

**RELATION BETWEEN SEROTONIN IN THE CENTRAL
NERVOUS SYSTEM, OBESITY AND GLUCOSE
METABOLISM STUDIED WITH CENTRAL NERVOUS
SYSTEM SEROTONIN AGONIST Ro60-0175 IN OBESE
AND DIABETIC WISTAR RATS**

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Abstract

Many regulatory pathways for food intake, including those that use serotonin as a neurotransmitter, are affected by obesity or by hypercaloric diets leading to obesity. Summarizing the data known so far, it can be said that reduced serotonergic signalling and low availability of SERT are associated with hyperphagia and obesity. Because of these facts, it is important to know how serotonin mediation in the brain works and how it affects eating behaviour and glucose metabolism. Ro60-0175 is a selective serotonin agonist in the central nervous system that we use in our study. Forty Wistar rats were separated into two groups – rats with obesity and diabetes and healthy rats (control group). Each of these groups was separated into other two – one with a daily intraperitoneal injection (i.p.) of Ro60-0175 (3 mg/kg) and one without. For four weeks, we were tracking blood glucose levels, insulin secretion and rats' weight. It was shown that after the application of Ro60-0175, the weight of the rats in the diabetic and obese group decreased by 5.5% ($p < 0.05$), and by 2.56% in the control group, and there was no significant change in the groups without daily intraperitoneal injection of Ro60-0175. In the diabetic and obese rats group in which Ro60-0175 was applied, we registered a reduction of hyperglycemia by 35.4% ($p < 0.05$) comparing the results before the start of using Ro60-0175, which is greater in the rats which lost more body weight. The research also

shows decreasing of insulin resistance by 42.1% ($p < 0.01$) in diabetic and obese rats group using Ro60-0175. Using Ro60-0175 for treatment of obesity and obesity-induced diabetes in male Wistar rats, leads to significant reduction of body weight and hyperglycemia and peripheral insulin resistance.

Key words: obesity, diabetes, serotonin, central nervous system, agonist

Introduction. Many of the food intake regulatory pathways, including those using serotonin (5-hydroxytryptamine, 5-HT) as neurotransmitters, are affected by obesity or by hypercaloric diets leading to obesity. Concerning serotonin, the earliest studies from the 1970s and 1980s showed that depleting hypothalamic serotonin results in hyperphagia and obesity in rodents [1] and infusion of serotonin in the hypothalamus results in weight loss [2]. In addition, knocking out whole body SERT (sodium-dependent serotonin transporter) in female rats leads to increased abdominal fat and obesity [3] but it is unknown whether this is a purely hypothalamic effect. In humans, fenfluramine, a drug that increases serotonergic transmission, results in weight loss and reduced appetite [4], while several other serotonergic drugs such as selective serotonin reuptake inhibitors (SSRIs) and the 5-HT_{2C} receptor agonist lorcaserin result in reduced food intake and weight loss [5] although long term SSRI use is associated with (re)gain of weight [6]. Moreover, a genetic study in humans demonstrated that the short allele of the SERT gene promoter, associated with reduced transcription of the gene, is a risk factor for obesity [7]. An in vivo study demonstrated an inverse relationship between BMI (body mass index) and SERT availability in the subcortical region [8] indicating that in humans, the brain serotonin system is potentially involved in body weight regulation and might be affected in obesity.

The ability of fenfluramine and lorcaserin to reduce food intake depends mainly on 5-HT_{2C} receptors [9] and because of that selective 5-HT_{2C} receptor agonists lead to weight reduction [10]. Activation of 5-HT_{2C} receptors in the arcuate nucleus leads to the production of proopiomelanocortin (POMC) which is then cleaved into α -melanocyte stimulating hormone (α -MSH). α -MSH acts on the melanocortin 4 receptor in the paraventricular nucleus of the hypothalamus to induce satiety [10]. Because of that, 5-HT_{2C} receptor agonists can act directly in the hypothalamus and control different metabolic aspects and feeding. 5-HT_{2C} receptors located in the prefrontal cortex can also have an effect on food behaviour. Because of that 5-HT_{2C} receptor agonists have the ability to influence both 'metabolic' and 'cognitive-hedonic' aspects of feeding [11].

Several lines of evidence exist showing a relationship between central serotonin and peripheral glucose metabolism. In rodents, extracellular serotonin levels in the hypothalamic suprachiasmatic nucleus (SCN) are associated with glucose intolerance [12] and agonizing 5-HT_{2C} receptors improve insulin sensitivity while knockout of 5-HT_{2C} receptors results in hepatic insulin resistance [12]. Moreover, the latter could be reversed by re-expressing these receptors specifically in hypothalamic POMC neurons [13]. Besides, SERT deficient mice are hyper-

glycemic and hyperinsulinemic and have reduced insulin signalling in the liver before the onset of obesity [3]. In humans, genetic studies have indicated a link between the whole body's serotonin system and insulin resistance as relationships were shown with polymorphisms in the SERT promoter region [14] and in the 5-HT_{2C} receptor promoter region [15]. Also, pharmacologically antagonizing 5-HT_{2C} receptors in humans deteriorate insulin sensitivity [16] while pharmacologically agonizing the serotonin receptor improves insulin sensitivity in type 2 diabetic patients [17], and long-term pharmacological inhibition of SERT with SSRIs causes hyperglycemia and a trend towards diabetes [6].

Ro 60-0175 is a selective agonist for 5-HT_{2C} serotonin receptor subtypes. Because of this activity, we use this agent to study its effect and, respectively, the effect of 5-HT_{2C} receptor agonists, not only on body weight but also on glucose metabolism by measuring blood glucose levels, levels of immunoreactive insulin, and HOMA-index in male Wistar rats.

Materials and methods. *Experimental animals.* We used sexually mature male Wistar rats for the purposes and tasks of the experimental studies. Experiments were conducted under standard rearing, food and water conditions. The experiments were performed according to the International Guiding Principles for Animal Research, as well as the ethical principles in planning and conducting the experiments according to the Commission on Ethics of Scientific Research at Medical University – Sofia.

Investigating the role of central brain serotonin, in obese and diabetic Wistar rats, using a selective brain serotonin agonist Ro60-0175 (3 mg/kg). The in vivo experiments were carried out on forty male Wistar rats with an average weight of 240–260 g, at the time animals were taken from a central vivarium. The rats were divided into two groups – rats with induced obesity and diabetes and healthy rats (control group). Induced obesity was achieved by administering a high-calorie diet that included high-fat, high-carbohydrate food and high fructose corn syrup for 8 weeks, compared to a standard diet regime from the control group. In the obese group, all animals developed fasting blood glucose values (after at least 8 h of fasting) above 7.0 mmol/l, which is defined as impaired fasting glycemia, and 9 of them above 7.5 mmol/l, which is defined as diabetes mellitus [18]. All rats in the obese group reached a BMI (body mass index) above 0.68 g/cm², which is accepted for obesity in Wistar adult male rats [19]. All rats in the control group had a BMI between 0.48 g/cm² to 0.68 g/cm², which is defined as the normal value for adult Wistar rats [19].

BMI was determined according to the formula

$$\text{BMI} = \text{weight (g)} / \text{length}^2 \text{ (cm}^2\text{)}.$$

Each of these two groups was divided into other two – one with a daily intraperitoneal injection of Ro60-0175 in a dose of 3 mg/kg, within 4 weeks, and one without.

The animals were observed for 4 weeks. They were divided into groups of three or five per cage. The body mass of all animals was measured weekly, along with blood glucose and immunoreactive insulin.

Biochemical methods. *Determination of glucose in the blood of rats.* Blood glucose was determined from tail blood using a glucometer (FreeStyle Optium Neo, Abbott Diabetes Care Ltd., UK). The drop of blood is placed on the strip and the result is obtained after 3 s.

Determination of insulin resistance by calculating the HOMA-index. To calculate the insulin sensitivity in the experimental animals, HOMA-index (Homeostatic Model for the Assessment of Insulin Sensitivity) was used. Assessment data are based on results from physiological studies that describe the feedback loop of glucose regulation and insulin secretion. The mathematical formula created in this way allows us to calculate the function of the beta cells and from there to determine the insulin resistance.

The index is calculated according to the following formula:

$$\text{HOMA} = (\text{Glucose} \times \text{Insulin}) / 22.5.$$

ELISA (enzyme immunosorbent assay) method. ELISA method for the determination of rat insulin is an immunological method for the quantitative determination of insulin in rat serum.

Statistical method. Data from all experiments were analyzed with SPSS software, v. 13. The obtained results were processed statistically and presented as mean value $\pm$ mean error using the methods of variance analysis (one-way ANOVA) and presented graphically using the statistical program SPSS 28.0.0.0 and Microsoft Excel, 2003.

Descriptive methods. Analysis of Variance of Quantitative Variables – Mean, Standard Deviation, Standard Error of Mean at 95% Confidence Interval; graphical representation of the data.

Statistical hypothesis testing methods. The level of significance used to reject the null hypothesis was $p < 0.05$, $p < 0.01$, or $p < 0.001$. Parametric (with normal data distribution): Student *t*-test for two independent samples – to compare means between two independent groups. Non-parametric – (with normal data distribution): Kolmogorov–Smirnov method for testing the normality of the distribution of a quantitative variable. Hypothesis testing methods were used: Student *t*-test parametric method for comparing two mean values at a significance level of $p < 0.05$ or $p < 0.001$.

Results. Effect on body weight in obese and diabetic Wistar rats, using a selective brain serotonin agonist Ro60-0175 (3 mg/kg). The body weight of the experimental animals was monitored weekly in all groups. From the measurements, a significant decrease in body weight was found in the groups with daily intraperitoneal administration of Ro60-0175 (3 mg/kg). In group 1 – the

group with obesity and diabetes with the daily intraperitoneal application of Ro60-0175 (3 mg/kg), a significant reduction in body weight was observed at the end of the four-week study by 5.5%, $p < 0.05^*$ from 460 g in the beginning to 446 g. There is also a statistically significant difference when comparing the body weight at the end of the study between group 1 and group 2, $p < 0.01^{**}$. In addition, a reduction in body weight was also observed in the control group with the intraperitoneal application of Ro60-0175 (3 mg/kg) – group 3, with 1.3%, from 360 g in the beginning to 355 g at week four, which was not a statistically significant change. Comparing the two control groups 3 and 4, on the fourth week, a statistically significant difference in body weight values was found between the group using Ro60-0175 (3 mg/kg) and the one without daily application ($p < 0.05^*$) (Fig. 1).

Effect on glucose levels in obese and diabetic Wistar rats, using a selective brain serotonin agonist Ro60-0175 (3 mg/kg). A decrease in the fasting blood glucose levels was found in group 1 – rats with obesity and diabetes with the daily intraperitoneal application of Ro60-0175 (3 mg/kg). The mean blood glucose level at the beginning of the study in group 1 was 11.4 mmol/l, and the reduction in levels of blood glucose at the end of the four-week follow-up was 15.4% to 9.64 mmol/l, which is a statistically significant decrease, $p < 0.05^*$. The difference in blood glucose levels between group 1 and group 2, the groups in which at the beginning of the study were obese and with impaired carbohydrate metabolism, at the end of the study was statistically significant, $p < 0.05^*$. No statistically significant decrease in blood sugar levels was observed in the other three groups. During the study period, no hypoglycemic levels were recorded in any of the groups (Fig. 2).

Effect on the levels of immunoreactive insulin and insulin resistance, calculated by the HOMA-index, in obese and diabetic Wistar

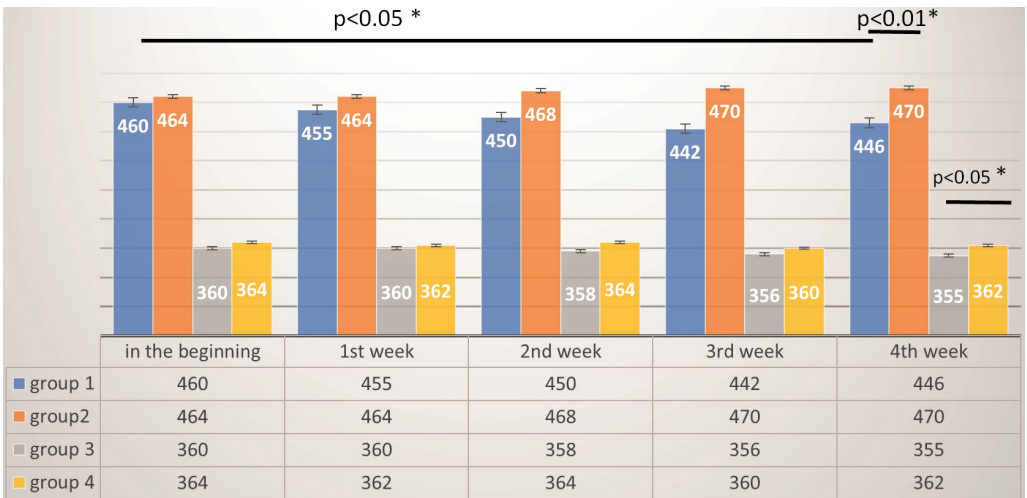


Fig. 1. Average body weight loss, measured in grams, in the different groups, presented by weeks

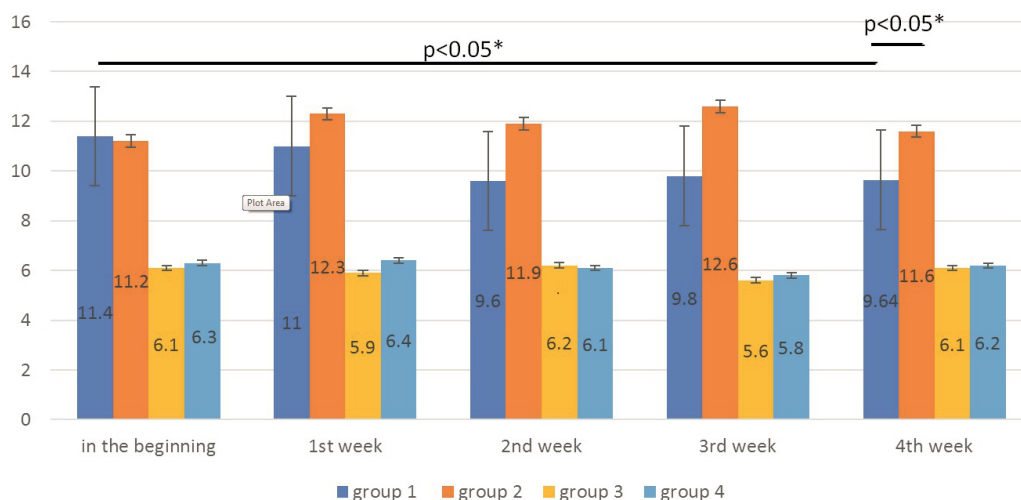


Fig. 2. Average levels of blood glucose, measured in mmol/l every week in the different groups

rats, using a selective brain serotonin agonist Ro60-0175 (3 mg/kg). Levels of immunoreactive insulin in the different groups were monitored weekly. A significant decrease in immunoreactive insulin was found in group 1, the group with obesity and diabetes with the daily intraperitoneal application of Ro60-0175 (3 mg/kg) by 29.4%, $p < 0.01^{**}$ from 18.0 mIU/ml at the beginning to 12.4 mIU/ml at week four (Fig. 3). In addition, a statistically significant difference was also found when comparing the values of immunoreactive insulin in groups 1 and 2, $p < 0.01^{**}$ in the fourth week (Fig. 3). No significant changes were observed in the level of immunoreactive insulin in the other groups (Fig. 3). The same constellation was observed in the follow-up of the HOMA-index, with a

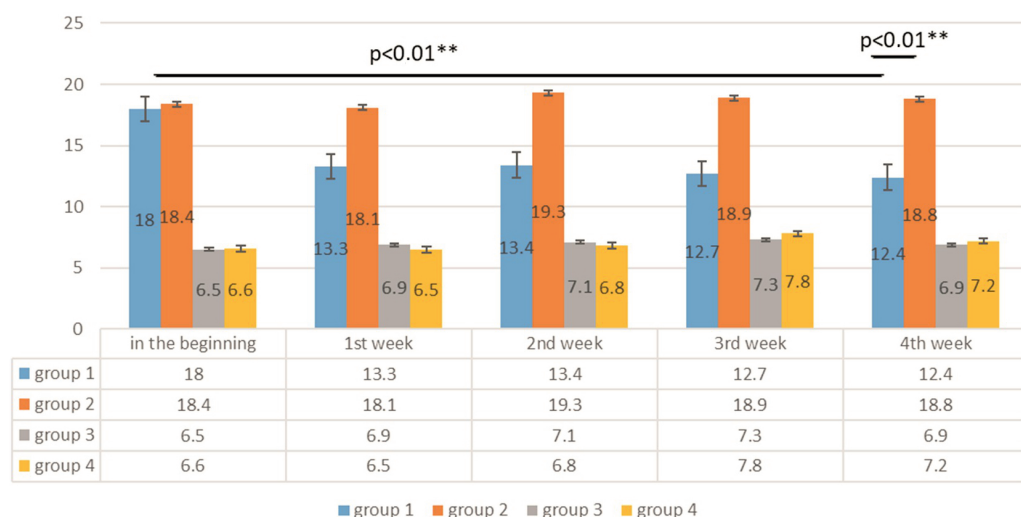


Fig. 3. Average levels of immunoreactive insulin, measured in mIU/ml during the study

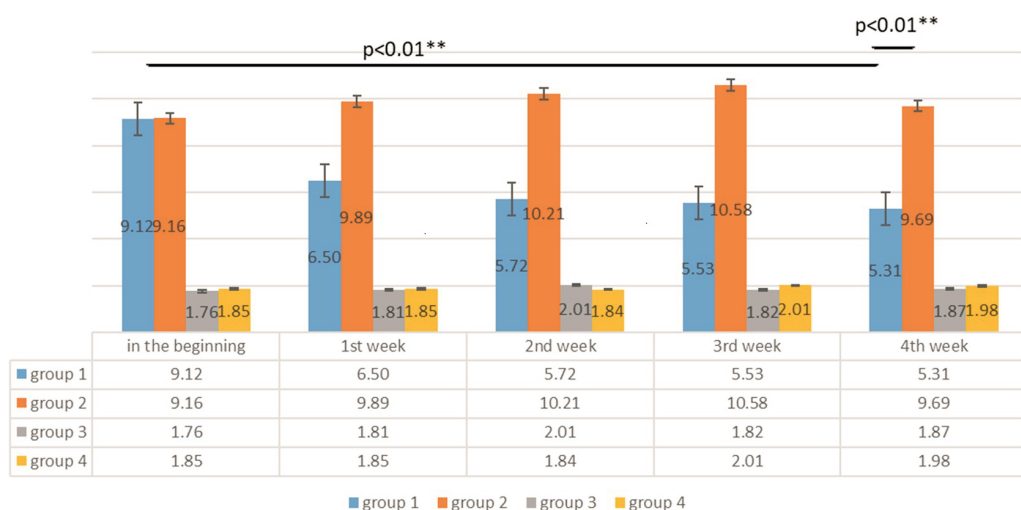


Fig. 4. HOMA-index levels during the study

statistically significant difference in HOMA-index values between groups 1 and 2 at the end of the 4-week observation period, which speaks of a decrease in insulin resistance values in the group using the central brain serotonin agonist (Fig. 4). This was due to the decreased blood glucose levels described above and the decreased immunoreactive insulin levels.

Discussion. Central brain serotonin mediation has long been the focus of scientific interest in therapeutic strategies for obesity treatment and appetite regulation. Many different agents that affect the central brain regulation of appetite have been studied over the years. Lorcaserin, a 5-HT_{2C} receptor agonist, for example, is an FDA-approved drug for the treatment of obesity. The search for new molecules with appetite control effects related to serotonin mediation in the central nervous system continues.

Ro60-0175 is a selective agonist for 5-HT_{2C} serotonin receptor subtypes that we used in our study. In the shown experiments, a significant reduction in body weight was found in male Wistar rats with induced obesity and type 2 diabetes after intraperitoneal administration of Ro60-0175 (3 mg/kg), which correlates with the data available so far from the world literature. However, until now, the effect of selective serotonin agonists on blood glucose levels and insulin resistance has not been studied, or data from such studies have been presented rather as an observed effect without being systematically reviewed.

A significant reduction in blood glucose levels was found in obese and diabetic male Wistar rats with daily intraperitoneal application of Ro60-0175 (3 mg/kg). In the control groups using Ro60-0175 (3 mg/kg), no hypoglycemic values were recorded, suggesting that the reduction of blood glucose levels is in a glucose-dependent manner. Here, the main question is whether a reduction in blood glucose is due to a reduction in the food intake from the experimental animals, or

whether there are other mechanisms that can lead to a correction in carbohydrate metabolism disorders.

When examining the levels of immunoreactive insulin and HOMA-index, a significant reduction was also observed in the groups using central brain selective serotonin agonists and with baseline insulin resistance. The reduction of the values of immunoreactive insulin and of the HOMA-index correlates with the reduction of body weight. The question is, whether this reduction is a consequence of food intake restriction, a phenomenon that was observed in the groups using Ro60-0175 (3 mg/kg), or whether this reduction in insulin levels has another biochemical and physiological basis that should be investigated.

Examining serotonin mediation through the prism of metabolic disorders is a topic that will be relevant in the next few years. The numerous pieces of evidence about the biochemical, physiological and pathophysiological processes in which serotonin mediation is involved are a main point for the search for new pharmacological agents affecting these processes. In our research, for the first time, we systematically presented the action of central brain serotonin mediation, regarding its importance in metabolic processes. Of course, more detailed research is needed on the action of serotonin, both in the periphery and in the central nervous system, in order to be able to make new classes of medications for the treatment of diabetes, dyslipidemia, obesity, and other metabolic disorders.

Conclusions. In this study Ro60-0175 (3 mg/kg) was used for the treatment of obesity and obesity-induced diabetes in male Wistar rats. Daily intraperitoneal application of Ro60-0175 (3 mg/kg) significantly reduces body weight, hyperglycemia and peripheral insulin resistance in obese and diabetic Wistar rats. A reduction of body weight was registered not only in the obese and diabetic rats group but also in the control group.

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