REVIEW

Beta 3 adrenergic receptors: molecular, histological, functional and pharmacological approaches

OANA ANDREIA COMAN[1)], H. PĂUNESCU[1)], ISABEL GHIŢĂ[1)], L. COMAN[2)], ANCA BĂDĂRĂRU[2)], I. FULGA[1)]

[1)]*Department of Pharmacology and Pharmacotherapy*
[2)]*Department of Physiology*
Faculty of Medicine,
"Carol Davila" University of Medicine and Pharmacy, Bucharest

Abstract

Different classes of receptors mediate norepinephrine and epinephrine effects, one of the most recently discovered being the beta 3 adrenergic ones. The paper has proposed itself to present the history of the discovery of beta 3 adrenergic receptors, different techniques for their identification, their structure, localization, genetic data and also the mechanism of regulation of their functions. It also contains an exhaustive approach regarding the histological localization and functions of beta 3 adrenergic receptors in different apparatus and systems, making evident their effect on glucidic, lipidic and energetic metabolism. The substances that influence beta 3 adrenergic receptors activities, especially the agonists, have been studied regarding their practical applications in the treatment of diabetes mellitus and of the disturbances of lipid metabolism.

***Keywords*:** beta 3 adrenergic receptors, discovery, histology, molecular biology, physiology, pharmacology.

Discovery of beta 3 adrenergic receptors

A class of membrane proteins called adrenergic receptors mediates the multiple metabolic and neuroendocrine effects of epinephrine and norepinephrine. These receptors are coupled with G proteins, which in turn interact with intracellular effectors (secondary messengers) such as adenylyl cyclase or phospholipase C.

These receptors were initially divided in two types: alpha and beta [1]. Nevertheless, in some tissues as the white and brown fat tissue and the digestive tract some effects mediated by beta-receptors such as lipolysis, oxygen consumption and smooth muscle relaxation are not compatible with the implication only of beta 1 or beta 2 receptors. Harms HH *et al.*, in 1977, issued the hypothesis of the existence of some "atypical" receptors, by describing them on the white adipocytes of rats [2]. In the same way, atypical beta-receptors are implicated in the mediation of insulin secretion [3, 4] and in the relaxation of the digestive tract [5–7]. Tan S and Curtis-Prior PB proposed, in 1983, that the beta-receptors existent on the white adipocytes would be called beta 3 [8].

The major proof for the existence of beta 3 receptors was given by Arch JR *et al.*, in 1984, by reporting that a serial of new beta adrenergic ligands (BRL 26830A, BRL 33725A, BRL 35135A), which were not specific ligands on classical beta-receptors, had remarkable anti-obesity actions on mice with severe obesity and diabetes [9]. The beta 3 receptor was cloned by Emorine LJ *et al.* in 1989 [10].

Methods of identification and characterization of adrenergic receptors' structure and localization

Adrenergic receptors are membranous proteins. The discovery of the genes that encode membranous proteins was made by identifying some nucleotide sequences in the structure of these genes, which correspond to some hydrophobic amino acids (the transmembrane portions of these proteins). Later on, using the technique for obtaining cDNA (complementary DNA), these proteins were synthesized on some microorganisms in several steps:

- attainment of a precursor of mRNA by the aid of DNA-dependent RNA-polymerase following the nucleotide sequence of a membrane protein;
- removal of introns and obtainment of mRNA;
- synthesis of a single-chain DNA on the model of RNA by the aid of a reverse transcriptase;
- obtainment of double-chain DNA by coupling the nitrogen bases of single-chain DNA, which will be introduced in the prokaryote cell. This cell will synthesize the requested protein using its own enzymes for transcription and translation.

Membranous proteins are impossible to crystallize in order to determine the structure of organic substances. Nevertheless, this structure can be determined by the analysis of the relation structure-function of the proteins which structures is modified by genetic engineering. The functional consequences of these modifications can thus be determined. If it is postulated that a certain amino acid is implicated in a certain function, for

example on a binding site of the ligand, a mutation in the receptor gene can be induced so that the interested amino acid could be replaced by another one, with a different spatial configuration. The functional consequences of these modifications could be then tested. There were also defined functional domains by substituting a part of a receptor with parts of another receptor. Thus were obtained "chimerical" receptors. This kind of receptors permitted the definition of the domains implicated in the intracellular transmission of signal (signal given by the binding of a ligand on the receptor situs from the extra cellular part of the receptor) [11].

Because there are not such tissues in which adrenergic receptors might be concentrated (unlike the nicotinic receptors for acetylcholine, which are very concentrated in the electric organ of torpedo fish), the chosen method for isolation and characterization of adrenergic receptors was the *affinity chromatography* of receptors obtained by solubilization with detergents. Beta 1-like receptors were isolated from the turkey erythrocytes [12] and beta 2-like receptors were isolated from hamster lungs [13].

Description of the amino acid sequence of some polypeptide segments obtained by tryptic lysis led to the synthesis of oligonucleotides that were used as probes to clone the corresponding hamster beta 2 cDNA [14], the turkey beta l-like cDNA [15] and the human beta 3 [10].

Although the adrenergic receptors have been cloned since the late 1980s and early 1990s, the lack of high-affinity and high-avidity antibodies hampers the analysis of the subtype's expression in specific tissues and cell types. Most of the antibodies currently available have limited use in transfected systems in which receptor density is high but give poor results when used to determine endogenous expression. This limitation in antibody avidity is common in membrane proteins and in G protein-coupled receptors because of poor protein purification and membrane epitope recognition. Although analysis of ligand binding to tissue homogenates yields an estimate of the tissue content of the various adrenergic receptor subtypes, it does not have sufficient resolution to determine cell type distribution. Currently, methods useful for determining receptor localization are *in situ hybridization histochemistry* (ISH), *receptor autoradiography* [16].

Northern blotting and *Western blotting* were also used to detect beta adrenoceptors. Northern blotting is a powerful method for the localization of mRNAs and the study of regulation of mRNA expression [17]. The Western blot (alternatively, immunoblot) is used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/non-denaturing conditions) [18].

In situ hybridization (ISH) is a type of hybridization that uses a labeled complementary DNA or RNA strand (i.e. probe) to localize a specific DNA or RNA sequence in a portion or section of a tissue (*in situ*) or, if the tissue is small enough, in the entire tissue. This is distinct from *immunohistochemistry*, which localizes proteins in tissue sections. RNA ISH (*hybridization histochemistry*) is used to measure and localize mRNAs and other transcripts within tissue sections or whole mounts.

For *hybridization histochemistry*, sample cells and tissues are usually treated to fix the target transcripts in place and to increase access of the probe. As noted above, the probe is either a labeled complementary DNA or, now most commonly, a complementary RNA (riboprobe). The probe hybridizes to the target sequence at elevated temperature and then the excess probe is washed away (after prior hydrolysis using RNase in the case of unhybridized, excess RNA probe). Solution parameters such as temperature, salt and/or detergent concentration can be manipulated to remove any non-identical interactions (i.e. only exact sequence matches will remain bound). Then, the probe that was labeled with either radio-, fluorescent- or antigen-labeled bases (e.g., digoxigenin) is localized and quantitated in the tissue using either autoradiography, fluorescence microscopy or immunohistochemistry, respectively. ISH can also use two or more probes, labeled with radioactivity, or the other non-radioactive labels in order to simultaneously detect two or more transcripts [19].

ISH combines the power of precise cellular localization with the ability to perform semiquantitative analysis of the mRNA level, whereas Northern blotting has the ability to identify genetic splice variants or to study multiple RNA molecules sequentially in the same tissue samples [17].

The development of *radioligand binding technique* was an important breakthrough in bringing about a better understanding of the nature of beta-adrenergic receptors. This technology for direct identification of beta-adrenergic receptors was used first for [^{3}H]catecholamines as ligands but these studies did not display the characteristics expected of beta-adrenergic receptors. The older radioligands, such as [^{3}H]dihydro-alprenolol and (+/-)[^{3}H]propranolol or the newer (-) [^{125}I]iodocyanopindolol and (-) [^{125}I] iodopindolol, were all four used most extensively to characterize beta-adrenergic receptors. A characteristic of these ligands described above is their hydrophobic nature. This property limits their usefulness in experiments employing intact cells because of the potential permeation of the radioligand into the cell. A hydrophilic antagonist radioligand (+/-)[^{3}H]CGP–12177 has been synthesized [20].

Lack of high affinity radioligands made difficult the detection of beta 3 adrenoceptors in different tissues. Difficulty also occurs in the correct pharmacological characterization of beta 3 adrenoceptor in tissue that have a high number of beta 1 and beta 2 adrenoceptors, but a low number of beta 3 adrenoceptors.

The structure of beta 3 adrenoceptors

Beta 3 receptors, just like beta 1 and beta 2 receptors belong to the family of serpentine receptors and have seven transmembrane segments (TM), each of 22–28 amino acids, with three intracellular and three extracellular loops. The beta 3 receptor contains 396

amino acids. The N-terminal ending is extracellular and is glycosylated. The C-terminal ending is intracellular but does not possess a site, which can be phosphorylated by a protein kinase A (PKA) or beta receptors kinase (betaARK), site present at the beta 1 and beta 2 type receptors. The disulfide bond between the second and the third extracellular loop between Cys110 and Cys361 is essential for the receptor activity and for interacting with the ligands (Figure 1).

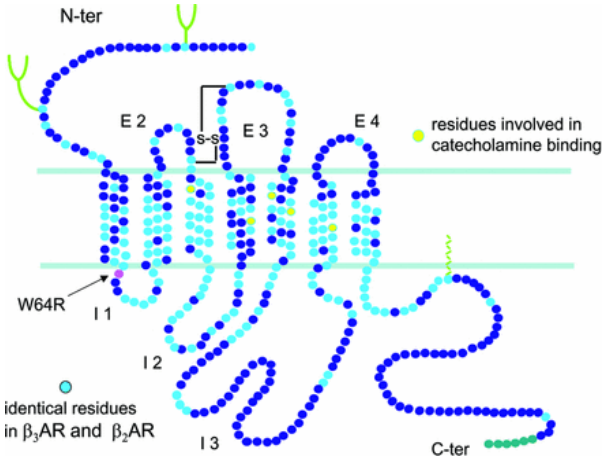

Figure 1 – ***The beta 3 adrenoceptor structure. Source: http://www.biochemsoctrans.org/bst/035/0023/bst0350023.htm.***

In addition, transmembrane domains TM3, TM4, TM5 and TM6 are essential for the interaction with the ligand. Transmembrane domains TM2 and TM7 are implicated in G-protein activation and thus initiation of an effect [21].

The computer modeling defined an image of the ligand-binding site of the beta 3 receptor (Figure 2).

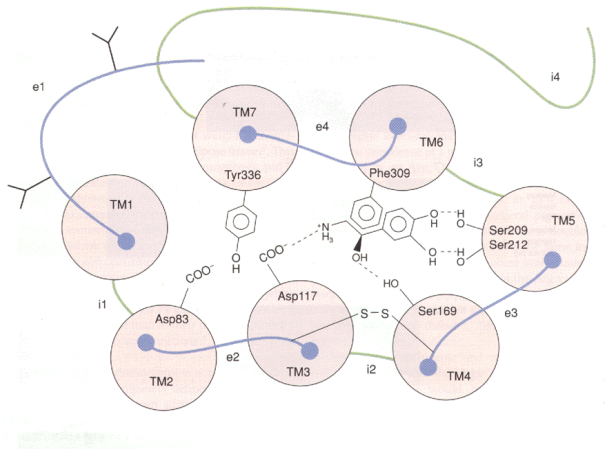

Figure 2 – ***The beta 3 adrenoceptor ligand binding sites. Source: http://www.uni-graz.at/~binder/science/b3adrenoceptors.html.***

The amino acids implicated were identified by direct mutagenesis or photoaffinity. These amino acids were: aspartate (position 117 in TM3), serine (position 169 in TM4, and positions 209 and 212 in TM5), and phenylalanine (position 309 in TM6).

Transmembrane domains TM2 and TM7 are implicated in G protein activation, probably by mean of aspartate from position 83 (TM2) and of tyrosine from position 336 (TM7) [22].

The beta 3 receptor differs from the beta 1 and beta 2 receptors by structure as well as by pharmacological profile. Structural homology between the beta 3 and beta 1 receptors is 51%, between beta 3 and beta 2 receptors is 46% and between beta 1 and beta 2 receptors is 54%. The homology between the human, bovine, monkey, hamster, guinea pig, rat and mouse beta 3 receptors is considerably higher (80–90%) than the different subtypes of beta-receptors. There is an alternative splicing of mRNA (different reading of mRNA depending on the tissue where this mRNA is produced). Thus, in humans three different forms of the beta 3 adrenergic receptor were described: form A with 396 amino acids, form B and C that have beside the C-terminal 12 and 6 amino acids respectively [23].

Post-synthesis modifications of adrenergic receptors

The adrenergic receptors may suffer post-synthesis modifications of proteins, as N-glycosylation at the previous presented sites, palmitoylation or disulfide bond formation.

N-linked glycosylation

All adrenergic receptor subtypes, except the alpha 2b of rat and man, display one or most frequently two Asn-X-Ser/Thr consensus sites for N-glycosylation in the amino-terminal region.

N-linked carbohydrates may account for as much as a quarter of the apparent weight of the adrenergic receptor proteins.

Carbohydrates may thus play a role in receptor trafficking (this signifies modifications in the proportion of receptors expressed on the cell surface and in the proportion of receptors stocked in some intracellular vesicles, from where a part of them come back at the cell surface and a part are destroyed).

Palmitoylation

Palmitoylation consists in binding a rest of palmitic acid to cysteine situated immediately after the TM7 domain. This is possible for all adrenergic receptors subtypes except the alpha 2c.

Lack of palmitoylation has been associated with constitutively increased phosphorylation under the influence of regulatory proteins.

Palmitoylation might be considered a regulatory mechanism to control the functionality of the receptor by the ligand.

Disulfide bond formation

G protein-coupled receptors often contain cysteine residues in their second and third extracellular loops that can form disulphide bonds to stabilize the receptor conformation. All three beta adrenoceptor subtypes contain one cysteine residue in their second extracellular loop and three cysteine residues in their third extracellular loop. As was shown for the beta 1 and the beta 2 adrenoceptor, the cysteine residues in the human beta 3 adrenoceptor are likely to form a functional disulphide bond [24, 25].

Genetics of beta 3 adrenoceptors

The gene that encodes the beta 3 receptors is found on the short arm of chromosome 8 (8p12–p11.2). Human beta 3 adrenoceptor gene has unique functional properties in comparison with that of rodents. Beta 3 and alpha 1b receptors are the only ones from the family of adrenergic receptors that possess introns. The gene of beta 3 receptors in humans has two exons and one intron and differs from this gene in rats (it contains three exons and two introns). There is alternative splicing of mRNA in men and rats. This alternative splicing signifies a different reading of mRNA depending on the tissue where this mRNA is produced. In men, there are described three different forms of beta 3 receptors: A, B and C respectively. They have 396 amino acids (for the A form), 396 + 12 amino acids at the C-terminal ending (for the B form) and 396 + 6 amino acids at the C-terminal ending (for the C form) [26].

Regulation of beta 3 adrenoceptors activity

Beta 3 adrenoceptors are coupled with Gs proteins leading to an increase in the activity of adenylyl cyclase and consequently in the quantity of cAMP but also with Gi proteins, leading to a decrease of intracellular cAMP. Beta 3 adrenoceptors activate MAP kinase p38 (mitogen-activated protein kinase p38) via protein kinase A. cAMP dependent stimulation of UCP1 (uncoupling protein 1) transcription in brown adipocytes by beta 3 adrenoceptors needs MAP kinase p38 [27, 28].

It is well known that beta-receptors, such as beta 2 receptors, suffer important processes of desensitization at the prolonged action of specific agonists on these receptors. These processes suppose initially the phosphorylation of the receptor under the action of protein kinase A and of the specific kinase of the beta-receptor (beta ARK). Afterwards, the intracellular sequestration of the complex receptor beta-ligand takes place. Later on (after hours or days), the "down-regulation" phenomenon appears, with a decrease in the synthesis of mRNA of beta 2 receptor and consequently of the quantity of the beta-receptors [29].

Apart from this, the beta 3 receptors do not suffer the rapid desensitization induced by the binding of the agonist and not even the sequestration. As for the "down-regulation" phenomenon, the administration of norepinephrine to the brown adipocytes leads to a rapid but short decrease of the expression the beta 3 receptors gene. This process is mediated by the activation of cAMP and of protein kinase C but not by the acceleration of the degradation of mRNA of beta 3 receptors [30]. Other studies [31, 32] are controversial regarding the possibility of desensitization on long term of beta 3 receptors, especially of the human ones, when they are expressed in CHW cells (Chinese hamster fibroblast cells) [29, 33].

"Up-regulation" of beta 3 receptors, which appears in the process of differentiation of white adipocytes from pre-adipocytes (cells that do not express beta 3 receptors), is realized in association with other adipocytes specific genes and uses C/EBPα (CCAAT – enhancer-binding proteins). A second candidate for the regulation of the expression of beta 3 receptors in adipocytes could be WATSF-1 (white fat tissue specific factor-1), a protein that binds CRE (cAMP response elements), and is preferentially expressed in white fat tissue. It seems that phosphorylation plays an important part in modulating the activity of this factor [34, 35].

Localization of beta 3 adrenoceptors and the effects of their stimulation upon different tissues

It is now evident that specific mRNA for beta 3 receptors is present in a variety of human tissues (Table 1).

Table 1 – ***Human tissues expressing beta 3 adrenoceptors***

White adipose tissue	Brain
Brown adipose tissue	Gallbladder
Heart	Prostate gland
Colon	Small intestine
Urinary bladder	Stomach
Ureters	Myometrium
Skeletal muscle	Penis cavernous bodies

The expression of mRNA has been documented with various techniques such as reverstranscription-PCR (RT–PCR), RNase protection, Northern blotting [36, 37].

Beta 3 adrenoceptors from the myocardium

In atrial myocytes activation of beta 3 adrenoceptors led to the phosphorylation of calcium channels with the rise in the transmembrane current of calcium [38]. Beta 3 adrenoceptor transcripts were detected in human ventricle by a polymerase chain reaction assay. Recent studies showed that beta 3 adrenoceptors had *a negative inotropic action on the ventricles*, action mediated through the activation of Gi proteins. Beta 3 receptors from the human ventricular muscle stimulated the production of NO through the activation of endothelial NO synthase, which was discovered on the ventricular myocytes. In this way, NO generates a rise of cGMP with the consequent inhibition of phosphodiesterase 3 and/or activation of phosphodiesterase 2, which can reduce the contractile force of the myocardium. In the human ventricle, in case of cardiac insufficiency, a rise of 2 to 3 folds than normal in the number of beta 3 adrenoceptors was produced [39].

Beta 3 adrenoceptors from the vessels

Beta 3 adrenoceptors were found on isolated canine pulmonary arterial rings under isometric conditions in vitro and their stimulation induced a cAMP dependent *vasodilatation* [40]. The putative presence of beta-3 adrenoceptors in peripheral microvascular muscle was studied in dogs through measurement of cutaneous blood flow and skin temperature changes. Their stimulation induced vasodilatation in this territory [41]. In anesthetized rhesus monkeys, a vasodilator effect was observed also in the cutaneous and fat territories and led to a decrease of the blood pressure and a reflex increase in cardiac frequency [42].

In rats, beta 3 adrenoceptors were found on the endothelium of the thoracic aorta and act synergistic with beta 1 and beta 2 adrenoceptors in mediating vascular relaxation by activation of NO synthase and NO production, with increase of cGMP [43].

Relaxation of aorta through beta 3 adrenoceptors proved to be independent of the stimulation of Gi/o proteins, but was given by the activation of some potassium channels: K_{Ca}, K_{ATP}, K_v [44].

In vitro studies showed that beta 3 agonists induced a relaxation of the carotid artery in rats, which was not antagonized by propranolol [45].

In humans, beta 3 adrenoceptors were found, using reverse transcription-polymerase chain reaction analysis and Western Blotting, in the endothelium of the internal mammary artery producing NO induced vasodilatation – a possible practical implication in coronary artery bypass surgery [46].

Beta 3 adrenoceptors were also found in the human coronary arteries using reverse transcription-polymerase chain reaction and immunostaining. They mediated adrenergic vasodilatation by two mechanisms: increase in the NO synthesis and cellular hyperpolarization (through K channels-calcium dependent) [47].

Cerebral beta 3 adrenoceptors

By reverse transcription/PCR the presence of beta 3 adrenoceptors in different regions of the rat brain was demonstrated. mRNA levels for beta 3 adrenoceptors were greater in the hippocampus, cortex and striatum and lower in hypothalamus, brain stem and cerebellum [48]. The intracerebroventricular administration of a beta 3 agonist to a rat led to the neuronal activation of the hypothalamic areas which are responsible for *the central regulation of appetite* through an effect mediated by beta 3 adrenoceptors [49].

The rise in tryptophane level in the rat brain, which can be produced by numerous treatments that induce stress, could also be determined by the activation of beta 3 adrenoceptors [50].

By using a PCR technique, the existence of mRNA for beta 3 adrenoceptors was discovered in the human brain. It was also found that in very little children brain the quantity of mRNA for beta 3 adrenoceptors is 100 times greater than in adults [36].

Ocular beta 3 adrenoceptors

By using pharmacological approaches it was determined that beta adrenergic *relaxation of the cattle iris muscles* was mediated by a mix population of beta receptors, with the predominance of atypical receptors, type beta 3 [51]. The beta 3-adrenergic receptor protein was detected in lysates of human retinal endothelial cells by Western blotting. In addition, the hypothesis that beta 3 adrenoceptors might play a part in the proliferation and migration of human culture retinal endothelial cells was issued [52].

Beta 3 adrenoceptors from the gastro-intestinal tract, gallbladder and pancreas

By the use of selective beta 3 agonists beta atypical (type beta 3) receptors were described on the intestinal tract, which could play a role in the modulation of gastrointestinal motility [53]. These receptors might be identical to those producing lipolysis in the white and brown fat tissue. These results were sustained by the methods of identification of mRNA of beta 3 receptors in different regions of the intestinal tract of different species and by the relative similar potency of beta 3 agonists in the mediation of lipolysis in adipocytes and in inhibiting gastrointestinal motility in vitro and in vivo. These results suggest that the effects of beta 3 selective agonists are produced by the activation of such receptors present on the gastrointestinal tract [54, 55].

Beta 3 adrenoceptors produced *a slowing down of gastric emptying and of intestinal transit*. In guinea pigs beta 3 stimulation induced a cAMP dependent relaxation of stomach fundus and a cAMP independent relaxation of duodenum [56].

In rats, beta 3 adrenoceptors had an *inhibitory role in the control of acidic secretion* induced by indirect stimuli (pentagastrine, deoxiglucose) [57].

SR 586111A, a beta 3 selective agonist, possessed *significant gastroprotective properties*. This effect could be due either to inhibition of gastric acid secretion or to the rise in mucous content of the gastric wall or even to the protective effect on the vascular integrity of the gastric wall [58].

Selective beta 3 agonists (BRL 35135, CL 316243) and to a lesser level other beta agonists reduced the incidence of antral ulcerations to rats normally fed treated with indometacin [59].

Beta 1, 2 and 3 receptors are functionally detectable in the human colon and stimulation of each type by agonists induced the relaxation of the longitudinal musculature of the colon (*tinea coli*). Uncoupling protein 1 (UCP1) seemed to be involved in a novel pathway leading from increased cAMP levels to relaxation in organs exhibiting peristalsis [60].

Beta 3 adrenoceptors are detectable in the gallbladder [61]. A population of neuroendocrine cells in the human pancreas and duodenum that express beta 3-adrenergic receptors was identified using immunohistochemical staining with polyclonal antibodies raised against a 15 amino acid sequence of the human receptor and double-immunostained with anti-insulin, -glucagon, -somatostatin and -pancreatic polypeptide antibodies. These cells appeared to be somatostatin D-cells [62].

Beta 3 adrenoceptors from the urinary apparatus

On the vesical detrusor, the most abundant of the mRNA that encode beta-receptors was represented by the mRNA of beta 3 receptors (99% of the total of mRNA). Stimulation of beta adrenoceptors induced *relaxation of the vesical detrusor* [63]. Beta 3 receptors were also found in the external vesical sphincter using radioligand-binding techniques [64].

Another study brought the first proof of the existence of beta 3 subtype of the beta adrenoceptors on the ureters smooth muscle by reverse transcription polymerase chain reaction assay and this subtype, together with the beta 2 and beta 1 (less) receptors,

mediate *ureteral relaxation* induced by adrenergic stimulation [65].

Beta 3 adrenoceptors from the genital apparatus

Beta 3 receptors were present in the cavernous body of the penis, on the smooth muscle cells, as showed by immunohistochemistry and Western Blot analysis. After activation of these receptors by BRL 37344, a vascular relaxation cGMP dependent but NO-independent was produced. It was issued that a *vasorelaxant tonus in the cavernous bodies* mediated by beta 3 receptors was present and that the activity of these receptors is related to the inhibition of Rho/Rho kinase. RhoA is a GTP-dependent protein that is inactive when GTP is free and activated when it bounds GTP. RhoA stimulates Rho kinase and facilitates the interaction of actin with myosin and thus muscular contraction. Inhibition of RhoA/Rho kinase by the stimulation of beta 3 receptors induced relaxation of the smooth muscles of the cavernous body [66]. Using RNase protection assays without previous PCR amplification mRNA of beta 3 receptors was also described in the prostate [67].

Using radioligand binding, RT–PCR analysis, Western blotting analysis found that in human myometrium beta 2 and beta 3 receptors were present. They were identified both in non-pregnant and pregnant myometrial tissues. In both cases beta 3 receptors were prevailing [68]. The expression of beta 3 receptors and the immune reactivity of receptor protein were greater in the pregnant myometrium by rapport to the non-pregnant one. *Inhibition of spontaneous contractions* induced by a beta 3 adrenoceptor agonist, SR 59119A, was significantly greater in pregnant, compared with nonpregnant myometrium [69].

Beta 3 adrenoceptors from the respiratory apparatus

In rabbits, existence of beta 3 receptors was demonstrated on the nasal epithelium and these receptors were implicated in the control of water and salt movement through this epithelium [70]. Beta 3 receptors also exist in the dog smooth bronchial muscle and their stimulation induced cAMP dependent bronchodilatation [71]. Stimulation of atypical receptors (probably beta 3) increased albumin active transport through the tracheal ferret epithelium [72] and raised the frequency of cilia movements in the dog bronchial epithelium, effect proved also in rabbits [73].

The presence of functional beta 3 receptors on the bronchial muscles seems to be species-dependent. Thus, after the administration of beta 3 specific agonists a *bronchial relaxation* produced in dogs but not in men, guinea pigs or sheep [72].

Beta 3 adrenoceptors from the skeletal muscles

A monoclonal antibody was raised against the human beta 3-adrenoceptor expressed on a transfected mammalian cell line. The use of Mab 72c demonstrated the expression of the beta 3-adrenoceptor in a variety of human tissues, including gall bladder, prostate and colon, where a mRNA signal had been detected in another studies. A direct demonstration of the expression of beta 3-adrenoceptors in human skeletal muscle was also provided using a high affinity monoclonal antibody, Mab72c [61]. Beta 3 receptors mediated inhibition of calcium-dependent proteolysis, participating to the *antiproteolytic effect* of catecholamines *on the skeletal muscles* deprived of nutrients [74]. Glucose capture in skeletal muscles, directly stimulated by beta 3 agonists, did not seem to involve the direct stimulation of adenylyl cyclase [75].

Metabolic effects due to the stimulation of beta 3 adrenoceptors

The brown fat tissue and thermogenesis by activation of beta 3 adrenoceptors

The brown fat tissue (Figure 3) is able to react at different stimuli and produce a great quantity of energy dissipated as heat. This tissue is present in newborn humans (interscapular) and during the whole life at some animals such as rodents and animals that hibernate. Mitochondria from this fat tissue possess a specialized protein called thermogenin or "uncoupling protein" (UCP).

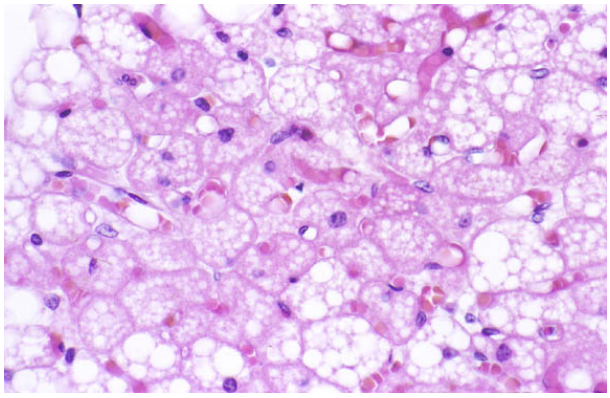

Figure 3 – ***The brown adipose tissue. Source: pathology.mc.duke.edu/research/PTH225.html.***

Its main function is to produce energy under the form of heat by a complex mechanism, not entirely known, consisting in the transport of protons, activated by fatty acids. *In vivo* experiments showed that the expression of UCP gene was physiologically stimulated when the lab animals had been exposed to cold conditions. The rise in the level of UCP-mRNA was mimed by injecting these animals with norepinephrine. Such effects have the selective beta 3 agonists (BRL 37344 and CGP 12177). These facts might suggest that induction of UCP1 expression is mediated by beta 3 receptors, but probably also by beta 1 and beta 2 receptors, which also have been described on the mature brown adipocytes [76].

Recently, other "uncoupling proteins", UCP2 and UCP3, were discovered. UCP 1 was detected only in the brown fat tissue, while UCP2 was present in many tissues and cellular types. UCP3 was mainly expressed in the skeletal muscle. It was supposed that UCP2 and UCP3 could be implicated in basal thermogenesis [77]. Beta 3 agonists also induced a rise in the quantity of

UCP2 and UCP3 expression in the mouse skeletal muscle directly or by the aid of free fatty acids. It was supposed that the anti-obesity effect of beta 3 agonists was produced also by thermogenesis due to UCP2 and UCP3 function [78]. There are experimental proofs that show that thermogenesis mediated by beta 3 adrenoceptors in the brown fat tissue is damaged at older animals. However, after exposing to cold, this response to beta-adrenergic stimulation is recovered [79].

White fat tissue (Figure 4) also plays a part in thermogenesis. UCP1 knockout mice express only 20–40% of the thermogenesis expressed by wild mice [80].

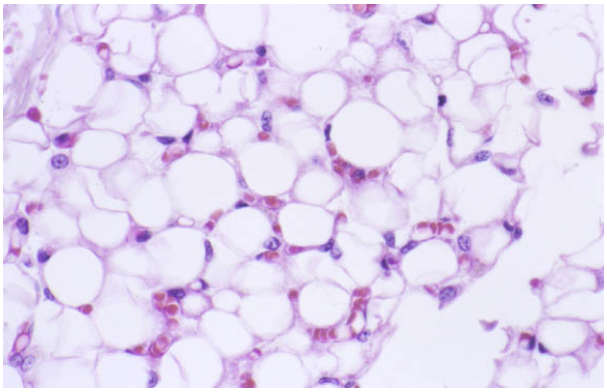

Figure 4 – ***The white adipose tissue. Source: pathology.mc.duke.edu/research/PTH225.html.***

A significant level of expression of the beta 3 adrenoceptor was demonstrated in human brown adipose tissue, suggesting that this receptor might play an important role in the biological response of this tissue to catecholamines [37].

Lipolysis

Sympathetic activation of lipolysis is a well-known fact. Murine adipocytes express especially beta 3 receptors, together with beta 1 and beta 2 receptors and also alpha 2 adrenoceptors [81].

Stimulation of beta 3 adrenergic receptors by specific agonists plays a central aspect in lipolysis in the white fat tissue and the consequent liberation of fatty acids in the blood. This effect is short lasting (24 hours). Free fatty acids are the main source for sustaining thermogenesis in brown fat tissue [82].

Chronic treatment with beta 3 agonists in obese insulinoresistant rodents led to a decrease in weight due to the reduction of fat tissue. An interesting fact was that this decrease of body weight appeared only in obese and not in normoponderal animals [83].

The beta 3-adrenoceptor was expressed in human white adipose tissue at a level which was insufficient to allow for its detection by Northern blot analysis of total RNA or by binding studies [37].

The strongest proof for the existence of functional beta 3 adrenoceptors in human white adipose tissue had been obtained from in vivo studies using microdialysis. This technique proved very useful in pharmacological investigations of in situ lipolysis in humans [84] as it permitted the monitoring and manipulation of local lipolysis and blood flow in vivo in human subcutaneous adipose tissue. The beta 3 adrenoceptor was shown to be the most important for lipid mobilization as only this receptor type stimulated lipolysis as well as local blood flow [85]. The beta 3 adrenergic receptors are responsible, at least in part, for the well-known regional variations in lipolytic activity between visceral and subcutaneous fat depots [86]. The increased lipolytic activity in visceral as compared with subcutaneous fat cells was largely explained by the higher lipolytic function of beta 3 adrenoceptors in visceral fat cells.

Cl-316243, a highly selective beta 3 agonist in rodents, produced to normoponderal men a 41% raise of the "à jeun" amount of free fatty acids after four weeks of treatment. After eight weeks of sustained treatment with this beta 3 agonist an increase with 23% in lipids oxidation over 24 hours appeared [87].

Action on glucidic metabolism

Different beta 3 agonists produced a normalization of glycemia at rodents suffering from type 2 diabetes mellitus, such as Zucker rats, OLETF (Otsuka Long-Evans Tokushima Fatty) rats, mice CRJ–ICR (Yellow KK mice), mice C57B1/6. These agonists could be efficacious in reducing hyperglycemia in chemically induced diabetes mellitus (with streptozotocin or alloxan), even if they do not modify glycemia when administrated to normoglycemic rodents.

The antihyperglycemic effect of beta 3 agonists was attributed to different mechanisms of action:

- stimulation of insulin secretion in beta-insular pancreatic cells;
- increase of the sensitiveness to insulin of peripheral tissues as a response to the increase of the expression of glucose carriers or of the insulin receptors and also to the rise of the blood-flow;
- diminished release of glucose from the liver;
- increase of the non-insulin-dependent capture of glucose and its utilization in tissues such as the brown and white fat tissue and the skeletal muscle [88].

Capture of glucose in the brown fat tissue is highly stimulated by exposure to cold and activation of the sympathetic nervous system. Norepinephrine and other adrenergic agonists, such as isoprenaline, stimulate *in vitro* glucose capture in the brown fat tissue. It was demonstrated that norepinephrine increased glucose transport mainly by beta 3 adrenergic stimulation.

It was supposed that beta 3 receptors might activate glucose transport by at least three means:

- protein Gs activation;
- interaction with other G proteins, such as Gi or Gq;
- direct interaction with an atypical signal molecule.

cAMP is very important for glucose capture stimulated by beta 3 receptors activation and its actions are mediated by protein kinase A activation [89].

The effect of cold exposure on glucidic metabolism and mimic of this effect by beta 3 agonists

Chronic cold exposure of rats activates the sympathetic nervous system, stimulates energetic metabolism, improves glucose tolerance, increases the insulin sensitivity and stimulates glucose uptake in the brown and white fat tissues, heart, diaphragm and skeletal muscles. Cold exposure exerts these

antidiabetic effects despite the fact that it lowers insulinemia and raises plasmatic concentration of norepinephrine.

In obese ZDF-rats, chronic administration of CL-316243, a selective beta 3 receptors agonist, significantly lowers the weight rise, the food ingestion and the weight of white fat tissue. CL-316243 normalizes glycemia, reduces hyperinsulinemia and the level of free fatty acids. It also improves glucose tolerance and reduces insulinemic response throughout a glucose tolerance test. It was suggested that chronic treatment with CL-316243 improves glucose tolerance and also the response to insulin of ZDF-obese rats through a mechanism similar to that induced by cold exposure [90].

Therapeutic perspectives by influencing beta 3 adrenergic receptors functions

Pre-clinical and clinical studies on laboratory animals and humans have been developed from nearly 30 years now. These were realized on animal models of diabetes mellitus and obesity and the transposition of pre-clinical results in clinical studies was tried. Major obstacles were represented by the pharmacological differences between beta 3 receptors in rodents and in men, by the lack of selectivity in men of beta 3 selective compounds in rodents and at finally yet importantly by their unsatisfactory pharmacokinetics [83].

Encouraging data were obtained with a high selective beta 3 agonist (CL 316,243) that proved a significant lipolytic effect in humans followed by an increase of lipidic oxidation after eight weeks of treatment [91]. Acute administration of another potent and specific beta 3 agonist (L757793) in *Rhesus* monkeys induced a raise of lipolysis and of basal metabolism at doses, which did not produce beta 1 or beta 2 adverse effects [92].

Beta 3 receptors were cloned in 1989 and have been intensively studied because of their involvement in glucidic and lipidic metabolism. They are serpentine receptors and have seven transmembrane segments.

Currently, methods useful for determining receptor localization are *in situ hybridization histochemistry* (ISH), *Northern and Western blotting* and *radioligand binding technique*.

The beta 3 adrenergic receptors are localized on different tissues and organs, their stimulation inducing different effects in mammals. The expression of mRNA of these receptors on human tissues has been documented with various techniques.

Beta 3 adrenoceptor transcripts were detected in many locations in humans and in mammals: human ventricle, vasculature, brain, in lysates of human retinal endothelial cells, in the gastrointestinal tract, at the level of the urogenital apparatus, in human skeletal muscle, in the brown and white adipose tissues.

The metabolic effects may be considered the most interesting effects of the stimulation of beta 3 adrenoceptors. Therapeutic perspectives include the utilization of beta 3 agonist as anti diabetic and anti obesity drugs.

References

[1] LANDS A. M., LUDUENA F. P., BUZZO H. J., *Differentiation of receptors responsive to isoproterenol*, Life Sci, 1967, 6(21):2241–2249.

[2] HARMS H. H., ZAAGSMA J., DE VENTE J., *Differentiation of beta-adrenoceptors in right atrium, diaphragm and adipose tissue of the rat, using stereoisomers of propranolol, alprenolol, nifenalol and practolol*, Life Sci, 1977, 21(1):123–128.

[3] FURMAN B. L., TAYO F. M., *Effect of some beta adrenoceptors blocking drugs on insulin secretion in the rat*, J Pharm Pharmacol, 1974, 26:512–517.

[4] AHRÉN B., LUNDQUIST I., *Effects of selective and non-selective beta-adrenergic agents on insulin secretion in vivo*, Eur J Pharmacol, 1981, 71(1):93–104.

[5] COLEMAN R. A., DENYER L. H., SHELDRICK K. E., *beta-Adrenoceptors in guinea-pig gastric fundus – are they the same as the 'atypical' β-adrenoceptors in rat adipocytes?*, Br J Pharmacol, 1987, 90:40P.

[6] BLUE D. R., BOND R. A., ADHAM N., DELMENDO R., MICHEL A. D, EGLEN R. M., WHITING R. L., CLARKE D. E., *Antagonist characterization of atypical beta adrenoceptors in guinea pig ileum: blockade by alprenolol and dihydroalprenolol*, J Pharmacol Exp Ther, 1990, 252(3):1034–1042.

[7] CROCI T., CECCHI R., TARANTINO A., AUREGGI G., BIANCHETTI A., BOIGEGRAIN R., MANARA L., *Inhibition of rat colon motility by stimulation of atypical beta-adrenoceptors with new gut-specific agents*, Pharmacol Res Commun, 1988, 20(2):147–151.

[8] TAN S., CURTIS-PRIOR P. B., *Characterization of the beta-adrenoceptor of the adipose cell of the rat*, Int J Obes, 1983, 7(5):409–414.

[9] ARCH J. R., AINSWORTH A. T., CAWTHORNE M. A., PIERCY V., SENNITT M. V., THODY V. E., WILSON C., WILSON S., *Atypical beta-adrenoceptor on brown adipocytes as target for anti-obesity drugs*, Nature, 1984, 309(5964):163–165.

[10] EMORINE L. J., MARULLO S., BRIEND-SUTREN M. M., PATEY G., TATE K., DELAVIER-KLUTCHKO C., STROSBERG A. D., *Molecular characterization of the human beta 3-adrenergic receptor*, Science, 1989, 245(4922):1118–1121.

[11] SWARBRICK J., BOYLAN J. C., *Encyclopedia of Pharmaceutical Technology: A–D*, Vol. 1, Informa Health Care, New York, 2002, 212–213.

[12] VAUQUELIN G., GEYNET P., HANOUNE J., STROSBERG A. D., *Isolation of adenylate cyclase-free, beta-adrenergic receptor from turkey erythrocyte membranes by affinity chromatography*, Proc Natl Acad Sci U S A, 1977, 74(9):3710–3714.

[13] STILES G. L., BENOVIC J. L., CARON M. G., LEFKOWITZ R. J., *Mammalian beta-adrenergic receptors. Distinct glycoprotein populations containing high mannose or complex type carbohydrate chains*, J Biol Chem, 1984, 259(13):8655–8663.

[14] DIXON R. A., KOBILKA B. K., STRADER D. J., BENOVIC J. L., DOHLMAN H. G., FRIELLE T., BOLANOWSKI M. A., BENNETT C. D., RANDS E., DIEHL R. E., MUMFORD R. A., SLATER E. E., SIGAL I. S., CARON M. G., LEFKOWITZ R. J., STRADER C. D., *Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin*, Nature, 1986, 321(6065):75–79.

[15] YARDEN Y., RODRIGUEZ H., WONG S. K., BRANDT D. R., MAY D. C., BURNIER J., HARKINS R. N., CHEN E. Y., RAMACHANDRAN J., ULLRICH A. *et al.*, *The avian beta-adrenergic receptor: primary structure and membrane topology*, Proc Natl Acad Sci U S A, 1986, 83(18):6795–6799.

[16] PEREZ M. D. (ed), *The adrenergic receptors in the 21st century*, Humana Press, Inc., Totowa, New Jersey, 2005, 151–152.

[17] DONALDSON L. F., *Identification of G-protein-coupled receptor mRNA expression by Northern blotting and in situ hybridization*, Methods Mol Biol, 2004, 259:99–122.

[18] BURNETTE W. N., *"Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate–polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A*, Anal Biochem, 1981, 112(2):195–203.

[19] JIN L., LLOYD R. V., *In situ hybridization: methods and applications*, J Clin Lab Anal, 1997, 11(1):2–9.

[20] PERKINS J. P. (ed), *The beta-adrenergic receptors series: the receptors*, Humana Press, Inc., Clifton, New Jersey, 1991, 4–7.

[21] SKEBERDIS V. A., *Structure and function of beta3-adrenergic receptors*, Medicina (Kaunas), 2004, 40(5):407–413.

[22] ***, *β3-Adrenoceptors*, website http://www.uni-graz.at/~binder/science/b3adrenoceptors.html, 2009.

[23] NAHMIAS C., BLIN N., ELALOUF J. M., MATTEI M. G., STROSBERG A. D., EMORINE L. J., *Molecular characterization of the mouse beta 3-adrenergic receptor: relationship with the atypical receptor of adipocytes*, EMBO J, 1991, 10(12):3721–3727.

[24] STROSBERG A. D. (ed), *The β_3-adrenoceptor*, Taylor & Francis, CRC Press, London, 2000.

[25] STROSBERG A. D., *Structure, function, and regulation of adrenergic receptors*, Protein Sci, 1993, 2(8):1198–1209.

[26] EVANS B. A., PAPAIOANNOU M., HAMILTON S., SUMMERS R. J., *Alternative splicing generates two isoforms of the beta3-adrenoceptor which are differentially expressed in mouse tissues*, Br J Pharmacol, 1999, 127(6):1525–1531.

[27] SHIMIZU Y., TANISHITA T., MINOKOSHI Y., SHIMAZU T., *Activation of mitogen-activated protein kinase by norepinephrine in brown adipocytes from rats*, Endocrinology, 1997, 138(1):248–253.

[28] CAO W., MEDVEDEV A. V., DANIEL K. W., COLLINS S., *beta-Adrenergic activation of p38 MAP kinase in adipocytes: cAMP induction of the uncoupling protein 1 (UCP1) gene requires p38 MAP kinase*, J Biol Chem, 2001, 276(29):27077–27082.

[29] NANTEL F., MARULLO S., KRIEF S., STROSBERG A. D., BOUVIER M., *Cell-specific down-regulation of the beta 3-adrenergic receptor*, J Biol Chem, 1994, 269(18):13148–13155.

[30] BENGTSSON T., CANNON B., NEDERGAARD J., *Differential adrenergic regulation of the gene expression of the beta-adrenoceptor subtypes beta1, beta2 and beta3 in brown adipocytes*, Biochem J, 2000, 347(Pt 3):643–651.

[31] REVELLI J. P., MUZZIN P., GIACOBINO J. P., *Modulation in vivo of beta-adrenergic-receptor subtypes in rat brown adipose tissue by the thermogenic agonist Ro 16-8714*, Biochem J, 1992, 286(Pt 3):743–746.

[32] GRANNEMAN J. G., *Effects of agonist exposure on the coupling of beta 1 and beta 3 adrenergic receptors to adenylyl cyclase in isolated adipocytes*, J Pharmacol Exp Ther, 1992, 261(2):638–642.

[33] LIGGETT S. B., FREEDMAN N. J., SCHWINN D. A., LEFKOWITZ R. J., *Structural basis for receptor subtype-specific regulation revealed by a chimeric beta 3/beta 2-adrenergic receptor*, Proc Natl Acad Sci U S A, 1993, 90(8):3665–3669.

[34] DIXON T. M., DANIEL K. W., FARMER S. R., COLLINS S., *CCAAT/enhancer-binding protein alpha is required for transcription of the beta 3-adrenergic receptor gene during adipogenesis*, J Biol Chem, 2001, 276(1):722–728.

[35] KUTOH E., ONGENAE N., CLAESKENS A., VERHEYEN W., CHEYNS P., NEEFS J., KAIJEN P., *A putative white adipose tissue specific nuclear orphan receptor that interacts with the cAMP-response element of the human beta3-adrenergic receptor gene*, Mol Cell Endocrinol, 2000, 165(1–2):85–95.

[36] RODRIGUEZ M., CARILLON C., COQUEREL A., LE FUR G., FERRARA P., CAPUT D., SHIRE D., *Evidence for the presence of beta 3-adrenergic receptor mRNA in the human brain*, Brain Res Mol Brain Res, 1995, 29(2):369–375.

[37] DENG C., PAOLONI-GIACOBINO A., KUEHNE F., BOSS O., REVELLI J. P., MOINAT M., CAWTHORNE M. A., MUZZIN P., GIACOBINO J. P., *Respective degree of expression of beta 1-, beta 2- and beta 3-adrenoceptors in human brown and white adipose tissues*, Br J Pharmacol, 1996, 118(4):929–934.

[38] KAUMANN A. J., *(-)-CGP 12177-induced increase of human atrial contraction through a putative third beta-adrenoceptor*, Br J Pharmacol, 1996, 117(1):93–98.

[39] GAUTHIER C., TAVERNIER G., CHARPENTIER F., LANGIN D., LE MAREC H., *Functional beta 3-adrenoceptor in the human heart*, J Clin Invest, 1996, 98(2):556–562.

[40] TAGAYA E., TAMAOKI J., TAKEMURA H., ISONO K., NAGAI A., *Atypical adrenoceptor-mediated relaxation of canine pulmonary artery through a cyclic adenosine monophosphate-dependent pathway*, Lung, 1999, 177(5):321–332.

[41] BERLAN M., GALITZKY J., BOUSQUET-MELOU A., LAFONTAN M., MONTASTRUC J. L., *Beta-3 adrenoceptor-mediated increase in cutaneous blood flow in the dog*, J Pharmacol Exp Ther, 1994, 268(3):1444–1451.

[42] HOM G. J., FORREST M. J., BACH T. J., BRADY E., CANDELORE M. R., CASCIERI M. A., FLETCHER D. J., FISHER M. H., ILIFF S. A., MATHVINK R., METZGER J., PECORE V., SAPERSTEIN R., SHIH T., WEBER A. E., WYVRATT M., ZAFIAN P., MACINTYRE D. E., *Beta(3)-adrenoceptor agonist-induced increases in lipolysis, metabolic rate, facial flushing, and reflex tachycardia in anesthetized rhesus monkeys*, J Pharmacol Exp Ther, 2001, 297(1):299–307.

[43] TROCHU J. N., LEBLAIS V., RAUTUREAU Y., BÉVÉRELLI F., LE MAREC H., BERDEAUX A., GAUTHIER C., *Beta 3-adrenoceptor stimulation induces vasorelaxation mediated essentially by endothelium-derived nitric oxide in rat thoracic aorta*, Br J Pharmacol, 1999, 128(1):69–76.

[44] RAUTUREAU Y., TOUMANIANTZ G., SERPILLON S., JOURDON P., TROCHU J. N., GAUTHIER C., *Beta 3-adrenoceptor in rat aorta: molecular and biochemical characterization and signalling pathway*, Br J Pharmacol, 2002, 137(2):153–161.

[45] BERLAN M., GALITZKY J., MONTASTRUC J. L., *Beta 3-adrenoceptors in the cardiovascular system*, Fundam Clin Pharmacol, 1995, 9(3):234–239.

[46] ROZEC B., SERPILLON S., TOUMANIANTZ G., SÈZE C., RAUTUREAU Y., BARON O., NOIREAUD J., GAUTHIER C., *Characterization of beta3-adrenoceptors in human internal mammary artery and putative involvement in coronary artery bypass management*, J Am Coll Cardiol, 2005, 46(2):351–359.

[47] DESSY C., MONIOTTE S., GHISDAL P., HAVAUX X., NOIRHOMME P., BALLIGAND J. L., *Endothelial beta3-adrenoceptors mediate vasorelaxation of human coronary microarteries through nitric oxide and endothelium-dependent hyperpolarization*, Circulation, 2004, 110(8):948–954.

[48] SUMMERS R. J., PAPAIOANNOU M., HARRIS S., EVANS B. A., *Expression of beta 3-adrenoceptor mRNA in rat brain*, Br J Pharmacol, 1995, 116(6):2547–2548.

[49] CASTILLO-MELÉNDEZ M., MCKINLEY M. J., SUMMERS R. J., *Intracerebroventricular administration of the beta(3)-adrenoceptor agonist CL 316243 causes Fos immunoreactivity in discrete regions of rat hypothalamus*, Neurosci Lett, 2000, 290(3):161–164.

[50] LENARD N. R., GETTYS T. W., DUNN A. J., *Activation of beta2- and beta3-adrenergic receptors increases brain tryptophan*, J Pharmacol Exp Ther, 2003, 305(2):653–659.

[51] GEYER O., BAR-ILAN A., NACHMAN R., LAZAR M., ORON Y., *Beta3-adrenergic relaxation of bovine iris sphincter*, FEBS Lett, 1998, 429(3):356–358.

[52] STEINLE J. J., BOOZ G. W., MEININGER C. J., DAY J. N., GRANGER H. J., *Beta 3-adrenergic receptors regulate retinal endothelial cell migration and proliferation*, J Biol Chem, 2003, 278(23):20681–20686.

[53] ARCH J. R., KAUMANN A. J., *Beta 3 and atypical beta-adrenoceptors*, Med Res Rev, 1993, 13(6):663–729.

[54] LEZAMA E. J., KONKAR A. A., SALAZAR-BOOKAMAN M. M., MILLER D. D., FELLER D. R., *Pharmacological study of atypical beta-adrenoceptors in rat esophageal smooth muscle*, Eur J Pharmacol, 1996, 308(1):69–80.

[55] MANARA L., CROCI T., LANDI M., *Beta 3-adrenoceptors and intestinal motility*, Fundam Clin Pharmacol, 1995, 9(4):332–342.

[56] HORINOUCHI T., TANAKA Y., KOIKE K., *Beta 3-adrenoceptor-mediated relaxation of guinea-pig gastric funds smooth muscle: cAMP-independent characteristics and a primary role of 4-aminopyridine-sensitive voltage-dependent K+ (Kv) channels*, Nippon Yakurigaku Zasshi, 2002, 120(1):109P–111P.

[57] ADAMI M., CORUZZI G., SOTIROV E., BERTINI S., SOLDANI G., *Pharmacological evidence for beta3 adrenoceptors in the control of rat gastric acid secretion*, Dig Dis Sci, 2003, 48(2):334–339.

[58] VINAY H. K., PAUL A., GOSWAMI S. S., SANTANI D., *Effect of SR 58611A, a beta-3 receptor agonist, against experimental gastro-duodenal ulcers*, Indian J Physiol Pharmacol, 2002, 46(1):36–44.

[59] KURATANI K., KODAMA H., YAMAGUCHI I., *Enhancement of gastric mucosal blood flow by beta-3 adrenergic agonists prevents indomethacin-induced antral ulcer in the rat*, J Pharmacol Exp Ther, 1994, 270(2):559–565.

[60] SHABALINA I., WIKLUND C., BENGTSSON T., JACOBSSON A., CANNON B., NEDERGAARD J., *Uncoupling protein-1: involvement in a novel pathway for beta-adrenergic, cAMP-mediated intestinal relaxation*, Am J Physiol Gastrointest Liver Physiol, 2002, 283(5):G1107–G1116.

[61] CHAMBERLAIN P. D., JENNINGS K. H., PAUL F., CORDELL J., BERRY A., HOLMES S. D., PARK J., CHAMBERS J., SENNITT M. V., STOCK M. J., CAWTHORNE M. A., YOUNG P. W., MURPHY G. J., *The tissue distribution of the human beta3-adrenoceptor studied using a monoclonal antibody: direct evidence of the beta3-adrenoceptor in human adipose tissue, atrium and skeletal muscle*, Int J Obes Relat Metab Disord, 1999, 23(10):1057–1065.

[62] ANTHONY A., SIM R., GUILLAUME J. L., STROSBERG A. D., DHILLON A. P., POUNDER R. E., WAKEFIELD A. J., *Beta(beta)3-adrenergic receptors in human pancreatic islet and duodenal somatostatin neuroendocrine cells*, Aliment Pharmacol Ther, 2000, 14(5):579–585.

[63] YAMAGUCHI O., *Beta3-adrenoceptors in human detrusor muscle*, Urology, 2002, 59(5 Suppl 1):25–29.

[64] MORITA T., IIZUKA H., IWATA T., KONDO S., *Function and distribution of beta3-adrenoceptors in rat, rabbit and human urinary bladder and external urethral sphincter*, J Smooth Muscle Res, 2000, 36(1):21–32.

[65] PARK Y. C., TOMIYAMA Y., HAYAKAWA K., AKAHANE M., AJISAWA Y., MIYATAKE R., KIWAMOTO H., SUGIYAMA T., KURITA T., *Existence of a beta3-adrenoceptor and its functional role in the human ureter*, J Urol, 2000, 164(4):1364–1370.

[66] CIRINO G., SORRENTINO R., DI VILLA BIANCA R., POPOLO A., PALMIERI A., IMBIMBO C., FUSCO F., LONGO N., TAJANA G., IGNARRO L. J., MIRONE V., *Involvement of beta 3-adrenergic receptor activation via cyclic GMP- but not NO-dependent mechanisms in human corpus cavernosum function*, Proc Natl Acad Sci U S A, 2003, 100(9):5531–5536.

[67] BERKOWITZ D. E., NARDONE N. A., SMILEY R. M., PRICE D. T., KREUTTER D. K., FREMEAU R. T., SCHWINN D. A., *Distribution of beta 3-adrenoceptor mRNA in human tissues*, Eur J Pharmacol, 1995, 289(2):223–228.

[68] ROUGET C., BARDOU M., BREUILLER-FOUCHÉ M., LOUSTALOT C., QI H., NALINE E., CROCI T., CABROL D., ADVENIER C., LEROY M. J., *Beta3-adrenoceptor is the predominant beta-adrenoceptor subtype in human myometrium and its expression is up-regulated in pregnancy*, J Clin Endocrinol Metab, 2005, 90(3):1644–1650.

[69] BARDOU M., LOUSTALOT C., CORTIJO J., SIMON B., NALINE E., DUMAS M., ESTEVE S., CROCI T., CHALON P., FRYDMAN R., SAGOT P., MANARA L., MORCILLO E. J., ADVENIER C., *Functional, biochemical and molecular biological evidence for a possible beta(3)-adrenoceptor in human near-term myometrium*, Br J Pharmacol, 2000, 130(8):1960–1966.

[70] DANNER I., ESCANDE D., GAUTHIER C., *Beta(3)-adrenoceptors control Cl(-) conductance in rabbit nasal epithelium*, Eur J Pharmacol, 2001, 422(1–3):203–207.

[71] TAMAOKI J., YAMAUCHI F., CHIYOTANI A., YAMAWAKI I., TAKEUCHI S., KONNO K., *Atypical beta-adrenoceptor-(beta 3-adrenoceptor) mediated relaxation of canine isolated bronchial smooth muscle*, J Appl Physiol, 1993, 74(1):297–302.

[72] MARTIN C. A., ADVENIER C., *Beta 3-adrenoceptors and airways*, Fundam Clin Pharmacol, 1995, 9(2):114–118.

[73] TAKEYAMA K., TAMAOKI J., CHIYOTANI A., SAKAI N., KANEMURA T., KONNO K., *Stimulation of ciliary motility by beta 3-adrenoceptor agonist rabbit tracheal epithelium*, Kokyu To Junkan, 1993, 41(10):993–997.

[74] NAVEGANTES L. C., RESANO N. M., BAVIERA A. M., MIGLIORINI R. H., KETTELHUT I. C., *CL 316,243, a selective beta3-adrenergic agonist, inhibits protein breakdown in rat skeletal muscle*, Pflugers Arch, 2006, 451(5):617–624.

[75] ROBERTS S. J., SUMMERS R. J., *Cyclic AMP accumulation in rat soleus muscle: stimulation by beta2- but not beta3-adrenoceptors*, Eur J Pharmacol, 1998, 348(1):53–60.

[76] REHNMARK S., KOPECKÝ J., JACOBSSON A., NÉCHAD M., HERRON D., NELSON B. D., OBREGON M. J., NEDERGAARD J., CANNON B., *Brown adipocytes differentiated in vitro can express the gene for the uncoupling protein thermogenin: effects of hypothyroidism and norepinephrine*, Exp Cell Res, 1989, 182(1):75–83.

[77] NEDERGAARD J., CANNON B., *The 'novel' 'uncoupling' proteins UCP2 and UCP3: what do they really do? Pros and cons for suggested functions*, Exp Physiol, 2003, 88(1):65–84.

[78] NAKAMURA Y., NAGASE I., ASANO A., SASAKI N., YOSHIDA T., UMEKAWA T., SAKANE N., SAITO M., *Beta 3-adrenergic agonist up-regulates uncoupling proteins 2 and 3 in skeletal muscle of the mouse*, J Vet Med Sci, 2001, 63(3):309–314.

[79] SCARPACE P. J., TSE C., MATHENY M., *Thermoregulation with age: restoration of beta(3)-adrenergic responsiveness in brown adipose tissue by cold exposure*, Proc Soc Exp Biol Med, 1996, 211(4):374–380.

[80] GRANNEMAN J. G., BURNAZI M., ZHU Z., SCHWAMB L. A., *White adipose tissue contributes to UCP1-independent thermogenesis*, Am J Physiol Endocrinol Metab, 2003, 285(6):E1230–E1236.

[81] VALET P., GRUJIC D., WADE J., ITO M., ZINGARETTI M. C., SOLOVEVA V., ROSS S. R., GRAVES R. A., CINTI S., LAFONTAN M., LOWELL B. B., *Expression of human alpha 2-adrenergic receptors in adipose tissue of beta 3-adrenergic receptor-deficient mice promotes diet-induced obesity*, J Biol Chem, 2000, 275(44):34797–34802.

[82] COLLINS S., CAO W., ROBIDOUX J., *Learning new tricks from old dogs: beta-adrenergic receptors teach new lessons on firing up adipose tissue metabolism*, Mol Endocrinol, 2004, 18(9):2123–2131.

[83] DE SOUZA C. J., BURKEY B. F., *Beta 3-adrenoceptor agonists as anti-diabetic and anti-obesity drugs in humans*, Curr Pharm Des, 2001, 7(14):1433–1449.

[84] LAFONTAN M., ARNER P., *Application of in situ microdialysis to measure metabolic and vascular responses in adipose tissue*, Trends Pharmacol Sci, 1996, 17(9):309–313.

[85] ENOCKSSON S., SHIMIZU M., LÖNNQVIST F., NORDENSTRÖM J., ARNER P., *Demonstration of an in vivo functional beta 3-adrenoceptor in man*, J Clin Invest, 1995, 95(5):2239–2245.

[86] ARNER P., *Differences in lipolysis between human subcutaneous and omental adipose tissues*, Ann Med, 1995, 27(4):435–438.

[87] WEYER C., GAUTIER J. F., DANFORTH E. JR., *Development of beta 3-adrenoceptor agonists for the treatment of obesity and diabetes – an update*, Diabetes Metab, 1999, 25(1):11–21.

[88] MILAGRO F. I., GÓMEZ-AMBROSI J., FORGA L., MARTÍNEZ J. A., *A beta3-adrenergic agonist increases muscle GLUT1/GLUT4 ratio, and regulates liver glucose utilization in diabetic rats*, Diabetes Obes Metab, 1999, 1(2):97–104.

[89] CHERNOGUBOVA E., CANNON B., BENGTSSON T., *Norepinephrine increases glucose transport in brown adipocytes via beta3-adrenoceptors through a cAMP, PKA, and PI3-kinase-dependent pathway stimulating conventional and novel PKCs*, Endocrinology, 2004, 145(1):269–280.

[90] LIU X., PÉRUSSE F., BUKOWIECKI L. J., *Mechanisms of the antidiabetic effects of the beta 3-adrenergic agonist CL-316243 in obese Zucker-ZDF rats*, Am J Physiol, 1998, 274(5 Pt 2):R1212–R1219.

[91] WEYER C., TATARANNI P. A., SNITKER S., DANFORTH E. JR., RAVUSSIN E., *Increase in insulin action and fat oxidation after treatment with CL 316,243, a highly selective beta3-adrenoceptor agonist in humans*, Diabetes, 1998, 47(10):1555–1561.

[92] FISHER M. H., AMEND A. M., BACH T. J., BARKER J. M., BRADY E. J., CANDELORE M. R., CARROLL D., CASCIERI M. A., CHIU S. H., DENG L., FORREST M. J., HEGARTY-FRISCINO B., GUAN X. M., HOM G. J., HUTCHINS J. E., KELLY L. J., MATHVINK R. J., METZGER J. M., MILLER R. R., OK H. O., PARMEE E R., SAPERSTEIN R., STRADER C D., STEARNS R A., MACINTYRE D. E. *et al.*, *A selective human beta3 adrenergic receptor agonist increases metabolic rate in rhesus monkeys*, J Clin Invest, 1998, 101(11):2387–2393.

Corresponding author

Oana Andreia Coman, Associate Professor, MD, PhD, Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, 8 Eroilor Sanitari Avenue, 050474 Bucharest, Romania; Phone +4021–318 07 62, e-mail: andreiacoman@yahoo.com

Received: November 13th, 2008

Accepted: April 15th, 2009