtome RM2145, dried overnight at 37°C and then stored at room temperature. The day after, the slides were dewaxed and rehydrated by sequential immersion in a graded series of alcohols and transferred into water for 5 min for the endogenous peroxidase inhibition the slides were treated with 3% hydrogen peroxide in hydrated incubation enclosure at room temperature. Subsequently, the slides were transferred in PBS (Na_2HPO_4, KH_2PO_4, KCl, NaCl pH 7.4-7.6) at room temperature. The following protocol was realized using the kit DAB500 Chemicon by the standard streptavidin-biotin complex. After rinsing with PBS, the tissue sections were blocked with super block reagent (normal goat serum in phosphate buffered saline containing carrier protein) for 10 min and then rinsed with PBS for 4 min. Then, the sections were incubated overnight at 4°C with polyclonal anti-CB1 (Biosource Europe SA). After the incubation, any excess antibody was removed by washing and the slides were rinsed 2X with PBS, 5 min each. Next the polyvalent anti-serum (biotinylated goat anti-mouse IgG and goat anti-rabbit IgG in PBS) for 10 min at room temperature was added; unbound antibody was removed by washing (2X with PBS, 5 min each) and subsequently Streptavidin biotin peroxidase for 20 min at room temperature was applied. After incubation, unbound enzyme was removed by rinse procedure (2X with PBS, 5 min each). DAB chromogen in substrate buffer was then added for 5 min and stopped in distilled water. Then, the slides were removed from the water and one drop of aqueous mounting medium (DAKO Faramount) and a coverslip were applied to tissue sections.

Negative controls were performed by omission of primary antibody and by incubating sections with anti-serum saturated with omologous antigen. Finally, the sections have been observed with Leica DMLB microscopy. Densitometric quantification of CB1 expression was performed by image analysis using Image Pro Plus Software valuating five sections for animal.

RESULTS AND DISCUSSION

Researchers underline that the densitometria valutation of immunoreactivity CB1 performed by image analysis of the examinated fragments (stomach, jejunum-ileum and pancreas) evidence that it is bigger in obese rat compared with lean counter parts.

Stomach: In obese fa/fa Zucker rats the immunoreactivity of CB1 extensively interests the whole mucosal thickness of different gastric regions; the glandular epithelium is intensely but not uniformly reactive with evident increase in the lower half of the fundic glands in the higher mucosal region some cells display very strong CB1 immunoreactivity (Fig. 1 and 2).

In lean Zucker rats the immunoreactivity of CB1 is less intense in the glandular epithelium and essentially restricted in the lower half of the fundic glands (Fig. 3). In both animal models the immunoreactivity of glandular epithelium interests both the pepsinogen-secreting chief

Fig. 1: Obese Zucker rat; strong and not uniform CB1 immunoreactivity in the entire glandular epithelium of gastric mucosa; 20x

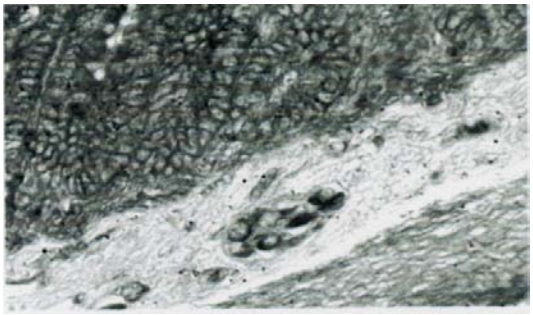

Fig. 4: Obese Zucker rat; strong CB1 immunoractivity in the gangliar neurons of stomach submucosal plexus; 40x

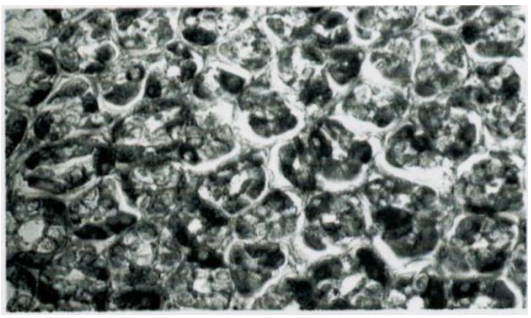

Fig. 2: Obese Zucker rat; strong CB1 immunoractivity in chief cells and endocrine cells of stomach oxintic glands; 40x

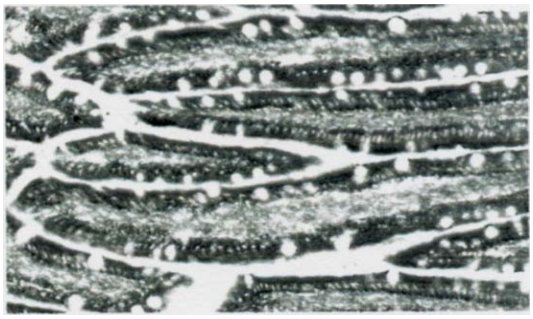

Fig. 5: Lean 16 Zucker rat; remarkable CB1 immunoreactivity in the mucosal epithelium of villi with increase in the surface membrane cell; 20x

Fig. 3: Lean Zucker rat; weak CB1 immunoreactivity in the stomach is restricted to the basal region of oxintic glands, vanished in the apical region; 20x

cells and endocrine cells (p cell type) while the parietal cells appear negative as confirmed by the use of toluidine blue (Fig. 2). In the muscular layer in the border between the circular and longitudinal myocellular sheet, some immunopositive gangliar neurons of myenteric plexus are found (Fig. 4).

Jejunum-ileum: Diffuse cytoplasmic CB1 immunoreactivity was observed in the mucosal epithelium of villi (enterocytes and M cells), sometimes with increase in the apical cytoplasmic region and in cell membrane (Fig. 5 and 6a). The globet cells aren't immunoreactivity. In the Galeazzi tubular glands the immunopositivity is more weak and restricted to the apical cytoplasm of cells (Fig. 7). In the lamina propria of villi and in the periglandular chorion numerous cytotypes are found that intensely express CB1 receptor immunoreactivity (Fig. 6a, b and 7), belonging to Immune-Secretory Intestinal System (GALT). This distrect is costituited by cytotipes residents both mucosal epithelium between the enterocytes (intraepithelial lymphocytes, IELS cells with Micropieghe, M; Dendritic cells, Dcs) both in the lamina propria periglandular and in the stroma of the villi (T and B lymphocytes, MC mast cells and macrophages. In the submucosal layer some gangliar neurons of Meissner plexus intensely positive are found intensely immunoreactive are found (Fig. 4) whose axon terminals follow at times since villous stroma axial.

Pancreas: In the exocrine pancreas of obese and lean Zucker rats, apart from the period of life, the acinar cells show weak immunoreactivity. The epithelial cells of excretory ducts show strong immunopositivity. In the

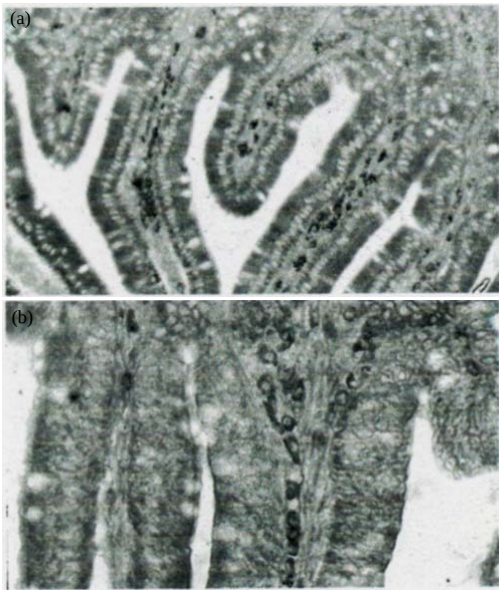

Fig. 6: Obese Zucker rat; strong CB1 immunoreactivity in the epithelium of villi; very strong immunoreactivity appears in cells of the villi stroma; Fig. 6a; 20x; Fig. 6b; 40x

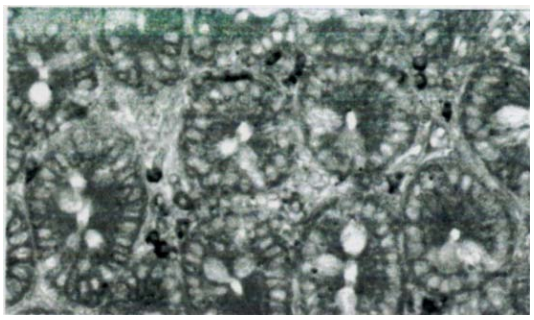

Fig. 7: Obese Zucker rat; weak CB1 immunoreactivity in Galeazzi tubular glands; very strong in cells of periglandular stroma; 40x

endocrine pancreas of normal rats we evidenced a good CB1 immunoreactivity restricted to the A and D cells that are in the periphery of the Langherans islet (Fig. 8). In obese and lean Zucker rats sacrificed at the 8 and 12 weeks old, the islets display intense CB1 immunoreactivity extended to all endocrine cells; the cells of insular periphery express more intensively the CB1 receptor (Fig. 9). Densitometric quantification of CB1 immuhistochemical expression confirm these findings.

In obese Zucker rats, sacrificed at 16 weeks old, morphological changes in the islets that have apoptotic damages caused by lipotoxicity (Shimabukuro *et al*., 1998)

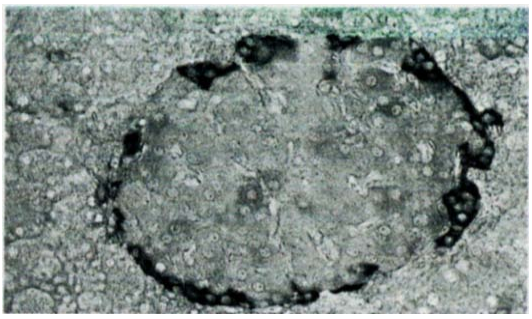

Fig. 8: Normal rat; very remarkable CB1 immunoreactivity is detected almost exclusively in A-D cells of pancreatic islet and very weak in some B cells; 40x

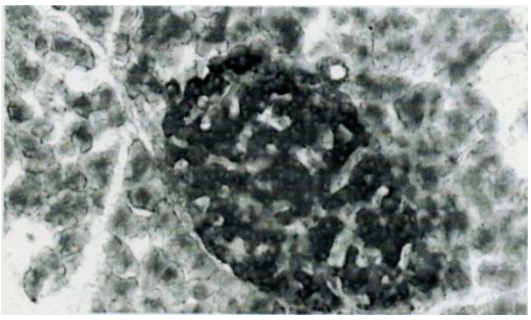

Fig. 9: Obese 8 weeks old Zucker rat; dicrete CB1 immunoreactivity in exocrine pancreas; intense CB1 immunoreactivity heterogeneously distributed in A and B cells of pancreatic islet; 40x

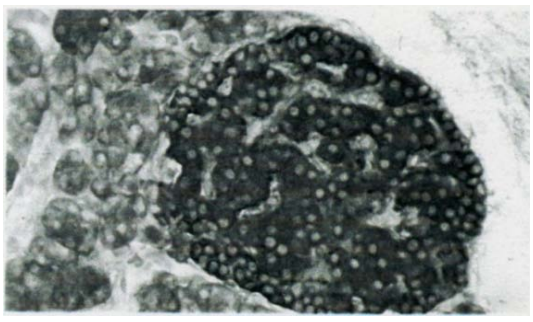

Fig. 10: Lean 16 weeks old Zucker rat; CB1 immunoreactivity in exocrine pancreas heterogenously distributed in the acinar cells and uniformly distributed in A and B cells of pancreatic islet; 40x

can be noticed. Consequently, the immunohistochemical reactivity is restricted to scattered cells remained not altered (Fig. 10). In lean Zucker rats, sacrificed at 16 weeks old, the islets are not apoptotic and the intensity of

immunoreactivity for CB1 is basically identical to that one, similarly intense, exhibited by lean younger rats, sacrificed at 8 and 12 weeks old (Fig. 11).

The immunohistochemical findings made in lean and obese Zucker rats show that the CB1 receptor is expressed from different structural components of Gastroentericpancreatic system (GEP) and they assume consequently che gli EC have a direct control on a lot of functions Gastroenteropancreatic system (GEP) which therefore constitutes a new peripheral target of the action of EC on regulation of nutritional balance and glycemic homeostasis. This target adds other peripheral tissue influenced by EC and already identified such as the adipose organ (Cota *et al*., 2003a), skeletal muscle (Liu *et al*., 2005) and mayor salivary glands. In particolar, in the gastrointestinal tract of animals object of study CB1 receptor was expressed. No only by enteric neurons as up to now documented by other researchers (Kulkarni-Narla and Brown, 2000; Pertwee, 1999; Pinto *et al*., 2002; Storr *et al*., 2004; Griffin *et al*., 1997) but many other structures: epithelium, glands, endocrine cells and immuno cells of villi stroma. The variety of these structures CB1 immunoreactive allows to assume that the direct control of the EC on the apparatus gastrointestinal practicing through different selective mechanisms:

- An endocrine: Paracrine direct control on the gastric glandula secretion
- An autocrine: Paracrine direct control on absorbing functions and ionic transport of nutrients made by mucosal epithelium of villi
- A neurocrine: Direct control already pointed out by some AA on the motility of gastroenteric tract (mioenteric plexus) and probably on general mucosal functions (submucosal plexus)

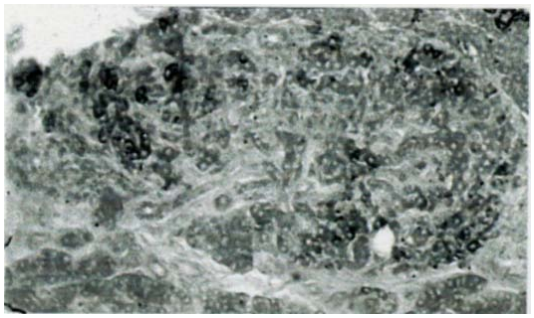

Fig. 11: Obese 16 weeks old Zucker rat; CB1 immunopositiveness in exocrine pancreas. In endocrine pancreas CB1 immunoreactivity is completely negative in apoptotic cells but present in residual non apoptotic cells; 40x

Particular significance does the intense CB1 immunoreactivity, researchers highlighted for the first time, expressed by different cytotypes that as previously mentioned, constitute the so-called Mucosal Intestinal Immune system (GALT) that represents as is known, the most important district of humoral and cellular defense against a wide variety of exogenous antigens and bacterial food which are recruited, internalized and neutralized. The immunohistochemical expression of CB1 receptor in cells in immune function, refutes the idea on the preferentiality histochemical traditionally assigned to the CB1 and CB2 receptors. The CB1 receptor was in fac initially considered as brain-specific; CB2 however exclusively expressed in immune function from tissues (spleen tonsils) (Munro *et al*., 1993). With reference to the results it is possible to envisage, instead, a new role for the CB1 and believe that the activation of CB1 expressed by cytotypes of the GALT may cooperate to modulate with autocrine-paracrine mechanism, together with other messengers of peptidic nature earlier identified in this district (Tessitore *et al*., 2005), the defensive response of the intestinal mucosal immune system.

In the pancreas: the CB1 immunohistochemical expression researchers detected by exocrine acinar and ductal cells nonche by A, B, D cells suggests that the EC may exercise an autocrine-paracrine direct control modulating the exocrine secretive functions and the production or the release of insular hormones, a part from the influence that the EC can have on the glucose metabolism in other peripheral targets (liver-skeletal muscle) (Osei-Hyiaman *et al*., 2005; Liu *et al*., 2005). Everything implicitly opens new therapeutic perspectives in the pathophysiology of pancreatic, even if at present, the results of studies on the exact role played by the EC in this organ are still fragmentary and somewhat controversial. E 'has been shown, in fact that the activation of the human CB1 and CB2 receptors with low doses of agonists of endocannabinoids, attenuates the inflammatory response in acute pancreatitis while high doses the increase (Michalski *et al*., 2007). For against the CB1 receptor antagonist AM251 prolongs the survival of rats suffering from severe acute pancreatitis (Matsuda *et al*., 2005). With regard to the influence that the EC exert on the endocrine pancreas has been documented that activation of the CB1 receptor in the rat induces glucose intolerance and decrease of insulin secretion by the B cells (Bernudez-Silva *et al*., 2006). Conversely the administration of an antagonist of CB1 (Rimonabant) in numerous clinical trials has produced in obese rats and in obese and diabetics patients not only a significant reduction in body weight but also a decrease

in insulin resistance and glycemic profile (Colombo *et al*., 1998; Fernandez and Allison, 2004; Van Gaal *et al*., 2005; Ravinet *et al*., 2003).

The densitometric evaluation of CB1 immunoreactivity in gastroenteric tract and in pancreas of obese and hyperfagic Zucker rats compared with lean Zucker rats show that CB1 receptor is overexpressed and so the endocannabinoids system is hyperactivated and modulated negatively by leptin, similarly to what it has been documented in the same animals and with other methods by different researchers in other central and peripheral targets of endocannabinoid system where the EC levels are permanently more elevated than usually (Osey-Hyiaman *et al*., 2005; Engeli *et al*., 2005; Di Marzo *et al*., 2001, 2008; Matias *et al*., 2006; Starowicz *et al*., 2008).

So, in gastroenteric tract of obese Zucker rats, the lack of block action of leptin, increase the trasduction of orexigenic signals through CB1 receptor from EC upregulated. Consequently, the increase of these signals produce an uncontrolled intake of a bigger quantity of nutrients made more digestive and absorbing for a CB1 receptor overexpression by already mentioned competent cells of gastroenteric tract.

In endocrine pancreas of the same animals, the protracted activation of CB1 receptor and consequent hyperfagia not counterbalanced by the activation of lacking leptin receptor can tardily produce because of the insular lipotoxicity and of the activation of iNOS (Shimabukuro *et al*., 1998), an apoptotic destruction of B cells. In this situation, the orexigenic hyperstimulus can cooperate to the apoptotic destruction of B cells and the consequent appearance of a glucidic dismetabolism.

The hyperactivation of endocannabinoids system in experimental and spontaneous obesity justifies the innovative therapeutical perspective made very recently by using an antagonist of CB1 (Rimonabant) to control both obesity and consequent comorbidity (diabetes) (Kirkham, 2003; Fernandez and Allison, 2004; Van Gaal *et al*., 2005; Despres *et al*., 2005; Di Marzo *et al*., 2000).

CONCLUSION

In short, it is very important to point out the considerable topographic extension of gastroentero-pancreatic apparatus of the adipose organ and of muscular scheletric apparatus, peripheral tissues made by cells not only able to express the CB1 but also to synthesized endocannabinoids and to degrade them (Mechoulam *et al*., 1995; Katayama *et al*., 1997). This fact asserts the existence in addition to a central endocannabinoid system of a peripheral endocannabinoid system whose roles in controlling the nutritional balance can complete each other: the control of appetite and hedonistic aspects linked to palatability toward more tasty food should belong to the central endocannabinoid system; the control on body weight and intake, assimilation and digestion of nutrients should belong to the peripheral endocannabinoid system through the intervention of adipose organ and of Gastroenteropancreatic system (GEP).

ACKNOWLEDGEMENT

This research was supported by MURST, ex 60%.

REFERENCES

Bermudez-Silva, F.J., A. Serrano, F.J. Diaz-Molina, I. Sanchez Vera and P. Juan-Pico *et al*., 2006. Activation of cannabinoid CB1 receptors induced glucose intolerance in rats. Eur. J. Pharmcol., 531: 282-284.

Bermudez-Silva, F.J., J. Suarez, E. Baixeras, N. Cobo and D. Bautista *et al*., 2008. Presence of functional cannabinoid receptors in human endocrine pancreas. Diabetology, 51: 476-487.

Bonaventura, G., D. Cucco, D. Lipari, G.F. Spatola, M.L. Uzzo and V. Tessitore, 2007a. Immunohistochemical expression of CB1 cannabinoids receptor in rat lung. Exp. Med. Rev., 1: 51-57.

Bonaventura, G., D. Cucco, G.F. Spatola, M.L. Uzzo and V. Tessitore, 2007b. An immunohistochemical study on the CB1 receptor expression in rat salivary glands. Exp. Med. Rev., 1: 281-285.

Bonaventura, G., V. Tessitore, M.L. Uzzo and G.F. Spatola, 2005. Immunohistochemical distribution of orphanin FQ in the rat gastrointestinal apparatus and pancreas. Ital. J. Anat. Embryol., 110: 262-264.

Borrelli, F., G. Aviello, B. Romano, P. Orlando and R. Capasso *et al*., 2009. Cannabidiol, a safe and non-psychotropic ingredient of the marijuana plant Cannabis sativa, is protective in a murine model of colitis. J. Mol. Med., 87: 1111-1121.

Calignano, A., G. La Rana, A. Makriyannis, S.Y. Lin, M. Beltramo and D. Piomelli, 1997. Inhibition of intestinal motility by anandamide, an endogenous cannabinoid. Eur. J. Pharmacol., 340: R7-8.

Cint, S., R. Maheis, C. Pico, E. Ceresi and A. Obrador *et al*., 2000. Secretory granules of endocrine and chief cells of human stomach mucosa contain leptin. Int. J. Obesity, 24: 789-793.

Colombo, G., R. Agabio, G. Diaz, C. Lobina, R. Reali and G.L. Gessa, 1998. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. Life Sci., 63: PL113-PL117.

Coruzzi, G., I.M. Adam, G. Coppelli, P. Frati and G. Soldani, 1999. Inhibitory effect of the cannabinoid receptor agonist WIN 55,212-2 on pentagastrin-induced gastric acid secretion in the anaesthetized rat. Arch. Pharmacol., 360: 715-718.

Cota, D. and S.C. Woods, 2005. The role of the endocannabinoid system in the regulation of energy homeostasis. Curr. Opin. Endocr. Diab., 12: 338-351.

Cota, D., 2007. CB1 receptors: Emerging evidence for central and peripheral mechanisms that regulate energy balance, metabolism, and cardiovascular health. Diabetes Metab. Res. Rev., 23: 507-517.

Cota, D., G. Marsicano, B. Lutz, V. Vicennati, G.K. Stalla, R. Pasquali and U. Pagotto, 2003a. Endogenous cannabinoid system as a modulator of food intake. Int. J. Obes. Relat. Metab. Disord, 27: 289-301.

Cota, D., G. Marsicano, M. Tschop, Y. Grubler and C. Flachskamm *et al*., 2003b. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J. Clin. Invest., 112: 423-431.

Cota, D., M.H. Tschop, T.L. Horvath and A.S. Levine, 2006. Cannabinoids, opioids and eating behavior: The molecular face of hedonism?. Brain Res. Rev., 51: 85-107.

Das, S.K., B.C. Paria, I. Chakraborty and S.K. Dey, 1995. Cannabinoid ligand-receptor signaling in the mouse uterus. Proc. Natl. Acad. Sci., 92: 4332-4336.

De Petrocellis, L., M.G. Cascio and V. Di Marzo, 2004. The endocannabinoid system: A general view and latest additions. Br. J. Pharmacol., 141: 765-774.

Despres, J.P., A. Golay and L. Sjostrom, 2005. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. New Engl. J. Med., 353: 2121-2134.

Di Marzo, L., T. Bisogno and L. De Petrocellis, 2000. Endocannabinoids: New targets for drug development. Curr. Pharm. Des., 6: 1361-1380.

Di Marzo, V. and I. Matias, 2005. Endocannabinoid control of food intake and energy balance. Nat. Neurosci., 8: 585-589.

Di Marzo, V., M. Bifulco and L. De Petrocellis, 2004. The endocannabinoid system and its therapeutic exploitation. Nat. Rev. Drug Discovery, 3: 771-784.

Di Marzo, V., R. Capasso, I. Matias, G. Aviello and S. Petrosino *et al*., 2008. The role of endocannabinoids in the regulation of gastric emptying: Alterations in mice fed a high-fat diet. Br. J. Pharmcol., 153: 1272-1280.

Di Marzo, V., S.K. Goparaju, L. Wang, J. Liu and S. Batkai *et al*., 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature, 410: 822-825.

Di Sabatino, A., N. Battista, P. Biancheri, C. Rapino and L. Rovedatti *et al*., 2011. The endogenous cannabinoid system in the gut of patientswith inflammatory bowel disease. Mucosal. Immunol., 4: 574-583.

Elmquist, J.K., C.F. Elias and C.B. Saper, 1999. From lesions to leptin: Hypothalamic control of food intake and body weight. Neuron, 22: 221-232.

Engeli, S., J. Bohnke, M. Feldpausch, K. Gorzelniak and J. Janke *et al*., 2005. Activation of the peripheral endocannabinoid system in human obesity. Diabetes, 54: 2838-2843.

Fernandez, J.R. and D.B. Allison, 2004. Rimonabant sanofi-synthelabo. Curr. Opin. Invest. Drugs, 5: 430-435.

Fox, P., P.G. Bain, S. Glickman, C. Carroll and J. Zajicek, 2004. The effect of cannabis on tremor in patients with multiple sclerosis. Neurology, 62: 1105-1109.

Germano, M.P., V. D'Angelo, M.R. Mondello, S. Pergolizzi and F. Capasso *et al*., 2001. Cannabinoid CB1-mediated inhibition of stress-induced gastric ulcers in rats. Arch. Pharmacol., 363: 241-244.

Griffin, G., S.R. Fernando, R.A. Ross, N.G. McKay and M.L.J. Ashford *et al*., 1997. Evidence for the presence of CB_2-like cannabinoid receptors on peripheral nerve terminals. Eur. J. Pharmacol., 339: 53-61.

Gye, M.C., H.H. Kang and H.J. Kang, 2005. Expression of cannabinoid receptor 1 in mouse testes. Arch. Androl., 51: 247-255.

Hao, S., Y. Avraham, R. Mechoulam and E.M. Berry, 2000. Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. Eur. J. Pharmacol., 392: 147-156.

Howlett, A.C., F. Barth, T.I. Bonner, G. Cabral and P. Casellas *et al*., 2002. International Union of Pharmacology. XXV11. Classification of cannabinoid receptors. Pharmacol. Rev., 54: 161-202.

Izzo, A.A., F. Fezza, R. Capasso, T. Bisogno and L. Pinto *et al*., 2001. Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. Br. J. Pharmacol., 134: 563-570.

Izzo, A.A., N. Mascolo, F. Borrelli and F. Capasso, 1999. Defaecation, intestinal fluid accumulation and motility in rodents: implications of cannabinoid CB1 receptors. Naunyn Schmiedebergs Arch. Pharmacol., 359: 65-70.

Izzo, A.A., R. Capasso, G. Aviello, F. Borrelli and B. Romano *et al*., 2012. Inhibitory effect of cannabichonene, a major nonpsychotropic cannabinoid extracted from cannabis sativa, on inflammation induced hypermotility in mice. Bir. J. Pharmacol., 144: 1444-1460.

Jamshidi, N. and D.A. Taylor, 2001. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. Br. J. Pharmacol., 134: 1151-1154.

Juan-Pico, P., E. Fuentes, F.J. Bermudez-Silva, F. Diaz-Molina and C. Ripoll, F.R. de Fonseca and A. Nadal, 2006. Cannabinoid receptors regulate Ca^{2+} signals and insulin secretion in pancreatic β-cell. Cell Calcium, 39: 155-162.

Kalra, S.P., M.G. Dube, S. Pu, B. Xu, T.L. Horvath and P.S. Kalra, 1999. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr. Rev., 20: 68-100.

Katayama, K., N. Ueda, Y. Kurahashi, H. Suzuki, S. Yamamoto and I. Kato, 1997. Distribution of anandamide amidohydrolase in rat tissues with special reference to small intestine. Biochim. Biophys. Acta, 1347: 212-218.

Kirkham, T.C., 2003. Endogenous cannabinoids: A new target in the treatment of obesity. Am. J. Physiol. Regul. Integr. Comp. Physiol., 284: 343-344.

Komorowski, J. and H. Stepien, 2007. The role of the endocannabinoid system in the regulation of endocrine function and in the control of energy balance in humans. Postepy Hig. Med. Dosw., 61: 99-105.

Kulkarni-Narla, A. and D.R. Brown, 2000. Localization of CB_1-cannabinoid receptor immunoreactivity in the porcine enteric nervous system. Cell Tissue Res., 302: 73-80.

Liu, Y.L., I.P. Connoley, C.A. Wilson and M.J. Stock, 2005. Effects of the cannabinoid CB1 receptor antagonist R141716 on oxygen consumption and soleus muscle glucose uptake in Lep(ob)/Lep(ob) mice. Int. J. Obes. Relat. Metab. Disord., 29: 183-187.

Matias, I., M.P. Gonthier, P. Orlando, V. Martiadis and L. de Petrocellis *et al*., 2006. Regulation, function and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. J. Clin. Endocrinol. Metab., 91: 3171-3180.

Matsuda, K., Y. Mikami, K. Takeda, S. Fukuyama and S. Egawa *et al*., 2005. The cannabinoid 1 receptor antagonist, AM251, prolongs the survival of rats with severe acute pancreatitis. Tohoku J. Exp. Med., 207: 99-107.

Matsuda, L.A., S.J. Lolait, M.J. Brownstein, A.C. Young and T.I. Bonner, 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature, 346: 561-564.

Mechoulam, R., S. Ben-Shabat, L. Hanus, M. Ligumsky and N.E. Kaminski *et al*., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol., 50: 83-90.

Michalski, C.W., T. Laukert, D. Sauliunaite, P. Pacher and F. Bergmann *et al*., 2007. Cannabinoids ameliorate pain and reduce disease pathology in cerulein-induced acute pancreatitis. Gastroenterology, 132: 1968-1978.

Munro, S., K.L. Thomas and M. Abu-Shaar, 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature, 365: 61-65.

Osei-Hyiaman, D., M. DePetrillo, P. Pacher, J. Liu and S. Radaeva *et al*., 2005. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J. Clin. Invest, 115: 1298-1305.

Pagotto, U., V. Vicennati and R. Pasquali, 2007. The endocannabinoid system in the physiopathology of metabolic disorders. Horm. Res., 67: 186-190.

Patton, G.C., C. Coffey, J.B. Carlin, L. Degenhardt, M. Lynskey and W. Hall, 2002. Cannabis use and mental health in young people: Cohort study. BMJ, 325: 1195-1198.

Pertwee, R.G., 1999. Evidence for the presence of CB1 cannabinoid receptors on peripheral neurones and for the existence of neuronal non-CB1 cannabinoid receptors. Life Sci., 65: 597-605.

Pinto, L., R. Capasso, G. Di Carlo and A.A. Izzo, 2002. Endocannabinoids and the gut. Prostaglandins Leukot Essent Fatty Acids, 66: 333-341.

Piomelli, D., 2003. The molecular logic of endocannabinoid signalling. Nat. Rev. Neurosci., 4: 873-884.

Plasse, T.F., R.W. Gorte, S.H. Krasnow, M. Lane, K.V. Shepard and R.G. Wadleigh, 1991. Recent clinical experience with dronabinol. Pharmacol. Biochem. Behav., 40: 695-700.

Ravinet, T.C., M. Arnone, C. Delgorge, N. Gonalons, P. Keane, J.P. Maffrand and P. Soubrie, 2003. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. Am. J. Physiol. Regul. Integr. Comp. Physiol., 284: 345-353.

Ruiz-Llorente, L., M.G. Sanchez, M.J. Carmena, J.C. Prieto, M. Sanchez-Chapado, A. Izquierdo and I. Diaz-Laviada, 2003. Expression of functionally active cannabinoid receptor CB_1 in the human prostate gland. Prostate, 54: 95-102.

Schicho, R. and M. Storr, 2011. Alternative targets within the endocannabinoid system for future treatment of gastrointestinal diseases. Can. J. Gastroenetrol., 25: 377-383.

Shimabukuro, M., Y.T. Zhou, Y. Lee and R.H. Unger, 1998. Troglitazone lowers islet fat and restores β cell function of Zucker diabetic fatty rats. J. Biol. Chem., 273: 3547-3550.

Starowicz, K.M., L. Cristino, I. Matias, R. Capasso, A. Racioppi, A.A. Izzo and V. Di Marzo, 2008. Endocannabinoid dysregulation in the pancreas and adipose tissue of mice fed with a high-fat diet. Obesity, 16: 553-565.

Storr, M., A. Sibaev, G. Marsicano, B. Lutz, V. Schusdziarra, J.P. Timmermans and H.D. Allescher, 2004. Cannabinoid receptor type 1 modulates excitatory and inhibitory neurotransmission in mouse colon. Am. J. Physiol. Gastrointest. Liver Physiol., 286: 110-117.

Tessitore, V., G. Bonaventura, G.F. Spatola and L. Uzzo, 2005. Immunohistochemical expression orexin a andorexin type 2receptors in the rat gastrointestinal apparatus and endocrine pancreas. E. J. H., 49: 1-19.

Tessitore, V., M.L. Uzzo, G.F. Spatola, D. Cucco and G. Bonaventura, 2006. Immunohistochemical studies on leptin, grelin, orexin A, orphanin FQ and endocannabinoids expression in the stomach of lean and obese (fa/fa) Zucker rats. Proceedings of the 1st World Congress on Therapies against Obesity, May 18-19, 2006, Institut Pasteur Paris, France.

Tessitore, V., M.L. Uzzo, G.F. Spatola, D. Cucco and G. Bonaventura, 2006. Immunohistochemical study and densitometric comparison on CB_1 receptor expression in the gastrointestinal tract and pancreas of obese (fa/fa) and lean Zucker rats. Ital. J. Anat Embriol., 111: 256-265.

Tharp, W.G., Y.H. Lee, R.L. Maple and R.E. Pratley, 2008. The cannabinoid CB_1 receptor is expressed in pancreatic δ-cells. Biochem. Biophys. Res. Communi., 372: 595-600.

Van Gaal, L.F., A.M. Rissanen, A.J. Scheen, O. Ziegler and S. Rossner, 2005. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. Lancet, 365: 1389-1397.

Volicer, L., M. Stelly, J. Morris, J. McLaughlin and B.J. Volicer, 1997. Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. Int. J. Geriatr. Psychiatry, 12: 913-919.

Wiley, J.L., J.J. Burston, D.C. Leggett, O.O. Alekseeva, R.K. Razdan, A. Mahadevan and B.R. Martin, 2005. CB_1 cannabinoid receptor-mediated modulation of food intake in mice. Br. J. Pharmacol., 145: 293-300.

Zajicek, J., P. Fox, H. Sanders, D. Wright, J. Vickery, A. Nunn and A. Thompson, 2003. On behalf of the UK MS Research Group Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): Multicentre randomised placebo-controlled trial. Lancet, 362: 1517-1526.