# EXPERIMENTAL PHARMACOLOGICAL RESEARCH REGARDING THE ANTI-OBESITY EFFECT AND THE MOTOR BEHAVIOR INDUCED BY SOME NEWLY SYNTHETIZED $\beta_3$ ADRENERGIC RECEPTORS AGONISTS IN NORMAL MICE

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**Abstract**

A series of newly synthesized compounds by the Romanian National Institute of Chemical-Pharmaceutical Research and Development Bucharest were studied. These compounds are structurally similar to a series of $\beta_3$ adrenergic receptors agonists, some of which being under clinical trials. The research was directed towards investigating the effect of these compounds on the eating behavior of mice and their body mass evolution, taking into account the fact that the scientific literature already mentions some anti-obesity effects of the $\beta_3$ agonists. Experimental trials were also performed in order to observe if the new compounds influence the activity of the central nervous system. Thus was determined the motor activity. The new substances were conventionally named C1, C2, C3, C6.

From all studied compounds, C3 showed one of the highest prevention of the weight gain in mice, without influencing the motor activity.

**Rezumat**

O serie de noi compuşi sintetizaţi de către Institutul de Cercetări Chimico-Farmaceutice din Bucureşti a fost studiată. Din punct de vedere aceşti compuşi sunt asemănători din punct de vedere structural cu o serie de agonişti $\beta_3$, dintre care unii se află în faza de cercetări clinice.

Cercetarea a fost orientată spre studierea efectelor asupra comportamentului alimentar al şoarecilor şi asupra evoluţiei masei corporale ţinând cont de faptul că literatura de specialitate descrie deja anumite efecte antiobezitate ale unor agonisti $\beta_3$.

Au fost efectuate şi cercetări experimentale pentru a observa dacă noii compuşi au efect asupra sistemului nervos central, determinându-se în acest scop activitatea motorie.

Cei patru compuşi au fost denumiţi convenţional C1, C2, C3, C6. Dintre toţi compuşii, C3 a împiedicat cel mai mult creşterea în greutate a şoarecilor, fără a influenţa activitatea motorie.

**Keywords**: new $\beta_3$ agonists, obesity, motor activity.

## Introduction

Obesity is one of the most commune metabolic disorders that affect people all over the world. It is well known the relationship between obesity and some other metabolic or endocrine disorders such as diabetes mellitus or cardiovascular diseases. This knowledge has encouraged researchers to try to manipulate the lipid metabolism in order to reduce the excessive fat accumulation in the body. This might be achieved by influencing the lipolysis process.

The adrenergic system modulates lipolysis with the help of α and β receptors. These receptors have opposite effects on the lipid metabolism, especially in the white fat tissue. The α-adrenergic system mostly inhibits the lipolysis process. The stimulation of α adrenoreceptors produces an increase of the phosphatidylinositol turnover and a decrease in glycerol synthesis [7,8]. On the other hand, the β adrenoreceptors, through a newly identified subtype ($\beta_3$), are implicated in the stimulation of lipolysis, both in white and brown fat tissue, in many animal species, including humans [3,6].

Experimental pharmacological research has shown the existence of a new series of $\beta_3$ agonists that have reduced the body mass of obese laboratory animals and their level of blood glucose [10,12,13]. It was emphasized that the activation of $\beta_3$ adrenergic receptors plays a major role in the treatment of both obesity and diabetes [2,4].

Studies conducted on obese rats treated with selective $\beta_3$ agonists, have shown a significant reduction in the body mass. [11,14].

As the anti-obesity effect of some $\beta_3$ agonists has already been mentioned by the scientific literature [1,6], we have investigated the effect of one newly synthetised $\beta_3$ agonists compounds on the eating behavior and body mass, in normal non-diabetic and non-obese mice.

A series of four newly synthesized compounds by the Romanian National Institute of Chemical-Pharmaceutical Research and Development – Bucharest were studied [5]. They were conventionally named C1, C2, C3, C6. Chemically, they are substituted phenylethylamines. The doses used in the experiments were calculated taking into account the $LD_{50}$ values previously determined on mice after oral administration [9].

Considering the fact that the selectivity of these new compounds for $\beta_3$ receptors is yet to be proven, we tried to determine whether their influence on the eating behavior and body mass may be based on a $\beta_1$ central mechanism. For this reason we determined the motor activity.

**Materials and Methods**

All researches were conducted in accordance with The European Directive 86/609/EEC/24.11.1986 and The Romanian Government Ordinance 37/30.01.2002 regarding the protection of animals used for experimental and other scientific purposes

A population consisting in 72 male, white mice having reached maturity and weighing 24±4 g has been used for the purpose of this experiment. They were divided in 6 groups each containing 12 individuals, in such a way that the average initial mass/individual in each group was 24±0.5 g. After being weighed, each group was placed on sawdust bedding in a separate Plexiglas cage ventilated by an Air Handling Unit. The mice were fed with nutrient granules for rodents and had free access to water.

After a few preliminary tests regarding food consumption, the food quantity given to the mice was calculated using the formula:

*5g food/ 10g mouse/ 24h*

The mice were weighed daily, at the same time of day. The average mass in each group was calculated, and the food consumption was carefully determined. After one week of monitoring these parameters, the treatment with the new compounds was initiated as it is shown below:

Group 1 (control group) – 0.1mL/10g-bw p.o., Tween 80 suspension 0.5%
Group 2 – 20 mg/kg-bw p.o., C1 suspension 0.2%
Group 3 – 50 mg/kg-bw p.o., C2 suspension 0.5%
Group 4 – 100 mg/kg-bw p.o., C3 suspension 1%
Group 5 – 100 mg/kg-bw p.o., C6 suspension 1%
Group 6 – 0.4 mg/kg-bw p.o., hexoprenaline suspension 0.004%

All suspensions were prepared using Tween 80 as a suspension agent. Hexoprenaline was used as a reference β adrenergic agonist.

The mice were kept without food 30 minute before and after being administrated and 60 minute after being weighed.

After 7, and 14 days of daily administration, the horizontal and vertical motor activity was measured using an Ugo Basile Activity cage device. Each mouse stayed in the device 4 minutes while the sensors picked up every horizontal and vertical movement. Any change in the external look and in the social or motor behavior was carefully observed.

The statistical evaluation of the results was performed using a special software – GraphPad Prism version 5.01. This software analyses two group populations, either with normal distribution using the Student t test, either with abnormal distribution using Mann-Whitney test. The D`Agostino – Pearson and Kolmogorov-Smirnov tests were used to determine whether the population is distributed normally or abnormally.

**Results and Discussion**

***1. The effect of the new compounds on the eating behavior and body mass of normal mice***

The results on the eating behavior and body mass are showed in tables I-IV

**Table I.**

The evaluation of the eating behavior in the 1st week

| Food intake | | | | | | |
|---|---|---|---|---|---|---|
| Group | Average ± SD (initial week) | Average ± SD (1st week of treatment) | Eff% vs initial week | Student t test (p) | Eff% vs control | Student t test (p) |
| Tween | 84.31 ± 6.716 | 78.87 ± 4.060 | -6.45% | p>0.05 | - | - |
| C1 | 72.82 ± 7.232 | 68.98 ± 10.40 | -5.27% | p>0.05 | +1.18% | p>0.05 |
| C2 | 75.27 ± 8.044 | 78.51 ± 8.706 | 4.30% | p>0.05 | +10.75% | p<0.05 |
| C3 | 78.59 ± 9.045 | 80.13 ± 10.57 | 1.96% | p>0.05 | +8.41% | p>0.05 |
| C6 | 79.72 ± 7.480 | 83.07 ± 15.62 | 4.20% | p>0.05 | +10.65% | p>0.05 |
| Hexoprenaline | 85.72 ± 7.030 | 84.51 ± 7.605 | -1.41% | p>0.05 | +5.04% | p>0.05 |

**Table II.**

The evaluation of the eating behavior in the 2nd week

| Food intake | | | | | | | |
|---|---|---|---|---|---|---|---|
| Group | Average ± SD (initial week) | Average ± SD (2nd week of treatment) | Eff% vs initial week | Student t test (p) | Mann-Whitney test (p) | Eff% vs control | Student t test (p) |
| Tween | 84.31± 6.716 | 69.73 ± 10.78 | -17.29% | p>0.05 | - | - | - |
| C1 | 72.82 ± 7.232 | 66.71 ± 10.38 | -8.39% | p>0.05 | - | +8.90% | p>0.05 |
| C2 | 75.27 ± 8.044 | 72.06 ± 12.49 | -4.26% | p>0.05 | - | +13.03% | p>0.05 |
| C3 | 78.59 ± 9.045 | 63.16 ± 17.82 | -19.63% | - | p>0.05 | -2.34% | p>0.05 |
| C6 | 79.72 ± 7.480 | 68.66 ± 26.62 | -13.87% | - | p>0.05 | +3.42% | p>0.05 |
| Hexoprenaline | 85.72 ± 7.030 | 71.08 ± 9.442 | -17.08% | p<0.001 | - | +0.21% | p>0.05 |

**Table III.**

The evaluation of the body mass variation in the 1st week

| Body mass evolution | | | | | | |
|---|---|---|---|---|---|---|
| Group | Average ± SD (initial week) | Average ± SD (1st week of treatment) | Eff% vs initial week | Student t test (p) | Eff% vs control | Student t test (p) |
| Tween | 28.37 ± 2.058 | 33.45 ± 0.9545 | +17.90% | p<0.001 | - | - |
| C1 | 27.04 ± 1.654 | 30.76 ± 0.7493 | +13.76% | p<0.001 | -4.14% | p<0.05 |
| C2 | 27.57 ± 2.208 | 30.96 ± 0.7564 | +12.29% | p<0.01 | -5.61% | p<0.01 |
| C3 | 27.65 ± 1.796 | 30.72 ± 0.2911 | +11.10% | p<0.001 | -6.80% | p<0.001 |
| C6 | 27.94 ± 1.806 | 30.65 ± 0.3675 | +9.70% | p<0.01 | -8.20% | p<0.001 |
| Hexoprenaline | 27.71 ± 1.747 | 32.31 ± 1.0160 | +16.60% | p<0.001 | -1.30% | p>0.05 |

**Table IV.**

The evaluation of the body mass variation in the 2nd week

| Body mass evolution | | | | | | |
|---|---|---|---|---|---|---|
| Group | Average ± SD (initial week) | Average ± SD (2nd week of treatment) | Eff% vs initial week | Student t test (p) | Eff% vs control | Student t test (p) |
| Tween | 28.37 ± 2.058 | 35.44 ± 0.9427 | +24.90% | p<0.001 | - | - |
| C1 | 27.04 ± 1.654 | 33.52 ± 0.6667 | +23.96% | p<0.001 | -0.94% | p>0.05 |
| C2 | 27.57 ± 2.208 | 33.60 ± 0.5268 | +21.87% | p<0.001 | -3.03% | p<0.05 |
| C3 | 27.65 ± 1.796 | 31.43 ± 0.9953 | +13.67% | p<0.001 | -11.23% | p<0.001 |
| C6 | 27.94 ± 1.806 | 31.69 ± 1.1090 | +13.42% | p<0.001 | -11.48% | p<0.001 |
| Hexoprenaline | 27.71 ± 1.747 | 35.67 ± 0.7058 | +28.73% | p<0.001 | +3.83% | p>0.05 |

The graphic below shows the variation of the average masses of the animals in each group during the experiment by comparing one week with the next one:

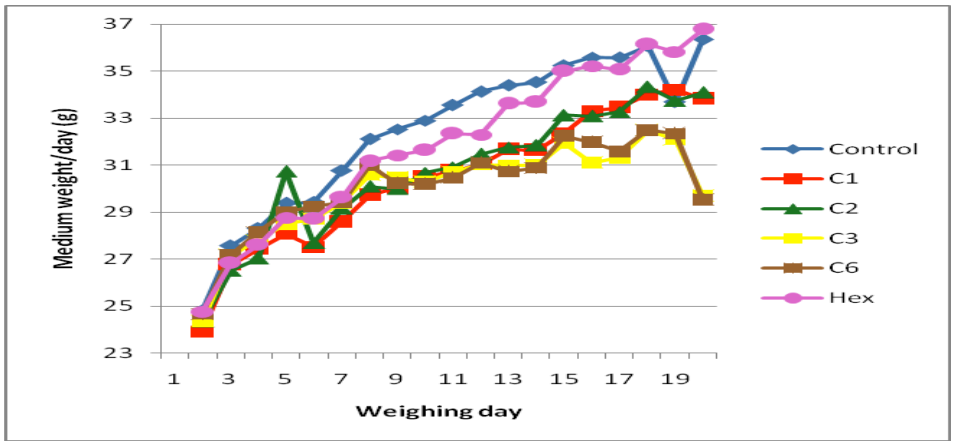


**Figure 1.**

Comparison of the body mass evolution in each group during the experiment

***2. The effect of the new compounds on the motor behavior of normal mice***

The results on the motor behavior and body mass are showed in tables V, VI.

**Table V.**

The evaluation of the motor activity/group in the 1st week

| **Horizontal motor activity (no. of movements in 4 minutes)** | | | | |
|---|---|---|---|---|
| Group | Average ± SD | Eff% vs control | Kolmogorov normality test | Student t test (p) |
| Tween | 458.7 ± 68.62 | - | YES | - |
| C1 | 541.5 ± 128.3 | +18.05% | YES | p>0.05 |

| | | | | |
|---|---|---|---|---|
| C2 | 485.4 ± 58.03 | +5.82% | YES | p>0.05 |
| C3 | 497.7 ± 51.97 | +8.5% | YES | p>0.05 |
| C6 | 540.8 ± 93.59 | +17.9% | YES | p<0.05 |
| Hexoprenaline | 419.3 ± 115.0 | -8.59% | YES | p>0.05 |
| **Vertical motor activity (no. of movements in 4 minutes)** | | | | |
| Group | Average ± SD | Eff% vs control | Kolmogorov normality test | Student t test (p) |
| Tween | 47.92 ± 21.95 | - | YES | - |
| C1 | 65.00 ± 27.97 | +35.64% | YES | p>0.05 |
| C2 | 46.25 ± 13.05 | -3.48% | YES | p>0.05 |
| C3 | 38.58 ± 12.02 | -1.95% | YES | p>0.05 |
| C6 | 48.08 ± 22.07 | +0.33% | YES | p>0.05 |
| Hexoprenaline | 47.75 ± 15.09 | -0.35% | YES | p>0.05 |

**Table VI.**

The evaluation of the motor activity/group in the 2nd week

| **Horizontal motor activity (no. of movements in 4 minutes)** | | | | |
|---|---|---|---|---|
| Group | Average ± SD | Eff% vs control | Kolmogorov normality test | Student t test (p) |
| Tween | 394.3 ± 53.30 | - | YES | - |
| C1 | 465.3 ± 141.6 | +18.00% | YES | p>0.05 |
| C2 | 436.9 ± 113.4 | +10.80% | YES | p>0.05 |
| C3 | 399.4 ± 83.28 | +1.29% | YES | p>0.05 |
| C6 | 357.5 ± 35.36 | -9.33% | YES | p>0.05 |
| Hexoprenaline | 416.7 ± 133.9 | +5.68% | YES | p>0.05 |
| **Vertical motor activity (no. of movements in 4 minutes)** | | | | |
| Group | Average ± SD | Eff% vs control | Kolmogorov normality test | Student t test (p) |
| Tween | 40.92 ± 23.86 | - | YES | - |
| C1 | 67.08 ± 36.15 | +63.93% | YES | p<0.05 |
| C2 | 48.08 ± 21.09 | +17.5% | YES | p>0.05 |
| C3 | 35.27 ± 17.37 | -13.81% | YES | p>0.05 |
| C6 | 37.27 ± 19.46 | -8.92% | YES | p>0.05 |
| Hexoprenaline | 58.00 ± 25.59 | +41.74% | YES | p>0.05 |

**Conclusions**

The activity of 4 new compounds (C1, C2, C3, C6), potential $\beta_3$ adrenergic receptors agonists, was studied.

The research was orientated towards investigating the eating behavior and the body mass evolution, taking into account the fact that a possible antiobesity effect of similar compounds was previously described in the scientific literature.

We have also investigated the effect of the new compounds on the horizontal and vertical motor activity, in order to observe if the new compounds influence the activity of the central nervous system, by a possible $\beta_1$ effect.

All four compounds prevented more or less the weight gain of the mice compared with the control group during the two weeks study. Compounds C3 and C6 had the most significant effect.

Compound C3 showed one of the highest prevention of the weight gain in mice compared with the control group (6.80% – 1st week; 11.23% – 2nd week; $p<0.001$). Because C3 didn`t influence the motor activity, we anticipate that this compound can be a selective agonist of the beta-3 receptors.

Compound C6 also reduced significantly the weight gain compared with the control group (8.20% – 1st week; 11.48% – 2nd week; $p<0.001$). Regarding the motor activity, C6 determined a significant increase in the first week (+17.9%; $p<0,05$), which, however, was not maintained in the second week.

In the case of C1, this compound also showed a prevention of the weight gain (4.14% – 1st week; $p<0.05$), but this effect can be associated with an increase in the motor activity (63.93% – 2nd week; $p<0.05$), which could suggest a central stimulation. Therefore, compound C1 might act as a non-selective beta-adrenergic agonist.

The trials showed that neither of the studied compounds altered significantly the food intake from one day to the next, during the two weeks study.

After 1 week of daily administration, the highest level of food intake compared with the control group was observed in the case of C2 (10.75%; $p<0.05$), but despite of this fact the compound prevented the weight gain.

After 2 weeks of daily administration the food intake of the mice treated with C2 was 13.03% higher than that of the control group ($p>0.05$).

The reference substance, hexoprenaline had no significant influence on the eating behavior, body mass evolution and motor activity of the mice.

This is consistent with the known selectivity of this drug on the beta-2 adrenoreceptors.

In conclusion, some of the studied compounds showed a good potential to prevent weight gain in normal mice, without affecting their food rations. This result is consistent to the idea already promoted by the scientific literature, that $\beta_3$ agonists might be used one day to prevent obesity.

**References**


1. Arch JR. Beta(3)-Adrenoceptor agonists: potential, pitfalls and progress. *Eur J Pharmacol.* 2002;440(2-3):99-107.
2. Danforth E Jr, Himms-Hagen JH. Obesity and diabetes and the beta-3 adrenergic receptor. *Eur J Endocrinol.* 1997;136(4):362-5.
3. 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.
4. Fumiki O, Hiroo T, Akane M, Satoshi A, Masayuki I, Masuo A. Adiponectin receptor 2 expression in liver and insulin resistance in db/db mice given a beta 3 adrenoreceptor agonist. *European J of Pharmacology*, 2005; 518:71-76.
5. Guţă R, Ilie C, Putină G, Nănău-Andreescu D, Negreş S, Căproiu MT, Compuşi cu potenţială activitate antidiabetică/antiobezitate din clasa receptorilor agonişti beta-3 adrenergici *Revista de Chimie* (Bucureşti), 2007, 58(10), 937-940
6. Himms-Hagen J, Cui J, Danforth E Jr, Taatjes DJ, Lang SS, Waters BL, Claus TH. Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. *Am J Physiol*. 1994;266(4 Pt 2):R1371-82
7. Hoffman BB, Lefkowitz RJ, Taylor P. The Autonomic and Somatic Motor Nervous Systems, in Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th edition, Hardman GJ, Limbird LE eds., 1996: 105-139.
8. Hoffman BB. Autonomic drugs, in Katzung GB, Basic & Clinical Pharmacology, 9th edition, 2004: 122-159.
9. Negres S, Chirita C, Zbarcea C, Cristea A.N., Morosan E., Mihele D, Putina G. Experimental pharmacological research regarding acute toxicity and effect on baseline glycaemia of some newly synthetized beta adrenergic receptors agonists. *Farmacia* 2007; 6:662-670
10. Sakura H, Togashi M, Iwamoto Y. Beta 3-adrenergic receptor agonists as anti-obese and anti-diabetic drugs. *Nihon Rinsho.* 2002;60(1):123-9
11. Tsujii S, Bray GA. A beta-3 adrenergic agonist (BRL-37,344) decreases food intake *Physiology & Behavior* 1998; 15;63(4):723-8
12. 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. *Eur J Endocrinol*., 1997;136:429.
13. Weyer C, Gautier JF, 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
14. 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.


*Manuscript received: February 1st 2011*