New Potential Beta-3 Adrenergic Agonists with Beta-Phenylethylamine Structure, Synthesized for the Treatment of Dyslipidemia and Obesity

Simona Negreș, Cornel Chiriță, Andreea Letiția Arsene, Denisa Margină, Elena Moroșan and Cristina Elena Zbârcea

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65328

Abstract

Beta-3 adrenergic receptors have important physiological implications, being expressed in many places in the body, including brown adipose tissue. Of the effects studied in preclinical research on lipid metabolism attributable to stimulation of these receptors, we can mention the increased thermogenesis and metabolic rate in the brown adipose tissue, reduction of body weight in obese diabetic rats, lowering of intra-abdominal and subepithelial fat in nonobese and nondiabetic rats, decrease of triglyceride, and increase of HDL cholesterol levels. Carbohydrate metabolism is also changed by beta-3 adrenergic agonists, the most prevalent effects being blood glucose lowering in diabetic rats, increasing insulin secretion of the pancreas, or increasing glucose tolerance. Metabolic effects of 13 newly synthesized compounds of beta-phenylethylamine structure and reference BRL 37344 were investigated in order to identify a potential affinity for beta-3 adrenergic receptors. The antidiabetic and hypolipemiant effects were investigated on a rat model of alloxan-induced diabetes. The results demonstrated that new beta-phenylethylamine derivatives produced marked biological activity over lipid profile. All compounds have markedly decreased the values of total cholesterol, LDL cholesterol, and triglycerides and also have increased the values of antiatherogenic HDL cholesterol. The effects were significantly more intense than the reference substance BRL 37344.

Keywords: beta-3 adrenergic agonists, antidiabetic, hypolipemiant, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, hexokinase, beta-phenylethylamine
1. Introduction

The sympathetic nervous system is part of the autonomic nervous system and innervates tissues in almost every organ system. Adrenergic system is important for maintaining the organism homeostasis and mediates the neuronal and hormonal stress response commonly known as the fight-or-flight response.

Central and peripheral adrenergic neurotransmitters are epinephrine and norepinephrine, which act on their specific adrenergic receptors.

| Adrenergic receptors types and subtypes | Tissue localization | Dominant effects |
|----------------------------------------|--------------------|-----------------|
| α-Adrenergic receptors | α₁A | Heart, blood vessels, smooth muscle, liver, lung, vas deferens, prostate, cerebellum, cortex, hippocampus | Contraction of vascular smooth muscle; vasoconstriction of large resistant arterioles in skeletal muscle |
| | α₁B | Heart, kidney, spleen, lung, blood vessels, cortex, brainstem | Promotes cardiac growth and structure |
| | α₁D | Aorta, coronary artery, platelets, prostate, cortex, hippocampus | Vasoconstriction in aorta and coronary artery |
| | α₂A | Sympathetic neurons, platelets, pancreas, locus coeruleus, brainstem, spinal cord | Main inhibitory receptor on sympathetic neurons |
| | α₂B | Liver, kidney, pancreas, blood vessels | Mediates α₂ vasoconstriction |
| | α₂C | Basal ganglia, cortex, cerebellum, hippocampus | Modulates dopamine neurotransmission Inhibits hormone release from adrenal medulla |
| β-Adrenergic receptors | β₁ | Heart, kidney, skeletal muscle, cortex, olfactory nucleus, brain stem | Positive inotropic and chronotropic effects |
| | β₂ | Bronchial and gastrointestinal smooth muscle, blood vessels, heart, lung, skeletal muscle, cortex | Smooth muscle relaxation, skeletal muscle hypertrophy |
| | β₃ | Adipose tissue, gastrointestinal tract, gallbladder, urinary bladder | Lipolysis, thermogenesis, relaxation of the bladder |

Tissue localization and dominant effects (after Goodman & Gillman’s 2011, modified).

Table 1. Types and subtypes of adrenergic receptors.

Adrenergic receptors were described for the first time by Ahlquist in 1948, who hypothesized the existence of two different types of receptors, α and β, based on the consideration that adrenaline, noradrenaline, and other pharmacological agonists regulate various physiological functions [1]. This differentiation of receptors was confirmed by the finding that there are antagonists, which selectively block α receptors (e.g., phenoxybenzamine or phentolamine) or
β receptors (e.g., propranolol). Every type of adrenergic receptors has different subtypes, which are mentioned in Table 1 [2–4].

1.1. Beta-3 adrenergic receptor discovery and structure

In the early 1980s, Tan S and Curtis-Prior PB proposed the term of beta-3 or beta-hybrid receptor for a new type of beta-adrenergic receptor, based on some studies of four beta-adrenergic agonists on isolated rat adipose cells. They observed that lipolytic potency decreased in the order: isoprenaline (beta-1 and beta-2 agonist) > noradrenaline (beta-1 >>> beta-2 agonist) > salbutamol (beta-2 agonist) > prenalterol (beta-1 agonist). They also studied the effects of some beta-antagonists on lipolysis induced by various agonists. Propranolol (nonselective beta-antagonist) was more potent than betaxolol (selective beta-1 antagonist) or ICI 118551 (selective beta-2 antagonist). All results conducted to the idea that lipolysis in adipose tissue is regulated by other adrenergic receptor than the classical ones, beta-1 and beta-2 [5].

In 1989, Emorine et al. first characterized beta-3 receptor by discovering the gene that encodes it [6]. Before that Arch et al. observed that some nonspecific classical beta-receptor agonists, named BRL 26830A, BRL 33725A, and BRL 35135A, had antiobesity actions on obese and diabetic mice [7].

Further studies have shown that beta-3 receptor is different than beta-1 and beta-2 by some important issues:

- the specific agonists CL 316243 and BRL37344 stimulate only beta-3 receptor [8, 9];
- lack of beta-3 receptor desensitization after agonists activation [10]; and
- the need to use larger quantities of catecholamines to stimulate beta-3 receptors [11].

The structures of beta-1, beta-2, and beta-3 receptors are similar, being all members of G protein-coupled receptors. Beta-3 receptor is a protein which contains 396 amino acids, found in seven transmembrane segments, with three intracellular and three extracellular loops. The amino-terminal region is extracellular, glycosylated, and with variable length. The carboxyl-terminal region is intracellular and it does not possess phosphorylation sites, which are present at beta-1 and beta-2 receptors. Essential for interaction with the ligands are the disulfide bond between the second and the third extracellular loops and also four of the seven transmembrane segments. Other two segments are implicated in G-protein stimulation, with adenylate cyclase and second messenger activation [12].

1.2. Metabolic effects of beta-3 adrenergic receptor stimulation

Some of the most important effects of beta-3 receptor activation are the metabolic ones, especially in the brown adipose tissue. This thermogenic tissue has the role of keeping constant body core temperature of small animals at cold ambient temperatures. Stimulation of beta-3 adrenergic receptors not only activates brown adipose tissue thermogenesis in the short term, but also increases mitochondrial biogenesis and the expression of thermogenin.
This protein mediates transport across the internal mitochondrial membrane and interrupts oxidative phosphorylation of the beta oxidation of fatty acids, increasing the use of energy [16]. There have been investigations about the effects of beta-3 adrenergic agonists on thermogenin. The results have shown that beta-3 agonists activated thermogenin, and also other uncoupling proteins, as follows:

- UCP2 that is found in many tissues.
- UCP3 that is found in skeletal muscle and has an important role in basal thermogenesis [17].

The stimulation of thermogenesis by beta-3 adrenergic agonists resulted in a number of experimental studies, which have shown that in animals, these substances lead to weight loss, a selective fat decrease, but without reducing food intake [18].

An in vitro study was performed on cells with high levels of beta-3 adrenoreceptors such as the adipocytes of the murine cell line 3T3-F442A. The study demonstrated that insulin and glucocorticoids downregulate beta-3 adrenoreceptor expression through a transcriptional effect. The impairment of beta-3 adrenoceptor gene expression in adipocytes of congenitally obese ob/ob mice could be related to the higher glucocorticoid plasma levels when compared to lean mice [19].

The main studied metabolic actions in preclinical research of beta-3 adrenergic agonists were the reduction of plasma insulin levels, increase glucose tolerance, and reducing body weight in obese diabetic rats. The major implication of beta-3 adrenergic receptor in glucose metabolism and hence in the release of insulin and in obesity has been demonstrated [20, 21].

The development of beta-3 adrenergic agonists was a step forward for the treatment of metabolic diseases by sympathetic activation, because norepinephrine and other relative nonselective derivatives have cardiovascular side effects which limit their use.

1.3. Preclinical studies of beta-3 agonists on carbohydrate and lipid metabolism

Numerous nonclinical studies have shown that administration of beta-3 agonists decreased glucose and lipids plasma concentrations in diabetic mice derived from genetically modified strains (kk, C57BL/KsJ- db/db) or in rats with experimentally induced diabetes [22, 23].

Several mechanisms of action were highlighted and reported:
- improving insulin resistance and increased tissue response to insulin [24],
- increasing insulin secretion in beta cells of the pancreas [25],
- decrease in glucose release from the liver, increase noninsulin-dependent uptake of glucose from white and brown adipose tissue and skeletal muscles [26], and
- increase of glucose tolerance at doses lower than those that stimulate lipolysis in adipose tissue, without affecting the amount of food intake or body weight [27].

The nonclinical research for proving the effects of beta-3 agonists on obesity were conducted on rodent species [28–30] from various strains, both normal and genetically modified to generate predisposition to obesity.
Studies in obese rats treated with the selective beta-3 adrenergic agonists have shown a significant reduction in body weight and reduction of adipose tissue, without food intake being affected by them. It was also demonstrated that an increase of two to three times of the mRNA level and of UCP-type protein, as well as of the guanidine 5'-diphosphate coupled, a relevant index of thermogenesis, in brown and white adipose tissues for the tested rats. In addition, it has been found, after the administration of beta-3 adrenergic agonists, an improvement of glucose tolerance and a decrease of hyperinsulinemia. The researchers suggested two possible mechanisms for defining this aspect, increase of the number of insulin receptors or decrease of glucose transporters in brown and white adipose tissues, which implies an increase in glucose uptake into muscle tissue [31].

Lorente Ferrer et al. investigated the effect of beta-3 receptors agonists on thermogenesis in deep adipose tissue. In general, these agonists increase energy consumption but their effects are quickly counteracted by glucocorticoids. Thus, their potential for long-term treatment of obesity is reduced. Since the metabolic effects of beta-3 receptor agonists (β_3A) overlap only partially with those of oleoyl-estrone (OE) (loss of appetite, weight change, loss of body fat), the possibility of combining them in the energy balance in order to accelerate the decrease of fat deposits was studied. Rats receiving OE or OE + β_3A significantly reduced weight compared with the control group, the maximum reduction corresponding to the group which received the combination [32].

The effect of beta-3 adrenergic receptor agonists was investigated on two strains of rats with different genetic predisposition to obesity: male rats aged 8 weeks Osborne Mendel (OM) strain and S5B/P1 (S5B) strain. Animals were treated with beta-3 adrenergic agonist CL316243 after they have been adapted to either a high fat diet (56% fat-based energy) or low fat (10% fat-based energy), but both equivalent diets in terms of protein content (24% based on protein energy). The animals were fed ad libitum and were injected with CL316243 in three doses: 0.1; 0.3; and 3 mg/kg at the beginning of the night. Food intake was measured at 1, 3, and 24 hours after injection. The results showed that CL316243 significantly reduced food intake for all measurements, in both types of rats. Inhibition of food intake was still higher in S5B-type mice. CL316243 significantly decreased serum leptin and serum glucose at both types of rats, especially at S5B. In OM rats, beta-3 adrenergic agonist increased serum insulin levels, while in S5B rats fed with a low-fat diet, the level of serum insulin decreased. In another experiment, CL316243 was administered to rats kept fasting overnight. It was observed after 30 minutes a significant reduction in insulin levels in both types, more pronounced in S5B. The glucose level in OM rats decreased after 30 and 60 minutes, while in rats S5B a decrease was observed only after 30 minutes from the administration. Experiments have shown that beta-3 agonist CL316243 has a much more obvious effect on rat strain resistant to obesity induced by high-fat diet [33].

Another study used a transgenic model of mice, lacking beta-3 adrenergic receptors. CL316243 blocked the activation of adenylate cyclase and lipolysis when it was administered to these mice. A modest growth of fat tissues especially in females was observed. These mice showed an increase in the level of mRNA for beta-1 receptor, but not for receptor beta-2. This showed a functional compensation between the genes for beta-1 and beta-3 receptors. Finally, a sharp
increase of insulin levels and lipolysis after administration of CL316243 in normal mice was noted; effects were not found after administration of beta-3 adrenergic agonists in beta-3 receptor-deficient mice [34].

In another model of knockout mice lacking functional beta-3 adrenoreceptors, there were no responses for food intake and insulin secretion in white and brown adipocytes after administration of beta-3 adrenergic agonist CL316243, indicating the implication of beta-3 receptors in these metabolic effects [35].

An increase in insulin levels during “fasted/fed” transition in rats has been demonstrated, associated with a decrease in the mRNA level of beta-3 adrenergic receptor and a decrease of the response in brown and white adipose tissues. It was concluded that there is a close relationship between the food intake, plasma levels of insulin, and beta-3 adrenergic receptors. Downregulation of the beta-3 receptors could be a possible mechanism by which insulin determines lipid storage and prevents lipid mobilization after food intake [36].

In another study, CL316243 was administered in obese diabetic KKAY mice for 2 weeks. The results showed a decrease of serum levels of glucose, insulin, triglyceride, free fatty acid, and tumor necrosis factor-alpha (TNF-alpha) and an increase of adiponectin. The beta-3 adrenergic receptor agonist recovered the mRNA expressions of adiponectin, adiponectin receptors, and beta-3 adrenoreceptor, which were reduced in epididymal white adipose tissue in KKAY mice. Also, CL316243 suppressed the overexpressed mRNA level of TNF-alpha in both epididymal white and brown adipose tissues. It was concluded that the normalization of adiponectin, adiponectin receptors and TNF-alpha could contribute at the amelioration of obesity-induced insulin resistance [28].

In the study conducted on nonobese/nondiabetic Sprague-Dawley rats, the selective beta-3 agonist CL 312243 increased food intake, metabolic rate, and body temperature after 7 days of treatment. The author also showed a decrease in intra-abdominal and subepithelial fat, a hepatic glucose level independent of variations in body weight, an increase in interscapular fat, and in total glucose, which stimulates the production of insulin. According to the results, only white and brown adipose tissues have been affected. A more important role of adipose tissue in glucose uptake underlining the potential role of beta-3 adrenergic agonist drugs for the treatment of obesity and insulin-resistant diabetes was suggested [8].

By a critical analysis of the published data in nonclinical research, it was concluded that beta-3 adrenergic receptor activation in the experiment-induced diabetes and obesity [37, 38] determines an increase of glucose tolerance and lipolysis activation in adipose tissue [22]. It seems that the effects are dose dependent and the selectivity of actions for glucose metabolism occurs at lower doses than those used for influencing lipid metabolism.

Comparing the effects of beta-3 agonists in rats and humans, Arch and Wilson stated that these compounds, with remarkable effects on rodents, have not convinced in clinical studies because of limited efficacy or serious side effects. The explanations mentioned by the authors included low pharmacokinetic properties and a low biotransformation to active compounds. A possible more important distinction between rats and humans is the different structure of beta-3 receptors, leading to lower efficacy of compounds in humans than in rats. In addition, it seems
that the number of beta-3 receptors is lower than beta-1 and beta-2 receptors in the tissues that mediate thermogenesis in humans [39]. This is one of the reasons why the clinical studies conducted on beta-3 selective agonists had some contradictory results.

Mirabegron, a currently approved drug for the treatment of overactive bladder, was recently studied in humans for its effects on brown adipose tissue. This drug has several advantages over other members of its class, including a higher bioavailability and a higher \textit{in vitro} affinity for the human beta-3 adrenoreceptor. Mirabegron was orally administered in the dose of 200 mg to 12 healthy male subjects with detectable brown adipose tissue. The results showed that all treated subjects had a higher brown adipose tissue metabolic activity, measured with $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) using positron emission tomography (PET) combined with computed tomography (CT). These are promising results for a possible future use of beta3 agonists for metabolic disease [40].

Based on all these preclinical and clinical considerations new chemical entities with beta-phenylethylamine nucleus, substituted in various positions on the nucleus or side chain, with potential action on diabetes and/or obesity were synthesized [41, 42]. Chemists led synthesis in order to obtain derivatives with increased beta-3 receptor selectivity. The compounds were conventionally named A1-\textit{$\beta$}PhEA–A13-\textit{$\beta$}PhEA (Figure 1).

![Figure 1. General structure of the newly synthesized compounds. X = H, alkoxy, halogen, dihalogen; Y = 4-carboproxy-phenoxy, 4-carbomethoxymethylene-phenoxy, 4-carbomethoxyethylene-phenox](image)

- $X = \text{H, alkoxy, halogen, dihalogen}$
- $Y = \text{4-carboproxy-phenoxy, 4-carbomethoxymethylene-phenoxy, 4-carbomethoxyethylene-phenox}$

### 2. Objectives

The purpose of this study was to test the effects of the newly synthesized compounds over lipid profile and body weight of rats to which alloxanic diabetes was induced, being a known fact that this metabolic disorder induces alterations in plasma lipids.

Alloxan was chosen for induction of type II diabetes mellitus because its pancreatic toxicity was demonstrated in nonclinical trials using isolated islet cell or entire perfused rat pancreas and afterwards by directly administering the substance to rodent or nonrodent animals. In the first stage, alloxan stimulates on short-term insulin secretion, which is followed by total suppression of the response of the islet cells to glucose, regardless of its concentration [43].
Alloxan is readily absorbed by beta pancreatic cell, a process that contributes to diabetogenic action. Its absorption also takes place in liver although hepatocytes are more resilient to its action compared to beta cells, therefore more protected against its toxicity [44].

2.1. Mechanism of alloxan toxicity

The mechanism of toxic action resides in formation of reactive species of oxygen [45], the alloxan exhibiting increased affinity to substrates containing thiolic groups (reduced glutathione, cysteine, proteins with sulfide groups, enzymes) [46]. The product of alloxan reduction, the dialuric acid, is reoxidized to alloxan, the redox cycle thus formed being responsible for releasing superoxide radicals—see Figure 2 [47].

\[
\begin{align*}
AH_2 + O_2 & \rightarrow AH^\cdot + O_2\cdot^- + H^+ \\
AH^\cdot + O_2 & \rightarrow A + O_2\cdot^- + H^+ \\
AH_2 + O_2\cdot^- + H^+ & \rightarrow AH^\cdot + H_2O_2 \\
AH^\cdot + O_2\cdot^- + H^+ & \rightarrow A + H_2O_2 \\
H_2O_2 + e^- & \rightarrow OH^\cdot + OH^- \\
Fe^{2+} + H_2O_2 & \rightarrow Fe^{3+} + OH^\cdot + OH^- \\
\text{Net: } O_2\cdot^- + H_2O_2 & \rightarrow O_2 + OH^\cdot + OH^-
\end{align*}
\]

Figure 2. Mechanism of diabetes induction by administration of alloxan [47]. A, Alloxan; AH^\cdot, alloxan radical; AH_2, dialuric acid; O_2\cdot^-, superoxide radical; OH^\cdot, hydroxyl radical.

An optimal protection against cytotoxic action of alloxan and dialuric acid is offered by an association between superoxide-dismutase (SOD) and catalase (CAT), in order to completely prevent the redox cycle and consecutively the formation of any reactive oxygen species [48].

Glucose confers complete protection against toxic effects of alloxan both in vivo and in vitro, by blocking glucokinase inhibition by it and also contributing to maintaining the antioxidant protection mechanism of the beta cells [49].

One of the targets for the reactive oxygen species is the DNA of the pancreatic islet cells. The DNA fragmentation occurs in beta cells exposed to alloxan [49, 50].

The DNA alteration stimulates poly-ADP-ribose, enzyme which contributes to affected DNA repair. In several trials it was stated that glucose administration contributes to counteracting the alloxan cytotoxicity. Such ability is the result not only of glucokinase protection but also of interaction with glucose carrier GLUT2 resulting in reduced alloxan absorption [51].
3. Materials and methods

Diabetes was induced to white Wistar male rats by intraperitoneal administration of extemporaneously prepared alloxan (Sigma-Aldrich) solution, in the dose of 130 mg/kg weight [52]. After 48 hours, glycemia was determined using ACCU-CHEK Active device (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). The blood was harvested from tail veins by vein puncture. The determination was used for the selection of diabetic animals, thus presenting a glycemia over 200 mg/dL.

From the total rat collectivity, a percentage of 66.34% became diabetic with the remaining 33.66% becoming hyperglycemic. Fifteen groups of diabetic animals were designated for experimental research (eight animals/group), being treated as follows:

- diabetic control group (D Control)—distilled water, 1 mL/100 g weight p.o.;
- reference group—BRL 37344, 50 mg/kg weight, p.o.;
- A1-βPhEA—20 mg/kg weight, p.o.;
- A2-βPhEA—50 mg/kg weight, p.o.; and
- A3-βPhEA through A13-βPhEA, 100 mg/kg weight, p.o.

At the same time, a nondiabetic control group was designated (ND control), treated with distilled water, 1 mL/100 g weight p.o. Administration of tested and reference substances (BRL 37344) continued for 14 days, once daily.

The doses chosen for investigating the metabolic effects of the newly synthesized derivatives were established following previous determinations of acute toxicity which allowed to set the LD$_{50}$ [53, 54].

At the end of the experiment, the animals were sacrificed and biochemical and enzymatic determinations were performed: glycemia, glucose-6-phosphate dehydrogenase (transforming glucose by pentose-phosphate pathway), glucose–6–phosphatase (catalyzes the hydrolysis of glucose–6–phosphate to glucose and inorganic phosphate), hexokinase (catalyzes the phosphorylation of glucose), total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.

4. Results and discussion

The model of alloxan-induced diabetes has produced alterations in the activity of the three enzymes [55–57] involved in glucose metabolism homeostasis (Figure 3).

Moreover, the lipid profile was altered by the administration of the pancreatic toxic, registering statistically significant increases in total cholesterol, LDL cholesterol, and triglycerides, together with decreases in HDL cholesterol (Figure 4).
Figure 3. Variations of the activity of the enzymes involved in glucose metabolism in diabetic animals compared to nondiabetic animals.

Figure 4. Variation of plasma lipids in diabetic animals compared to nondiabetic animals ** p<0.01; *** p<0.001.

To what concern the effect of new compounds with possible affinity for beta-3 adrenergic receptors, seven of those (A1-βPhEA, 20 mg/kg weight; A3-βPhEA, 100 mg/kg weight; A4-βPhEA, 100 mg/kg weight; A6-βPhEA, 100 mg/kg weight; A8-βPhEA, 100 mg/kg weight; A9-
\[ \beta \text{PhEA, 100 mg/kg weight; A13-} \beta \text{PhEA, 100 mg/kg weight) have markedly reduced the values of total cholesterol (Table 2, Figure 5).} \]

| Group                  | M ± SE       | ANOVA       | Dunnett posttest/ND | ANOVA       | Dunnett posttest/D |
|------------------------|-------------|-------------|---------------------|-------------|---------------------|
| Nondiabetic control    | 78.22 ± 9.553 | 0.0002***   |                     | <0.0001*** |                     |
| Diabetic control       | 119.3 ± 11.89 | **         |                     |             |                     |
| BRL 37344 50 mg/kg     | 77.65 ± 2.196 | ns          |                     |             |                     |
| A1-\beta \text{PhEA 20 mg/kg} | 62.03 ± 5.005 | ns          | ***                 |             |                     |
| A2-\beta \text{PhEA 50 mg/kg} | 74.83 ± 13.10 | ns          |                      |             |                     |
| A3-\beta \text{PhEA 100 mg/kg} | 70.42 ± 9.716 | ns          | **                  |             |                     |
| A4-\beta \text{PhEA 100 mg/kg} | 69.27 ± 6.423 | ns          | **                  |             |                     |
| A5-\beta \text{PhEA 100 mg/kg} | 113.0 ± 11.41 | ns          | ns                  |             |                     |
| A6-\beta \text{PhEA 100 mg/kg} | 61.00 ± 4.749 | ns          | ***                 |             |                     |
| A7-\beta \text{PhEA 100 mg/kg} | 75.05 ± 5.286 | ns          |                      |             |                     |
| A8-\beta \text{PhEA 100 mg/kg} | 72.31 ± 6.148 | ns          | **                  |             |                     |
| A9-\beta \text{PhEA 100 mg/kg} | 69.25 ± 6.542 | ns          | **                  |             |                     |
| A10-\beta \text{PhEA 100 mg/kg} | 77.73 ± 7.884 | ns          |                      |             |                     |
| A11-\beta \text{PhEA 100 mg/kg} | 66.60 ± 3.331 | ns          |                      |             |                     |
| A12-\beta \text{PhEA 100 mg/kg} | 75.32 ± 5.966 | ns          |                     |             |                     |
| A13-\beta \text{PhEA 100 mg/kg} | 62.15 ± 6.673 | ns          |                     |             |                     |

Table 2. The effect of new derivatives of beta-phenylethylamine on total cholesterol in rats with alloxan-induced diabetes * p<0.05; ** p<0.01; *** p<0.001.

Figure 5. Alterations of total cholesterol in diabetic animals treated with reference substance (BRL 37344) or newly synthesized derivatives of beta-phenyl ethylamine compared to diabetic control group * p<0.05; ** p<0.01; *** p<0.001.
The majority of the tested compounds (Table 3) have markedly reduced the values of LDL cholesterol (Figure 6), the effect probably due to increased plasma clearance for this lipid fraction as total cholesterol serum concentration decreased. Smaller reductions, still statistically significant (Table 3) in values of LDL cholesterol, and comparable to those of the beta-3adrenergic agonist, BRL 37344, were produced by the compounds: A7-βPhEA, A10-βPhEA, A12-βPhEA, and A13-βPhEA (Figure 6).

| Group                  | M ± SE     | ANOVA   | Dunnett posttest/ND control | ANOVA   | Dunnett posttest/D control |
|------------------------|------------|---------|-----------------------------|---------|---------------------------|
| Nondiabetic control    | 51.07 ± 3.610 | <0.0001*** | <0.0001*** ***              |         |                           |
| Diabetic Control       | 103.2 ± 7.899  |          |                             |         |                           |
| BRL 37344 50 mg/kg     | 78.71 ± 4.940  |          |                             |         |                           |
| A1-βPhEA 20 mg/kg      | 56.87 ± 1.887  |          |                             |         |                           |
| A2-βPhEA 50 mg/kg      | 57.47 ± 2.831  |          |                             |         |                           |
| A3-βPhEA 100 mg/kg     | 50.91 ± 1.205  |          |                             |         |                           |
| A4-βPhEA 100 mg/kg     | 57.08 ± 2.302  |          |                             |         |                           |
| A5-βPhEA 100 mg/kg     | 56.26 ± 2.134  |          |                             |         |                           |
| A6-βPhEA 100 mg/kg     | 56.88 ± 2.496  |          |                             |         |                           |
| A7-βPhEA 100 mg/kg     | 75.91 ± 6.447  |          |                             |         |                           |
| A8-βPhEA 100 mg/kg     | 54.62 ± 2.458  |          |                             |         |                           |
| A9-βPhEA 100 mg/kg     | 53.70 ± 2.128  |          |                             |         |                           |
| A10-βPhEA 100 mg/kg    | 76.00 ± 7.567  |          |                             |         |                           |
| A11-βPhEA 100 mg/kg    | 57.52 ± 3.112  |          |                             |         |                           |
| A12-βPhEA 100 mg/kg    | 66.77 ± 7.334  |          |                             |         |                           |
| A13-βPhEA 100 mg/kg    | 50.21 ± 1.188  |          |                             |         |                           |

Table 3. The effect of new derivatives of beta-phenylethylamine on LDL cholesterol values in alloxan-induced diabetic rats ** p<0.01; *** p<0.001.

Figure 6. Alterations of LDL cholesterol values in diabetic animals treated with reference substance (BRL 37344) or new derivatives of beta-phenylethylamine compared to diabetic control group ** p<0.01; *** p<0.001.
The results for lowered total cholesterol and LDL cholesterol are in line with other literature data showing that several beta-3adrenergic agonists have induced similar effects in mice with apolipoprotein E deficiency and in wild C57BL/6J strain animals. For such substances an increase in apolipoprotein A1 and PPARα and PPARγ receptors (peroxisome proliferator-activated receptor) expression in liver was demonstrated [58].

Other trials showed that, due to effects on lipid metabolism but also to glycemia reduction, the β3 adrenergic agonist BRL 37344 has induced a reduction in the process of formation of atherosclerotic plaque in ApoE(-/-) mice [59].

A pivotal role in slowing down the process of atherosclerosis stands with HDL cholesterol, which is a small size alpha-lipoprotein, formed in liver or shed from chylomicrons dismem-berment. These lipoproteins have a cholesterol-rich core with type 1 and 2 apolipoproteins at the surface, ensuring the reverse transport of cholesterol, from tissues to liver. The trial conducted by Shi et al. demonstrated the increases in mARN and apoA1 expression.

| Group                  | M ± SE   | ANOVA   | Dunnett posttest/ND control | ANOVA   | Dunnett posttest/D control |
|------------------------|----------|---------|----------------------------|---------|----------------------------|
| Nondiabetic control    | 46.36 ± 2.930 | <0.0001*** | <0.0001*** | *** |
| Diabetic control       | 29.34 ± 3.478 | ***     | ns                         | ns |
| BRL 37344 50 mg/kg     | 34.82 ± 3.216 | ns       |                           |     |
| A1-βPhEA 20 mg/kg      | 48.09 ± 3.621 | ns       |                           |     |
| A2-βPhEA 50 mg/kg      | 42.96 ± 2.102 | ns       |                           |     |
| A3-βPhEA 100 mg/kg     | 46.24 ± 4.104 | ns       |                           |     |
| A4-βPhEA 100 mg/kg     | 53.35 ± 4.875 | ns       |                           |     |
| A5-βPhEA 100 mg/kg     | 49.79 ± 2.926 | ns       |                           |     |
| A6-βPhEA 100 mg/kg     | 46.07 ± 3.679 | ns       |                           |     |
| A7-βPhEA 100 mg/kg     | 52.18 ± 3.653 | ns       |                           |     |
| A8-βPhEA 100 mg/kg     | 51.04 ± 1.662 | ns       |                           |     |
| A9-βPhEA 100 mg/kg     | 47.81 ± 4.664 | ns       |                           |     |
| A10-βPhEA 100 mg/kg    | 45.66 ± 4.880 | ns       |                           |     |
| A11-βPhEA 100 mg/kg    | 38.04 ± 2.596 | ns       |                           |     |
| A12-βPhEA 100 mg/kg    | 38.46 ± 2.095 | ns       |                           |     |
| A13-βPhEA 100 mg/kg    | 41.94 ± 1.330 | ns       |                           |     |

*Table 4. The effect of new derivatives of beta-phenyl ethylamine on HDL cholesterol in rats with alloxan-induced diabetes *p<0.05; ***p<0.001.

The results of the experimental research have shown that all tested compounds have induced statistically significant increases in HDL cholesterol, compared to diabetic control group (Table 4). A smaller effect of increase of the values for this lipid fraction was produced by the compounds A11-βPhEA and A12-βPhEA, still being similar to the one for beta3 adrenergic agonist BRL 37344 (Figure 7).
### Table 5. The effect of new derivatives of beta-phenyl ethylamine on serum TG in rats with alloxan-induced diabetes

| Group                   | M ± SE     | ANOVA Dunnett posttest/ND | ANOVA Dunnett posttest/D |
|-------------------------|------------|---------------------------|--------------------------|
| Nondiabetic control     | 81.74 ± 2.261 | 0.0002***                 | <0.0001***               |
| Diabetic control        | 166.3 ± 6.793  | ***                       |                         |
| BRL 37344 50 mg/kg      | 77.13 ± 3.639  | ns                        | ***                      |
| A1-βPhEA 20 mg/kg       | 91.25 ± 4.573  | ns                        | ***                      |
| A2-βPhEA 50 mg/kg       | 88.01 ± 4.158  | ns                        | ***                      |
| A3-βPhEA 100 mg/kg      | 90.23 ± 4.643  | ns                        | ***                      |
| A4-βPhEA 100 mg/kg      | 67.38 ± 8.630  | ns                        | ***                      |
| A5-βPhEA 100 mg/kg      | 66.32 ± 5.682  | ns                        | ***                      |
| A6-βPhEA 100 mg/kg      | 66.58 ± 9.860  | ns                        | ***                      |
| A7-βPhEA 100 mg/kg      | 80.60 ± 3.113  | ns                        | ***                      |
| A8-βPhEA 100 mg/kg      | 84.24 ± 2.831  | ns                        | ***                      |
| A9-βPhEA 100 mg/kg      | 87.80 ± 4.384  | ns                        | ***                      |
| A10-βPhEA 100 mg/kg     | 86.21 ± 2.935  | ns                        | ***                      |
| A11-βPhEA 100 mg/kg     | 87.86 ± 5.032  | ns                        | ***                      |
| A12-βPhEA 100 mg/kg     | 84.35 ± 3.656  | ns                        | ***                      |
| A13-βPhEA 100 mg/kg     | 91.25 ± 6.193  | ns                        | ***                      |

In Wistar rats alloxan has induced a high increase, statistically significant, of serum triglycerides (TG) (Table 5). Compared to diabetic control group, all the tested compounds have reduced the values of serum triglycerides with high statistical significance. These effects could...
be due to increased expression of PPARα (liver, kidney, muscle, adipose tissue) and PPAR γ receptors (subtypes 1, 2, 3 in adipose tissue) resulting in increased expression of the gene for lipoprotein lipase.

4.1. Effects on body weight

During the research, animals had free access to standard food and water. The body weight was determined initially, at 48 hours after alloxan administration and then in day 5, 10, and 14 of the experiment. For nondiabetic control group the same determination were performed as in the case of diabetic groups. The food was dispensed daily in same amounts and body weight was determined before the next feeding.

Throughout this determination it was apparent that alloxan-induced diabetes produces, in 48 hours from administration, a statistically nonsignificant reduction (Figure 8) of body weight in treated animals (203.4 ± 0.7004 vs. 205.9 ± 0.7078).

The research results showed that, for diabetic control group, animal body weight increases after alloxan administration, reaching a significant higher value in day 14 of the experiment (p = 0.0097**). For the groups treated with reference substance, the body weight varied statistically nonsignificantly in all moments of determination (Table 6). The amount of consumed food increased for the diabetic control group, while for the treated groups, it remained constant. In the determinations of day 14, for nondiabetic control group it was registered an increase of 1.07% in body weight against the initial measurement, while for the diabetic control group the increase reached 5.65%. The variation in body weight at the end of the experiment against initial and compared to diabetic control group was calculated using the formulas:
| Group        | Parameter | Body weight basal | Body weight 48 h after alloxan | Body weight Day 5 | Body weight Day 10 | Body weight Day 14 |
|--------------|-----------|-------------------|-------------------------------|-------------------|--------------------|-------------------|
| ND control   | M ± SE    | 203.8 ± 3.301     | 197.0 ± 3.967                 | 206.5 ± 2.363     | 209.7 ± 2.305      | 214.7 ± 1.382**   |
|              | ANOVA/48 h| 0.7341 ns         |                               |                   |                    |                   |
| D control    | M ± SE    | 203.2 ± 3.429     | 198.7 ± 3.920                 | 203.8 ± 2.868     | 202.7 ± 2.246      | 201.5 ± 2.513     |
|              | ANOVA/48 h after A| 0.0097**         |                               |                   |                    |                   |
| BRL 37344    | M ± SE    | 204.3 ± 2.963     | 201.8 ± 3.936                 | 206.0 ± 2.145     | 205.5 ± 2.247      | 205.0 ± 2.556     |
|              | ANOVA/48 h after A| 0.6778 ns         |                               |                   |                    |                   |
| A1-βPhEA     | M ± SE    | 202.5 ± 2.872     | 197.2 ± 3.640                 | 201.7 ± 2.836     | 200.8 ± 3.114      | 199.8 ± 3.092     |
|              | ANOVA/48 h after A| 0.7823 ns         |                               |                   |                    |                   |
| A2-βPhEA     | M ± SE    | 200.2 ± 3.781     | 195.5 ± 4.123                 | 198.5 ± 3.667     | 198.7 ± 3.356      | 199.0 ± 3.540     |
|              | ANOVA/48 h after A| 0.9238 ns         |                               |                   |                    |                   |
| A3-βPhEA     | M ± SE    | 208.0 ± 2.206     | 201.8 ± 3.936                 | 206.0 ± 2.145     | 205.5 ± 2.247      | 205.0 ± 2.556     |
|              | ANOVA/48 h after A| 0.6202 ns         |                               |                   |                    |                   |
| A4-βPhEA     | M ± SE    | 204.8 ± 2.701     | 199.5 ± 3.805                 | 202.8 ± 2.651     | 203.2 ± 2.151      | 202.0 ± 3.088     |
|              | ANOVA/48 h after A| 0.4047 ns         |                               |                   |                    |                   |
| A5-βPhEA     | M ± SE    | 207.2 ± 2.868     | 201.1 ± 4.083                 | 206.0 ± 3.044     | 204.7 ± 2.552      | 206.7 ± 3.232     |
|              | ANOVA/48 h after A| 0.6717 ns         |                               |                   |                    |                   |
| A6-βPhEA     | M ± SE    | 205.0 ± 2.966     | 199.1 ± 3.973                 | 203.2 ± 2.868     | 202.5 ± 2.754      | 202.0 ± 2.781     |
|              | ANOVA/48 h after A| 0.7505 ns         |                               |                   |                    |                   |
| A7-βPhEA     | M ± SE    | 206.7 ± 2.552     | 201.1 ± 3.989                 | 204.8 ± 2.358     | 204.2 ± 2.167      | 203.7 ± 2.290     |
|              | ANOVA/48 h after A| 0.7213 ns         |                               |                   |                    |                   |
| A8-βPhEA     | M ± SE    | 204.8 ± 1.990     | 201.7 ± 3.802                 | 203.5 ± 1.586     | 202.7 ± 1.926      | 203.7 ± 2.290     |
|              | ANOVA/48 h after A| 0.9261 ns         |                               |                   |                    |                   |
| A9-βPhEA     | M ± SE    | 208.2 ± 1.249     | 202.1 ± 3.667                 | 205.5 ± 0.7638    | 204.7 ± 1.022      | 203.8 ± 1.558     |
|              | ANOVA/48 h after A| 0.3638 ns         |                               |                   |                    |                   |
| A10-βPhEA    | M ± SE    | 206.3 ± 2.741     | 200.8 ± 4.036                 | 205.0 ± 2.477     | 204.8 ± 2.587      | 204.0 ± 2.671     |
|              | ANOVA/48 h after A| 0.7431 ns         |                               |                   |                    |                   |
| A11-βPhEA    | M ± SE    | 208.0 ± 3.066     | 202.2 ± 4.401                 | 206.2 ± 2.738     | 205.8 ± 2.713      | 205.7 ± 2.813     |
|              | ANOVA/48 h after A| 0.7922 ns         |                               |                   |                    |                   |
| A12-βPhEA    | M ± SE    | 207.3 ± 2.894     | 201.7 ± 4.234                 | 205.7 ± 2.565     | 205.3 ± 2.603      | 204.5 ± 2.320     |
|              | ANOVA/48 h after A| 0.7561 ns         |                               |                   |                    |                   |
| A13-βPhEA    | M ± SE    | 209.3 ± 2.591     | 203.0 ± 4.244                 | 206.7 ± 2.028     | 206.7 ± 2.390      | 205.0 ± 2.266     |
|              | ANOVA/48 h after A| 0.6257 ns         |                               |                   |                    |                   |

ns, nonsignificant; A, alloxan.

Table 6. The effect of new derivatives of beta-phenylethylamine on body weight in rats with alloxan-induced diabetes. ** p<0.01.
Taking into account that body weight increased for the animals in diabetic control group and that for all tested substances it has decreased against initial, the effect of the tested substances was determined compared to diabetic control group (Figure 9). It was therefore noted that newly synthesized derivatives of beta-phenyl ethylamine and the reference substance BRL 37344 had produced decreases in body weight between 5.89 and 7.76%, compared to diabetic control animals after 14 days of treatment.

**Figure 9.** Variation of body weight for animals treated with reference substance and tested substances compared to diabetic control group at day 14 determination.

5. Conclusions

The results of this experimental research have demonstrated that newly synthesized derivatives of beta-phenylethylamine produce marked biological activity over lipid profile which is altered in diabetes induced by alloxan administration in rats.

All tested compounds have markedly decreased the values of total cholesterol, LDL cholesterol, and triglycerides, the effect being more intense than with reference substance BRL 37344. They also have increased the values of antiatherogenic HDL cholesterol, significantly more...
than the reference substance. Overall, the activity on body weight was of reduction even if the food consumption of the animals was not altered. These experimental data suggest that the tested new chemical entities have high therapeutical potential in the treatment of dislipidemias and/or obesity.

Acknowledgements

The authors acknowledge the contribution of INDCF Bucharest scientific collective who performed the chemical synthesis for the tested compounds and with whom have collaborated previously in two Romanian national research projects: CEEX 51/2005 și PC NR. 1750/2008.

Author details

Simona Negreș, Cornel Chiriță*, Andreea Letiția Arsene, Denisa Margină, Elena Moroșan and Cristina Elena Zbârcea

*Address all correspondence to: chirita.cornel@gmail.com

University of Medicine and Pharmacy “Carol Davila”, Faculty of Pharmacy, Bucharest, Romania

References

[1] Ahlquist RP. A study of the adrenotropic receptors. The American Journal of Physiology, 1948; 153(3):586–600. PMID 18882199

[2] Westfall TC, Westfall DP. Neurotransmission: The autonomic and somatic motor nervous systems. Chap. 8, in: Goodman and Gilman’s The Pharmacological Basis of Therapeutics. 12th Ed., McGraw-Hill; 2011. pp. 203–204.

[3] Graham RM, Perez DM, Hwa J, Piascik MT. α1-Adrenergic receptor subtypes. Molecular structure, function, and signaling. Circulation Research, 1996; 78:737–749. DOI: 10.1161/01.RES.78.5.737

[4] Sawa M, Harada H. Recent developments in the design of orally bioavailable β3-adrenergic receptor agonists. Current Medicinal Chemistry, 2006; 13(1):25–37. DOI: 10.2174/092986706775198006

[5] Tan S, Curtis-Prior PB. Characterization of the beta-adrenoreceptor of the adipose cell of the rat. International Journal of Obesity, 1983; 7(5):409–414. PMID: 6139348
[6] Emorine LJ, Marullo S, Briend-Sutren MM, Patey G, Tate K, Delavier-Klutchko C, Strosberg AD. Molecular characterization of the human beta 3-adrenergic receptor. Science, 1989; 245(4922):1118–1121. DOI: 10.1126/science.2570461

[7] Arch JR, Ainsworth AT, Cawthorne MA, Piercy V, Sennit MV, Thody VE, Wilson C, Wilson S. Atypical beta-adrenoeceptor on brown adipocytes as target for anti-obesity drugs. Nature, 1984; 309(5964):163–165. PMID: 6325935

[8] de Souza CJ, Hirschman MF, Horton ES. CL-316,243, a beta3-specific adrenoceptor agonist, enhances insulin-stimulated glucose disposal in nonobese rats. Diabetes, 1997; 46(8):1257–1263. PMID: 9231648

[9] Martin CA, Naline E, Manara L, Advenier C. Effects of two beta 3-adrenoceptor agonists, SR 58611A and BRL 37344, and of salbutamol on cholinergic and NANC neural contraction in guinea-pig main bronchi in vitro. British Journal of Pharmacology, 1993; 110(4):1311–1316. PMC2175861

[10] Carpéné C, Galitzky J, Collon P, Esclapez F, Dauzats M, Lafontan M. Desensitization of beta-1 and beta-2, but not beta-3, adrenoceptor-mediated lipolytic responses of adipocytes after long-term norepinephrine infusion. Journal of Pharmacology and Experimental Therapeutics, 1993; 265(1):237–247. PMID: 8097243

[11] Galitzky J, Reverte M, Portillo M, Carpéné C, Lafontan M, Berlan M. Coexistence of beta 1-, beta 2-, and beta 3-adrenoceptors in dog fat cells and their differential activation by catecholamines. American Journal of Physiology, 1993; 264:E403–E412. PMID: 8096365

[12] Coman OA, Păunescu H, Ghită I, Coman L, Bădrău A, Fulga I. Beta 3 adrenergic receptors: Molecular, histological, functional and pharmacological approaches. Romanian Journal of Morphology and Embryology, 2009; 50(2):169–179. PMID: 19434307

[13] Betz MJ, Enerbäck S. Human brown adipose tissue: What we have learned so far. Diabetes, 2015; 64:2352–2360. DOI: 10.2337/db15-0146

[14] Fisher MH, Amend AM, Bach TJ, Barker JM, Brady EJ, Candelore MR, Carroll D, Cascieri MA, Chiu SH, Deng L, Forrest MJ, Hegarty-Friscino B, Guan XM, Hom GJ, Hutchins JE, Kelly LJ, Mathvink RJ, Metzger JM, Miller RR, Ok HO, Parmee ER, Saperstein R, Strader CD, Stearns RA, Macintyre DE. A selective human beta3 adrenergic receptor agonist increases metabolic rate in rhesus monkeys. Journal of Clinical Investigation, 1998; 101(11):2387–2393. DOI: 10.1172/JCI2496

[15] Klaus S, Seivert A, Boeuf S. Effect of beta 3 adrenergic agonist C1316,243 on functional differentiation of with and brown adipocytes in primary cell culture. Biochimica and Biophysica Acta, 2001; 1539:85–92. PMID: 11389970

[16] Ursino MG, Vasinnaa V, Raschia E, Crema F, De Pontia F. The beta 3- adrenoceptor as a therapeutic target: Current perspectives. Pharmacological Research, 2009; 59:221–234. DOI: 10.1016/j.phrs.2009.01.002
[17] Nedergaard J, Cannon B. The “novel” “uncoupling” proteins UCP2 and UCP3: What do they really do? Pros and cons for suggested functions. Experimental Physiology, 2003; 88(1):65–84. PMID: 12525856

[18] Sleigh SH, Barton CL. Repurposing strategies for therapeutics. Pharmaceutical Medicine, 2010; 24(3):151–159. DOI: 10.1007/BF03256811

[19] Langin D, Tavernier G, Lafontan M. Regulation of beta 3-adrenoreceptor expression in white fat cells. Fundamental and Clinical Pharmacology, 1995; 9(2):97–106. PMID: 7628838

[20] Oana F, Takeda H, Matsuzawa A, Akahane S, Isaji M, Akahane M. Adiponectin receptor 2 expression in liver and insulin resistance in db/db mice given a beta 3 adrenoreceptor agonist. European Journal of Pharmacology, 2005; 518:71–76. DOI:10.1016/j.ejphar.2005.06.004

[21] Bhadada SV, Patel BM, Mehta AA, Goyal RK. β(3) Receptors: Role in cardiometabolic disorders. Therapeutic Advances in Endocrinology and Metabolism, 2011; 2(2):65–79. DOI: 10.1177/2042018810390259

[22] Kenji O, Matsui H, Yasuhiro O, Ryotaro T, Kenichiro M, Hajime I, Akiko I, Tomofumi M, Michitaka T, Yoko K. The polymorphism of the beta 3 adrenergic receptor gene is associated with reduced low-density lipoprotein particle size. Metabolism, 2003; 52(3): 356–361. DOI: 10.1053/meta.2003.50056

[23] Oana F, Takeda H, Matsuzawa A, Akahane S, Hayashi M, Tamura T, Uehara M, Isaji M, Akahane M. KTO-7924, a Beta3-adrenergic receptor agonist, reduces hyperglycemia and protects beta-cells in the islets of Langerhans of db/db mice. Endocrine Research. 2010; 35(4):174–182. DOI: 10.3109/07435800.2010.507733

[24] Ghorbani M, Shafiee Ardestani M, Gigloo SH, Cohan RA, Inanlou DN, Ghorbani P. Antidiabetic effect of CL 316,243 (a β3-adrenergic agonist) by down regulation of tumour necrosis factor (TNF-α) expression. PLoS One. 2012;7(10):e45874. DOI: 10.1371/journal.pone.0045874

[25] Pang Z, Wu N, Zhang X, Avallone R, Croci T, Dressler H, Palejwala V, Ferrara P, Tocci M, Polites G. GPR40 is partially required for insulin secretion following activation of beta 3-adrenergic receptors. Molecular and Cellular Endocrinology, 2010; 325:18–25. DOI: 10.1016/j.mce.2010.04.014

[26] Watts VL, Sepulveda FM, Cingolani OH, Ho AS, Niu X, Kim R, Miller KL, Vandegaer K, Bedja D, Gabrielson KL, Rameau G, O’Rourke B, Kass DA, Barouch LA. Anti-hypertrophic and anti-oxidant effect of beta3-adrenergic stimulation in myocytes requires differential neuronal NOS phosphorylation. Journal of Molecular and Cellular Cardiology, 2013; 62:8–17. DOI: 10.1016/j.yjmcc.2013.04.025
[27] Sen A, Nichani VN. Exploring beta 3 adrenoreceptors for potential clinical applications. International Journal of Pharmaceutical Sciences Review and Research, 2010; 5(3):55–58.

[28] Fu L, Isobe K, Zeng Q, Suzukawa K, Takekoshi K, Kawakami Y. The effects of beta(3)-adrenoceptor agonist CL-316,243 on adiponectin, adiponectin receptors and tumor necrosis factor-alpha expressions in adipose tissues of obese diabetic KKAY mice. European Journal of Pharmacology, 2008; 584(1):202–206. DOI: 10.1016/j.ejphar.2008.01.028

[29] Kanzler SA, Januario AC, Paschoalini MA. Involvement of ß –adrenergic receptors in the control of food intake 3 in rats. Brazilian Journal of Medical and Biological Research, 2011; 44 (11):1141–1147.

[30] Kim-Motoyama H, Yasuda K, Yamaguchi T, Yamada N, Katakura T, Shuldiner AR, Akanuma Y, Ohashi Y, Yazaki Y, Kadowaki T. A mutation of the beta 3-adrenergic receptor is associated with visceral obesity but decreased serum triglyceride. Diabetologia, 1997; 40(4):469–472. PMID: 9112025

[31] Umekawa T, Yoshida T, Sakane N, Saito M, Kumamoto K, Kondo M. Anti-obesity and anti-diabetic effects of CL316,243, a highly specific beta 3-adrenoceptor agonist, in Otsuka Long-Evans Tokushima fatty rats: Induction of uncoupling protein and activation of glucose transporter 4 in white fat. European Journal of Endocrinology, 1997; 136:429–437. PMID: 9150705

[32] Ferrer-Lorente R, Cabot C, Fernandez Lopez LA, Alemanz M. Combined effects of oleoyl-estrone and beta 3 adrenergic agonist (CL316,243) on lipid stores of diet induced overweight male Wistar rats. Life Sciences, 2005; 77:2051–2058. DOI: 10.1016/j.lfs.2005.04.008

[33] White LC, Ishihara Y, Dotson LT, Hughes DA, Bray GA, York DA. Effect of beta 3 agonist on food intake in two strains of rats that differ in susceptibility to obesity. Physiology & Behavior, 2004; 82:489–496. DOI: 10.1016/j.physbeh.2004.04.059

[34] Susulic VS, Frederich RC, Lawitts J, et al. Targeted disruption of the beta 3-adrenergic receptor gene. J Biol Chem., 1995; 270:29483. PMID: 7493988

[35] Grujic D, Susulic VS, Harper ME, et al. Beta3-adrenergic receptors on white and brown adipocytes mediate beta3-selective agonist-induced effects on energy expenditure, insulin secretion, and food intake. A study using transgenic and gene knockout mice. Journal of Biological Chemistry, 1997; 272:17686. PMID: 9211919

[36] Hadri KE, Charon C, Pairault J, Hauguel-De MS, Quignard B, Feve B. Down-regulation of beta3-adrenergic receptor expression in rat adipose tissue during the fasted/fed transition: Evidence for a role of insulin. Biochemical Journal, 1997; 323:359. PMID: 9163324
[37] Mund RA, Frishman WH. Brown adipose tissue thermogenesis: β3-adrenoreceptors as a potential target for the treatment of obesity in humans. Cardiology in Review, 2013; 21(6):265–269. DOI: 10.1097/CRD.0b013e31829cabff

[38] Sacks H, Symonds ME. Anatomical locations of human brown adipose tissue: Functional relevance and implications in obesity and type 2 diabetes. Diabetes, 2013; 62(6): 1783–1790. DOI: 10.2337/db12-1430

[39] Arch JR, Wilson S. Prospects for beta 3-adrenoceptor agonists in the treatment of obesity and diabetes. International Journal of Obesity and Related Metabolic Disorders, 1996; 20(3):191–199. PMID: 8653138

[40] Cypess AM, Weiner LS, Roberts-Toler C, Elia EF, Kessler SH, Kahn PA, English J, Chatman K, Trauger SA, Doria A, Kolodny GM. Activation of human brown adipose tissue by a β3-adrenergic receptor agonist. Cell Metabolism, 2015; 21(1):33–38. DOI: 10.1016/j.cmet.2014.12.009

[41] Guță R, Ilie C, Putina G, Nănău-Andreeescu D, Negreș S, Caproiu MT. Potential antidiabetic/antiobesity compounds from the beta-3-adrenergic receptors agonists class. Revista de Chimie 2007; 58(10):937–940.

[42] Guță R, Putina G, Andreeescu D, Ghiță C, Ilie C, Căproiu MT, Negreș S, Chiriță C. Potential antidiabetes/antiobesity compounds from the beta-3-adrenergic receptors agonists class (II). Revista de Chimie 2012; 63(6): 565–570.

[43] Kliber A, Szkudelski T, Chichlowska J. Alloxan stimulation and subsequent inhibition of insulin release from in situ perfused rat pancreas. Journal of Physiology and Pharmacology, 1996; 47:321–328. PMID: 8807559

[44] Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes, 1997; 46:1733–1742. PMID: 9356019

[45] Heikkila RE, Winston B, Cohen G. Alloxan-induced diabetes-evidence for hydroxyl radical as a cytotoxic intermediate. Biochemical Pharmacology, 1976; 25:1085–1092.

[46] Lenzen S, Munday R. Thiol-group reactivity, lipophilicity, and stability of alloxan, its reduction products and its N-methyl derivatives and a comparison with ninhydrin. Biochemical Pharmacology, 1991; 42:1385–1391. PMID: 1930261

[47] Munday R. Generation of superoxide radical, hydrogen peroxide and hydroxyl radical during the autoxidation of N,N,N′,N′-tetramethyl-pphenylenediamine. Chemico-Biological Interactions, 1988; 65:133–143. PMID: 2835187

[48] Lenzen S. Oxidative stress: The vulnerable beta-cell. Biochemical Society Transactions, 2008; 36(Pt 3):343-347. DOI: 10.1042/BST0360343
[49] Sakurai K, Ogiso T. Effect of ferritin on ëDNA strand breaks in the reaction system of alloxan plus NADPH-cytochrome P450 reductase: Ferritin’s role in diabetogenic action of alloxan. Biological and Pharmaceutical Bulletin, 1995; 18:262–266. PMID: 7742795

[50] Takasu N, Komiya I, Asawa T. STZ and alloxan induced H₂O₂ generation and DNA fragmentation in pancreatic islets. Diabetes, 1991; 40:1141–1145. PMID: 1834504

[51] Jorns A, Munday R, Tiedge M, Lenzen S. Comparative toxicity of alloxan, N-alkylalloxans and ninhydrin to isolated pancreatic islets in vitro. Journal of Endocrinology, 1997; 155:283–293. PMID: 9415063

[52] Negreş S, Chiriţă C, Moroşan E, Arsene AL. Experimental pharmacological model of diabetes induction with alloxan in rat. Farmacia, 2013; 61(2):313–323.

[53] Negreş S, Chiriţă C, Zbârcea CE, Cristea AN, Moroşan E, Mihele D, Putina G. Experimental pharmacological researches regarding acute toxicity and the effect on baseline glycaemia of some newly synthetized beta 3 adrenergic receptors agonists. Farmacia, 2007; 6: 662–670.

[54] Negreş S, Dinu M, Ancuceanu RV, Olaru OT, Ghica MV, Ţeremot OC, Zbârcea CE, Velescu BŞ, Ștefănescu E, Chiriță C. Correlations in silico/in vitro/in vivo regarding determinating acute toxicity in non-clinical experimental trial, according to bioethical regulations enforced by European union. Farmacia, 2015; 63(6):877–885.

[55] Ansari MA, Gupta BL, Baquer NZ. Changes in insulin receptor, hexokinase and NADPH producing enzymes in Choroid plexus during experimental diabetes. Journal of Biosciences, 1993; 18(3):337–343.

[56] BugdaycI G, Altan N, Sancak B, Bukan N, Kosova F. The effect of the sulfonylurea glyburide on glutathione-S-transferase and glucose-6-phosphate dehydrogenase in streptozotocin-induced diabetic rat liver. Acta Diabetologica, 2006; 43(4):131–134. DOI: 10.1007/s00592-006-0228-0

[57] Ramesh B, Pugalendi KV. Anti hyperglycemic effect of umbelliferone in streptozotocin-diabetic rats. Journal of Medicinal Food, 2006; 9(4):562–566. DOI: 10.1089/jmf.2006.9.562

[58] Shi ST, Li YF, Guo YQ, Wang ZH. Effect of beta-3 adrenoceptor stimulation on the levels of ApoA-I, PPARα, and PPARγ in apolipoprotein E-deficient mice. Journal of Cardiovascular Pharmacology, 2014; 64(5):407–411. DOI: 10.1097/FJC.0000000000000133

[59] Wang ZH, Li YF, Guo YQ. β3-Adrenoceptor activation attenuates atherosclerotic plaque formation in ApoE(-/-) mice through lowering blood lipids and glucose. Acta Pharmacologica Sinica, 2013; 34(9):1156–1163. DOI: 10.1038/aps.2013.70
