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The correction of the metabolic parameters of msg-induced obesity in rats by 2-[4-(benzyloxy) phenoxy] acetic acid

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ABSTRACT

Epidemiological data showed that the number of obese people increases swiftly in all countries. Obesity can evoke metabolic syndrome or second type diabetes (T2D). So, the aim of our study was to investigate the influence of 2-[4-(benzyloxy) phenoxy] acetic acid on metabolic parameters of monosodium glutamate (MSG)-induced obesity in rats. We divided the rats as follows: 1- control group, 2 - MSG-group, 3 - MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. We investigated anthropometric parameters and blood biochemistry. It was established that MSG induced the development of visceral obesity in rats, in particular, it increased the Lee index, body mass index, deposits of subcutaneous, gonadal and visceral adipose tissue. The administration of 2-[4-(benzyloxy) phenoxy] acetic acid decreased metabolic parameters evoked by MSG. After obesity induction, there was recorded significant growth of cholesterol, triglycerides, and LDL cholesterol blood levels and significant decline in HDL cholesterol blood levels. There was a significant reduction in triglycerides, LDL cholesterol and VLDL, in 2-[4-(benzyloxy) phenoxy] acetic acid - treated group. Our results represent the basis for development of new treatment of obesity and associated conditions.

1. Introduction

The World Health Organization (WHO) [41] reported that overweight and obesity cause 2.8 million people deaths and 35.8 million people (about 2.3%) of disability-adjusted life-years. Globally, the prevalence of overweight and obesity has more than doubled since 1980 [40]. In 2016, more than 1.9 billion adults were either overweight or obese; of whom more than 650 million were obese [40]. Overweight and obesity is associated with a higher prevalence of cancer, respiratory disease, osteoarthritis, hypertension, dyslipidemia, cardiovascular morbidity and type 2 diabetes [6,7,16,23].

The study of the pathogenesis and therapy of obesity are being conducted with the use of appropriate animal models. Animal models of obesity can be partitioned into different categories. To screen obesity and its physiological effects one can use the models of hypothalamic obesity, diet-induced obesity, genetic, chemical agents-induced obesity, drug-induced obesity and surgical model [37]. Ventromedial hypothalamic nucleus (VMH) can be achieved by bilateral destruction of the hypothalamic nuclei using monosodium glutamate (MSG) [38]. So, MSG induces hypothalamic damage when given during neonatal period, leading to stunted growth and obesity. MSG is widely distributed and is naturally occurring in various foods [28]. Now, in Ukraine, it is hard to find industrially produced canned or semi-finished products that do not include MSG. In 2000 The MSG became a legal food additive in 2000 (Resolution of Cabinet of Ministers of Ukraine No 342, 2000) [1]. In this regard, the permissible limits of MSG in the food products might be significantly exceeded, which can lead to various diseases, including obesity.

Therefore, in this study we used a model of MSG-induced obesity in rats. MSG can be administered (4–10 doses) subcutaneously or intraperitoneally to the neonatal rats to induce the obesity, which causes increase in regular food intake and leads to metabolic disorder which increases the glucose, triglyceride, insulin and leptin blood levels.

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Most of medications that have been used to manage obesity are now withdrawn due to their serious adverse effects [17]. Orlistat is the only Food and Drug Administration (FDA) approved drug for long-term treatment of obesity but this drug has undesirable gastrointestinal side effects [8]. All modern industry and academic programs focus on developing potent and selective synthetic agonists of FFA1 (long-chain free fatty acid receptor 1, previously known as GPR40). FFA1 is considered as a new potential remedy for treatment of type 2 diabetes. FFA1 is widely represented in β-cells of pancreas and enhances glucose-stimulated insulin secretion. But it does not influence the insulin secretion at low glucose levels. This mechanism provides a potentially safe and efficient strategy for increasing insulin levels in patients suffering from type 2 diabetes without the risk of hypoglycemia [39]. As obesity is associated with type 2 diabetes through its ability to provoke insulin resistance leading to glucose intolerance and dyslipidemia it is important to study the application of selective synthetic agonist of FFA1. 2-[4-(benzyloxy) phenoxy] acetic acid is a newly synthesized substance to meet this need.

The purpose of our investigation is to study the influence of 2-[4-(benzyloxy) phenoxy] acetic acid on metabolic parameters of MSG-induced obesity in rats.

2. Material and methods

2.1. Ethics committee statement

The investigation was carried out in accordance with the standards of the Convention on Bioethics of the Council of Europe, 'Europe Convention for the Protection of Vertebrate Animals' used for Experimental and Other Scientific Purposes' (1997), the general ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001) and Other International Agreements and National Legislation in this field. The animals were kept in a vivarium that was accredited in accordance with the 'standard rules on ordering, equipment and maintenance of experimental biological clinics (vivarium)'. All equipment, which was used for research, had been subjected to metrological control.

2.2. Animals and housing conditions

In our investigation 18 newly born male rats were included, and were divided into 3 groups of 6 animals each. The animals of each study group were individually housed in polypropylene cages in an environmentally controlled clean air room, with a temperature of 22±3C, a 12 h light/12 h dark cycle and at relative humidity of 60 ± 5%.

2.3. Highly effective screening for finding G-protein receptor agonists (GPR40)

In order to develop innovative anti-diabetic drugs using highly effective screening in cellular systems, it is expected to identify low molecular weight organic compounds that are agonists of one or more representatives of a new group of pharmacological target-receptors conjugated to G-proteins (G-proteins -coupled receptors, GPCR). The most promising among these receptors is GPR40, which is expressed in beta-cells of the pancreas and regulates the formation and secretion of insulin in the blood [15,27]. Most known endogenous ligands of these receptors and some synthetic low molecular weight GPR40 ligands have a positive effect in cell and animal models of diabetes.

Primary samples were created using virtual chemistry screening methods, followed by experimental testing in specialized cell-based test systems. For the experimental identification of the agonist activity of the compounds on the GPR40 receptor, a screening assay based on the FLIPR (Fluorometric Imaging Plate Reader, Molecular Devices, USA) technology was developed and validated using reference agonists. Below is a brief description of the screening methodology in both test systems and the illustration of some of the results.

2.3.1. Cultivation of CHO-GPR40 cells (CHO cell line clones that have been transfected with this G protein-coupled receptor)

CHO-GPR40 cells were cultivated using 50% DMEM (E15-009 PAA), 50% F12 (E15-016 PAA), 10% calf serum (B15-001 PAA), 2 mM glutamine (M11-004 PAA), 1% penicillin and streptomycin (P11-010 PAA), 400 mcg/ml G418 (G8168 Sigma), 1% NEAA (M7145 Sigma), 10 mM HEPES (54457, Sigma). Cells were digested 1: 8 (2 × 104 cells/cm 2) with culture confidentiality 70–80% using trypsin/ETAA; 5% CO2; 37 °C.

2.3.2. Registration of fluorescence change in CHO-GPR40 cells

CHO-GPR40 at a concentration of 5 × 105 per ml was cultivated in a 384 well plate (25 μl per well) for 16–20 h. The activation of GPR40 was recorded using Fluorescence Fluorescence Fluorescence Dye Fluo-4 and FLIPR® Tetra (Molecular Devices, USA). To register the change in fluorescence, the maxima of the absorption spectra of 470–495 nm and the emission of 515–575 nm were used. After incubating Fluo-4 fluorescent dye cells for 1 h, the plates were transferred to the device for simultaneous addition of the test substances and reading the FLIPR Tetra signal. In the positive control, the GPR40 receptor was activated by the addition of the reference

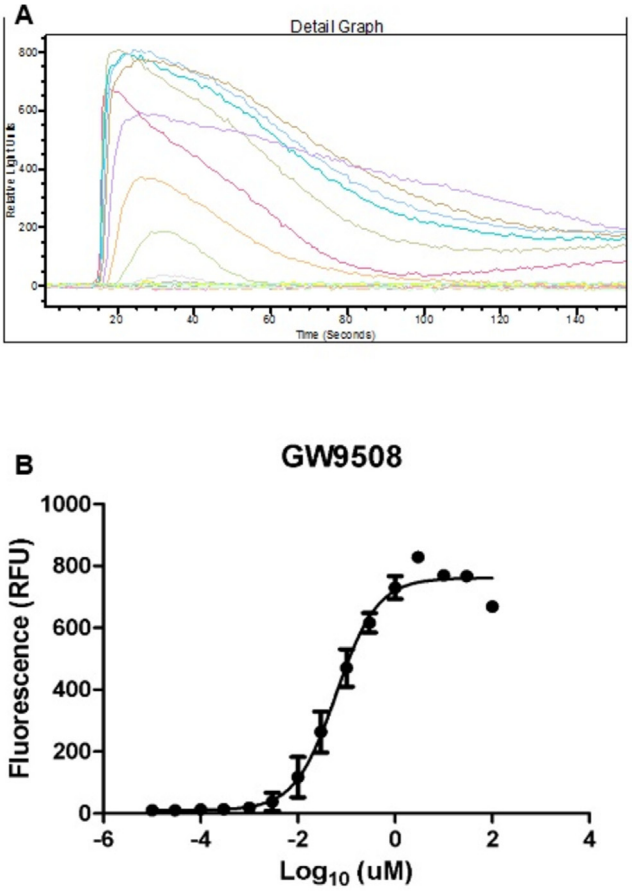

Fig. 1. Activation of GPR40 with a solution of NBSS Buffer containing GW9508 (A). The steady-state curve of the GPR40 (B).

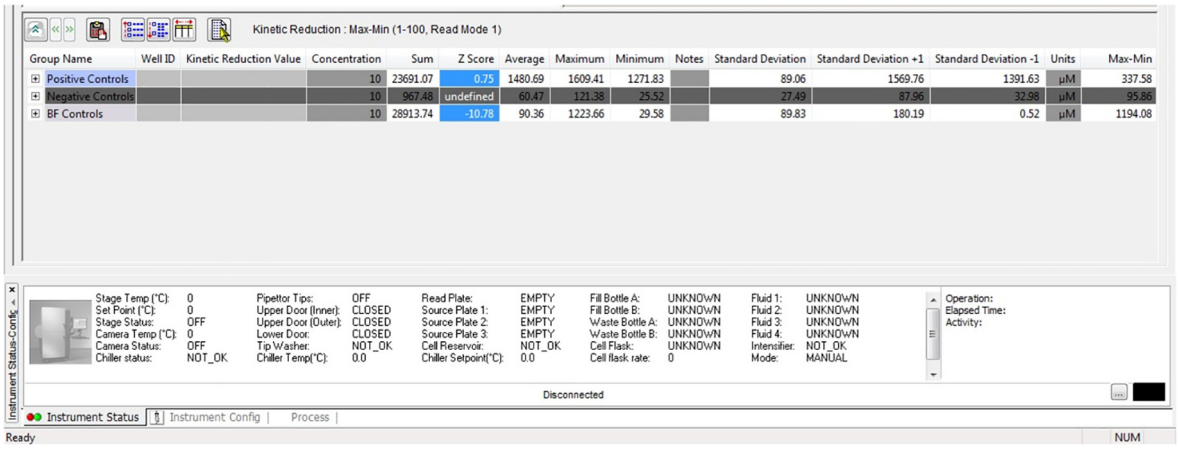

Fig. 2. Activation of GPR40 with a solution of the NBSS buffer containing GW9508 (blue), negative control (indicated by green), and potential activators (indicated by gray). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compound GW9508 (a potent GPR40 receptor agonist developed by the pharmaceutical company GlaxoSmithKline, USA) dissolved in the buffer of HVSS (H15-002 RAA). The signal was recorded in the range of 100–2500 RFU (relative fluorescence units).

2.3.3. Activation of CHO-GPR40

The activation results of the CHO-GPR40 solution of the HVSS buffer containing GW9508 (0.000003–100 µM) are presented in Fig. 1.

In Fig. 2 shows a partial illustration of a FLIPR screening method for 4983 chemical compounds of the primary screening sample in one of the 384-well microplate plates in which the CHO cells that were expressing the GPR40 were cultured. As a positive control, the NVSS buffer containing 40 µM GW9508 [[5,12,43]] was used, and the negative control contained only the buffer of the NVSS.

The detected potential activators were subsequently used to confirm the dose-effect curve. As a result of screening on the GPR40 receptor several different series of chemical compounds were obtained that activated GPR40 and thus caused the release of Ca2 + ions in CHO-GPR40 cells including 2-[4-(benzyloxy) phenoxy] acetic acid.

2.4. Synthesis Scheme (Fig. 3)

2.4.1. Synthesis procedure

Monobenzyl ether of hydroquinone (5 mmol, **Z1**) was dissolved in 20 ml of dry acetone followed by addition of 7.5 mmol of dry K_2CO_3. Then, α-bromoacetic acid ethyl ester (5.5 mmol) was added dropwise (**Z2**) and the mixture was refluxed for 4 h. The solvent was removed using rotary evaporator, and the residue was crystallized from ethanol, Yield: 1.35 g. (96%, **Z3**).

Compound **Z3** (1.35 g, 4.8 mmol) was dissolved with stirring in 10 ml of 30% aqueous solution of NaOH at 50°C. After **Z3** was completely dissolved, the reaction mixture was stirred for 1 h then acidified with 10% solution of HCl to pH = 2. The formed precipitate was filtered off, washed with water (3 × 10 ml), dried on air and crystallized from ethanol. Yield: 1.15 g (93%, 2-[4-(benzyloxy) phenoxy] acetic acid).

^{1}H NMR (400 MHz, DMSO-d_6): 4.62 (s, 2H, CH_2), 5.05 (s, 2H, CH_2), 6.84 (d, 2H, Ar), 6.94 (d, 2H, Ar), 7.38 (m, 5H, Ar). LC-MS (ESI): calculated for $C_{15}H_{13}O_4$, 258.3 $[M-H]^-$, found, 257.2. Spectra of 2-[4-(benzyloxy) phenoxy] acetic acid show on Fig. 4.

2.4.2. Study design

Newborn rats of MSG-group were injected subcutaneously (s.c.) in cervical region with MSG at a dose of 4 g/kg body weight (b.w.) dissolved in normal saline on alternate days 5 times, i.e., on postnatal day 2, 4, 6, 8 and 10, respectively [18,19]. Newborn rats of control group were administered with saline subcutaneously in the volume of 8 µl/g at 2nd, 4th, 6th, 8th and 10th postnatal days. Within 4 months after birth the rats were housed in polypropylene cages under controlled conditions and had free access to commercial pellet diet and water ad libitum. Six normal and 12 MSG-treated rats were used and were divided into groups of 6 rats in each as follows:

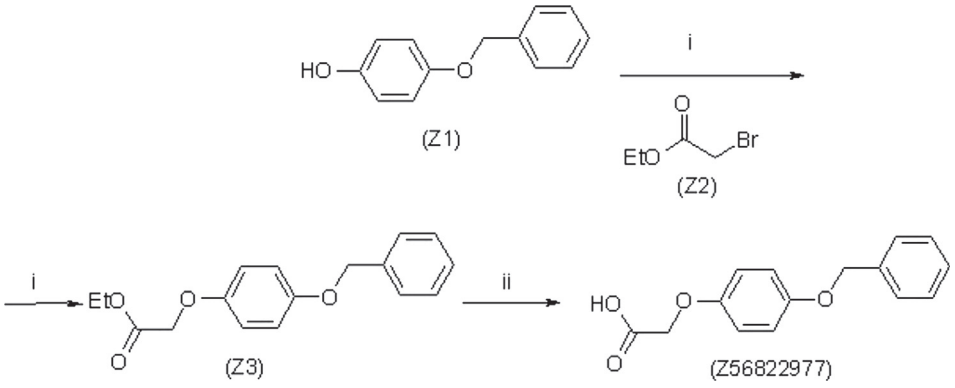

Fig. 3. Synthesis Scheme of 2-[4-(benzyloxy)phenoxy]acetic acid.

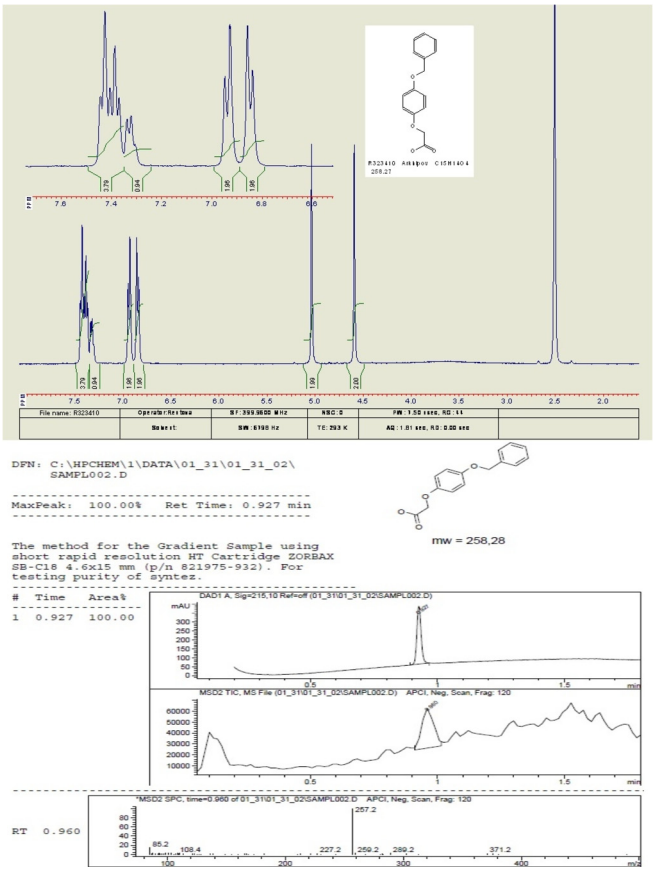

Fig. 4. Spectra of 2-[4-(benzyloxy) phenoxy] acetic acid.

Group I: Normal control rats administered normal saline [1ml/kg b.w., per os (p.o.)] - (intact rats);
Group II: MSG control rats administered normal saline [1 ml/kg b.w., per os (p.o.)] - (MSG-group);
Group III: MSG-treated rats administered -[4-(benzyloxy) phenoxy] acetic acid [25 mg/kg b.w., at volume 1 ml/kg per os (p.o.)] (MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group).

Administration was started at the end of the 4th week after birth and continued intermittently by alternating a one-week course with 3-weeks intervals of non-treatment. Within 4 months after birth rats were on a normal diet. All parameters of rats were measured at the age of 4months.

The doses of 2-[4-(benzyloxy) phenoxy] acetic acid (25 mg/kg b.w.) were selected on the basis of our unpublished preliminary studies of ED50.

2.4.3. Anthropometric measurements and obesity parameters assessment

During 4 months after birth we analyzed the changes of body weight in all groups. In adult age, rats from three experimental groups were weighed and sacrificed. We weighed all animals, measured their nose-to-anus length and calculated body mass index based on the formula below [29].

$$\text{Body mass index (BMI)} = \text{body weight (g)}/(\text{nose-to-anus length})^2\ (\text{cm}^2)$$

For each animal at month 4 after birth we determined the obesity status using Lee index [4].

$$\text{Lee index} = \text{cube root of body weight (g)}/\text{nose-to-anus length (cm)}.$$

2.4.4. Sample collection and blood biochemistry analysis

After the completion of the experimental period the animals were sacrificed. Rats of all groups were fasted for approximately 12 h prior sacrifice. The rats' blood was collected in tubes. Blood samples were kept at a temperature of 37 °C for at least 30 min and centrifuged for 15 min at 1000 × g, followed by collecting of serum.

After the collection of blood, the rats were sacrificed; the organs (heart, liver, kidneys, spleen, thymus, adrenals) and visceral adipose tissue (VAT) (mesenteric, epididymal and retroperitoneal) were removed, rinsed in ice cold physiological saline, and weighed. The serum and tissue samples were stored at −70 °C until analysis.

Liver function markers, such as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), indirect and direct bilirubin, triglycerides (TGs), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) were estimated by biochemical analyzer Microlab 300 (Elitech, France) and commercial kits from Elitech diagnostic (France) according to the standard protocols provided by manufacturers. Very low density lipoprotein cholesterol (VLDL-C) level was estimated using Friedewald's equation: VLDL-C = TGs/2,22 [10].

2.5. Statistical analysis

Statistical analysis performed by using Statistica 7 software. All data in this study were expressed as mean ± standard error (M ± SEM). Data distribution was analyzed using the Kolmogorov-Smirnov normality test. Continuous variables with parametric distribution were analyzed using Analysis of Variance (ANOVA) and if the results were significant, a post-hoc Turkey's test was performed. For data with non-parametric distribution Kruskall-Wallis and post-hoc Tukey's test were conducted for multiple comparisons. For comparisons of categorical variables, we conducted ×2 test. The difference between groups was defined as statistically significant when a p-value was less than 0.05.

3. Results and discussion

In our study, rats which were neonatally treated with MSG showed obesity with increased BMI and Lee's index. Also we observed decreased body weight, and massive amount of accumulated body fat. These observations are analogous with the results of earlier studies [2,20,24,25,26,33]. According to literature, the linear growth decreases in MSG-obese rats are attributed to depressed activity of growth and sexual hormones, leading to reduction in body weight as well as body length [42]. Our data is consistent with several reports, which described that MSG obese rats exhibited excessive visceral fat accumulation despite normophagia or hypophagia [3]; [14].

Fig. 5 shows our results of body weight measurements in rats. It was found that after 4 months of an experiment weight of the animals in the control group was 385,5 ± 28,402 g. In the group of rats injected with MSG, body weight was 339,1 ± 18,755 g. In our previous studies, analysis of the concentration of leptin in adipose tissue in rats that were administered in the neonatal period MSG showed an increase in this parameter compared to that of the intact animals [21,35]. According to the literature, the introduction of MSG leads to destruction of the ventromedial and arcuate nuclei of the hypothalamus in the newborn rats. This leads to interruption of leptin and insulin signals in this area, which results in

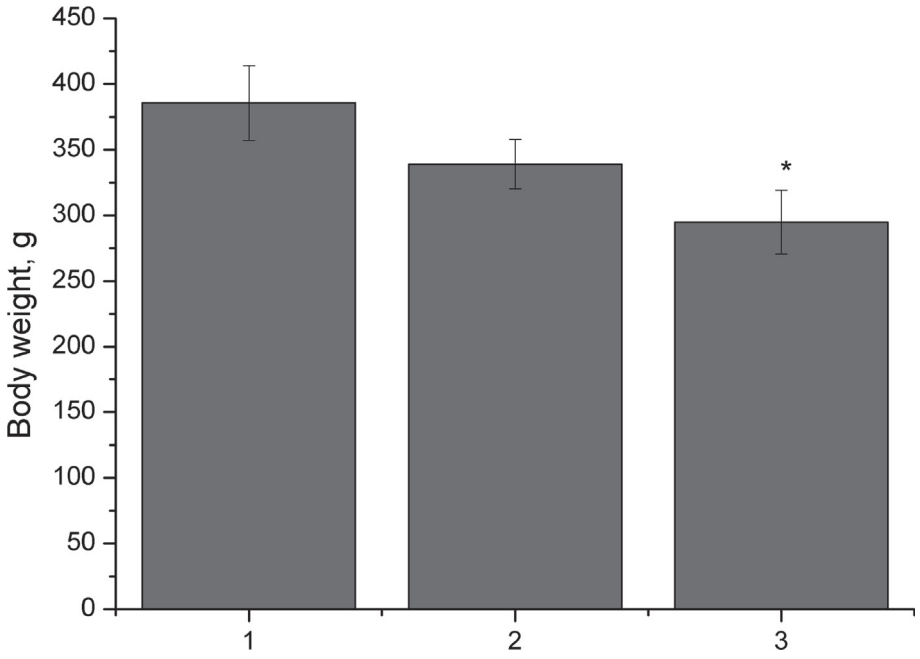

Fig. 5. Body weight in 4-month age rats in the condition of MSG-induced obesity and treatment with 2-[4-(benzyloxy) phenoxy] acetic acid (M ± SEM, n = 6 in each group). 1– intact rats, 2– MSG-group, 3– MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. *p < 0.05 compared with intact rats.

hyperinsulinemia and hyperleptynemia [30]. Under physiological conditions, leptin causes a decrease in food intake and body weight by acting on the hypothalamus [31], respectively.

After treatment with 2-[4-(benzyloxy) phenoxy] acetic acid body weight was significantly lower in comparison with control animals by 23.4% ($p < 0.05$) and reached 294,8 ± 24,383 g (Fig. 5). We revealed that the body mass of MSG + 2-[4-(benzyloxy) phenoxy] acetic acid rats did not differ significantly from the MSG rats.

Along with checking body weight we measured nose-to-anus length of animals in all research groups (Fig. 6). We observed the decrease of body length of MSG rats by 14% ($p < 0.05$) as compared with control animals (Fig. 6). The literature data shows that these body size changes may be related with the concentration of neuropeptide Y or its receptor in the hypothalamus [36], or with the content of growth hormone and growth hormone-releasing hormone [22].

But it was established that the treatment with 2-[4-(benzyloxy) phenoxy] acetic acid decreased the body length by 11.6% ($p < 0.05$) compared with control group.

The calculation of Lee index suggested the development of

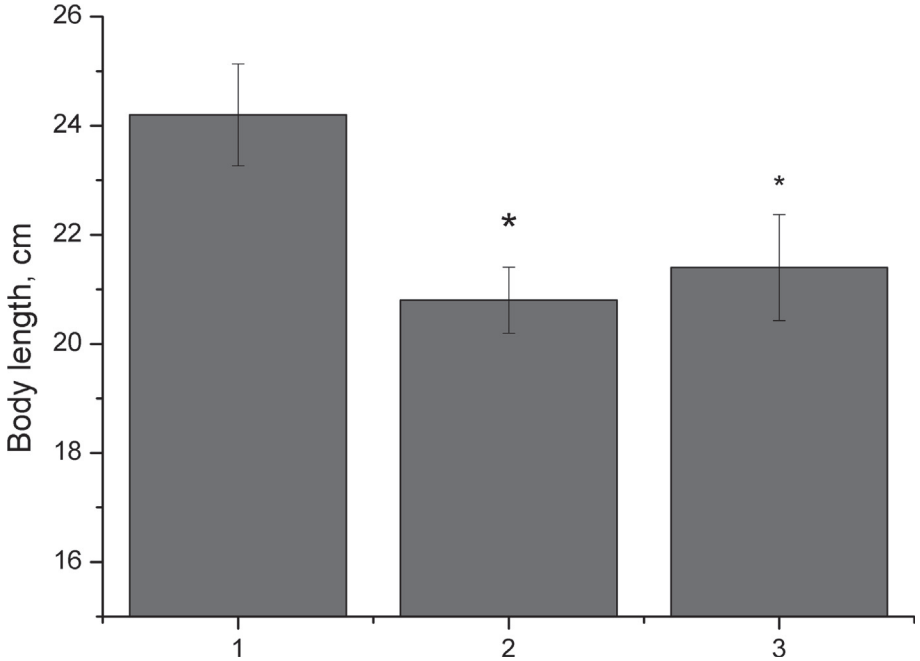

Fig. 6. Nose-to-anus length in 4-month age rats in the condition of MSG-induced obesity and treatment with 2-[4-(benzyloxy) phenoxy] acetic acid (M ± SEM, n = 6 in each group). 1–intact rats, 2– MSG-group, 3–MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. *p < 0.05 compared with intact rats.

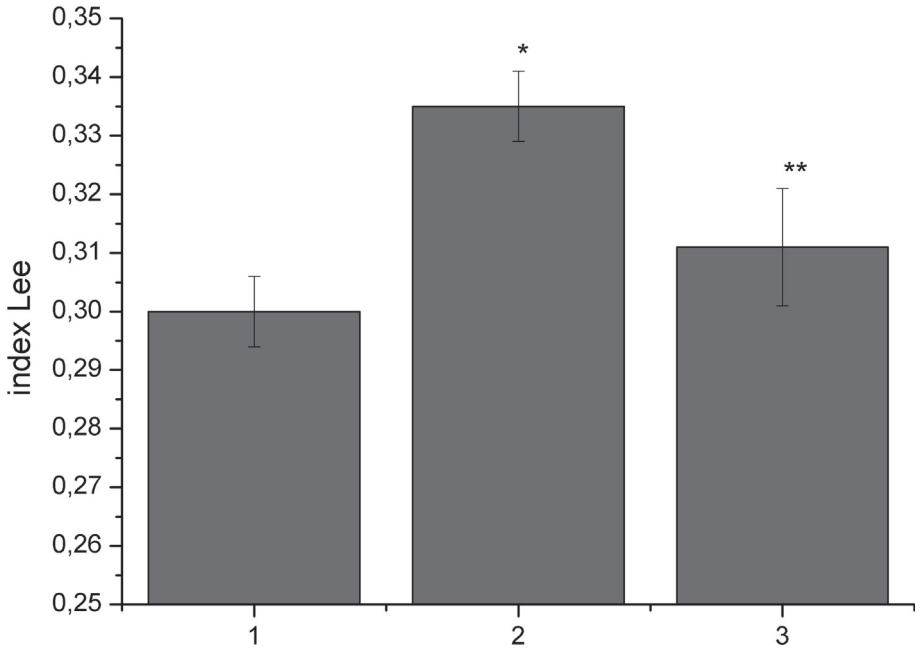

Fig. 7. Lee's index in 4-month age rats in the condition of MSG-induced obesity and treatment with 2-[4-(benzyloxy) phenoxy] acetic acid (M ± SEM, n = 6 in each group). 1—intact rats, 2— MSG-group, 3—MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. *p < 0.05 compared with intact rats. **p < 0.05 compared with MSG-group.

obesity in group 2 (MSG-group). This index was 0,335; 11,67% more compared to the control group. After administration of 2-[4-(benzyloxy) phenoxy] acetic acid led to significant reduction in Lee index compared with MSG-group (Fig. 7).

There were significant BMI differences found between the negative control group and the obesity-induced group (MSG-group) (Fig. 8). Our results are consistent with literature data that have shown the higher body mass in MSG group of rats, as inferred from Lee Index calculation [13]. There was BMI reduction detected in MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. After treating the obese animals body mass index decreased by 17.7% compared to MSG-group ($p < 0.05$). So, 2-[4-(benzyloxy) phenoxy] acetic acid restored the BMI and Lee Index to the control value. In the present study, 2-[4-(benzyloxy) phenoxy] acetic acid exhibited a significant decrease in body weight, Lee's index and BMI in MSG obese rats; suggesting a weight reducing effect.

Such metabolic alterations as lower metabolic rate in MSG-obese rats and adiposity in rats are due to reduction of sympathetic activity and low hormone sensitive lipase activity could be justified by the high depots of accumulated fat and reduced energy expenditure of these animals [11]. The significant increase in the mass of adipose tissue was also observed in animals injected with

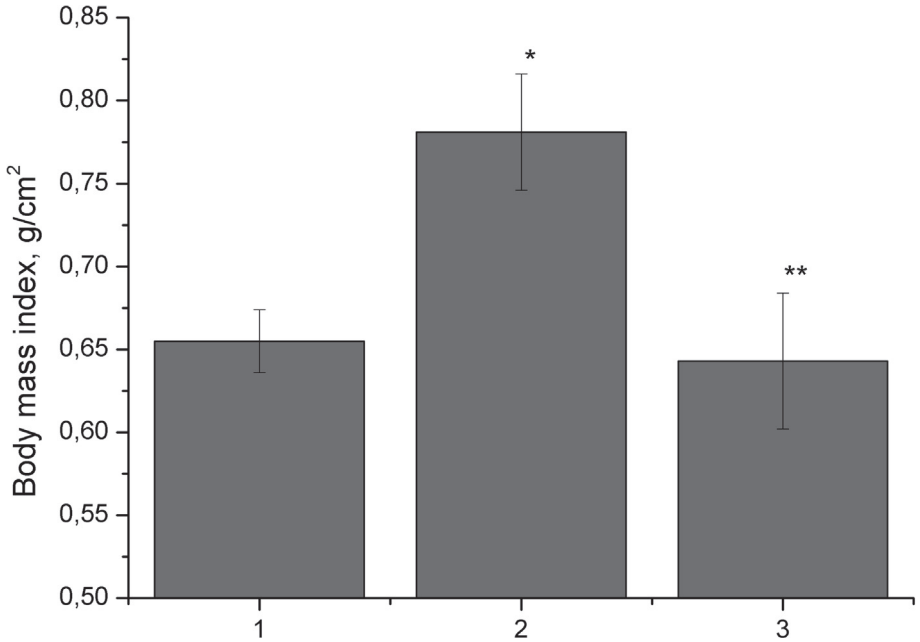

Fig. 8. Body mass index in 4-month age rats in the condition of MSG-induced obesity and treatment with 2-[4-(benzyloxy) phenoxy] acetic acid (M ± SEM, n = 6 in each group). 1—intact rats, 2— MSG-group, 3—MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. *p < 0.05 compared with intact rats. **p < 0.05 compared with MSG-group.

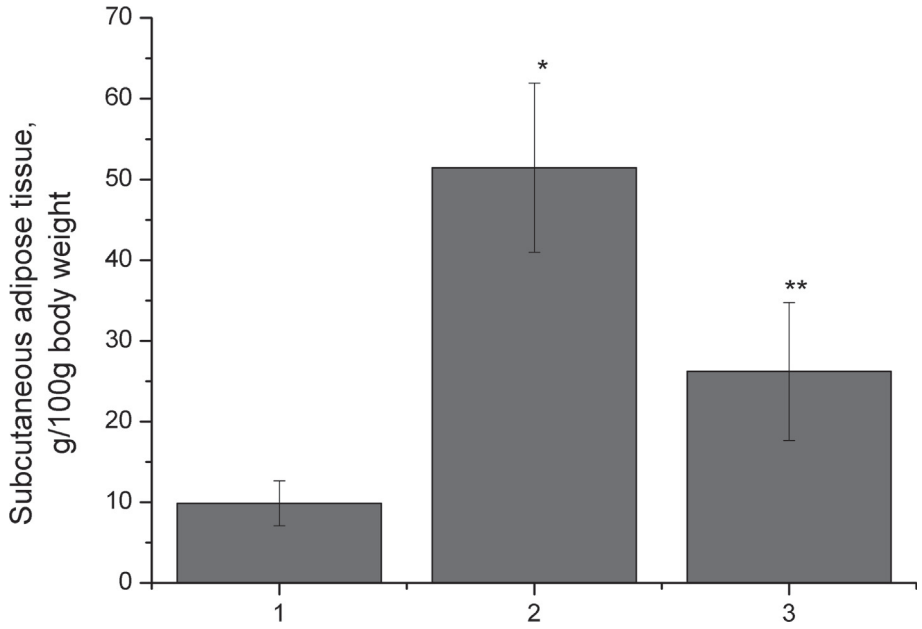

Fig. 9. Subcutaneous adipose tissue in 4-month age rats in the condition of MSG-induced obesity and treatment with 2-[4-(benzyloxy) phenoxy] acetic acid (M ± SEM, n = 6 in each group). 1—intact rats, 2— MSG-group, 3—MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. *p < 0.05 compared with intact rats. **p < 0.05 compared with MSG-group.

MSG in comparison with that of the control (Figs. 9—11). There is a significant increase in subcutaneous fat mass in the experimental group of animals. Mass of subcutaneous adipose tissue increased by 421,25% in the group of obese animals compared to controls (Fig. 9). Our results show a growth of gonadal fat mass in obese group of animals at 253,19% compared with control values (Fig. 10). The mass of visceral fat in control rats was 4759 ± 1011 g/100 g body weight. Mass of visceral fat in the experimental group of rats that were administered MSG was significantly increased by 492,12% (Fig. 11). These data confirm the findings that the introduction of MSG to newborn rodents induces the development of visceral obesity in adult animals and is a model of obesity in mice [32,34] and rats [13,38].

2-[4-(benzyloxy) phenoxy] acetic acid administration led to the decrease in fat mass. Mass of subcutaneous adipose tissue and VAT decreased significantly in MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group compared to the MSG-group by 49,1% ($p < 0.05$) (Fig. 9). Mass of gonadal adipose tissue also tended to decrease after the action of 2-[4-(benzyloxy) phenoxy] acetic acid by 36,1% ($p < 0.05$) (Fig. 10). 2-[4-(benzyloxy) phenoxy] acetic acid decreased also the mass of visceral fat in the MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group compared to MSG rats by 55% ($p < 0.05$) (Fig. 11). So 2-[4-(benzyloxy) phenoxy] acetic acid supplementation in MSG-obese rats reduced the VAT fat accumulation (mesenteric,

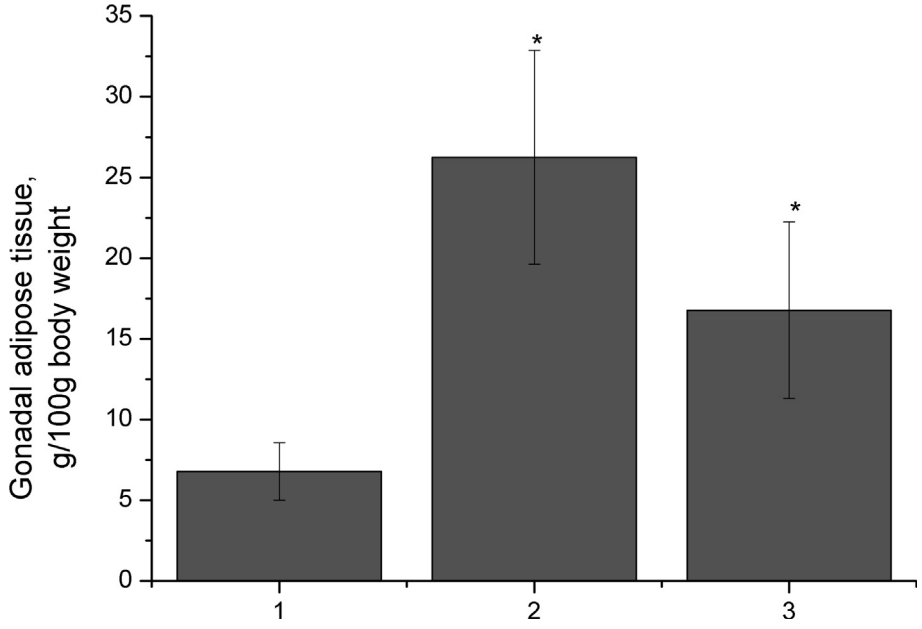

Fig. 10. Gonadal adipose tissue in 4-month age rats in the condition of MSG-induced obesity and treatment with 2-[4-(benzyloxy) phenoxy] acetic acid (M ± SEM, n = 6 in each group). 1—intact rats, 2— MSG-group, 3—MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. *p < 0.05 compared with intact rats.

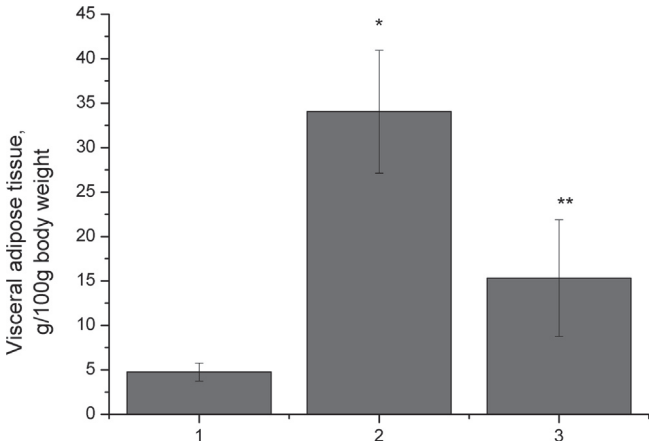

Fig. 11. Visceral adipose tissue in 4-month age rats in the condition of MSG-induced obesity and treatment with 2-[4-(benzyloxy) phenoxy] acetic acid (M ± SEM, n = 6 in each group). 1—intact rats, 2— MSG-group, 3—MSG + 2-[4-(benzyloxy) phenoxy] acetic acid group. *p < 0.05 compared with intact rats. **p < 0.05 compared with MSG-group.

epididymal and retroperitoneal).

The liver of rats with glutamate-induced obesity acquired visual changes, changed its color to dark, edges of liver got sharpened, organ became uneven in consistency but no change in liver weight could be observed (Table 1). As one can see from Table 1 there is a change in spleen and adrenal mass in a group of obese animals compared to the control group of animals. After treatment with 2-[4-(benzyloxy) phenoxy] acetic acid rats with obesity did not affect the mass of organs (Table 1).

MSG-induced obesity in our study was also associated with dyslipidemia. After obesity induction, there were significant increase of cholesterol, triglycerides, and LDL cholesterol and significant decrease of HDL cholesterol in the obesity-induced group as compared to control group (Table 2). The literature data showed that MSG evoked the metabolic shifting in hepatic tissue [9].

Following treatment, there was significant decrease of cholesterol and significant HDL cholesterol increase found in 2-[4-(benzyloxy) phenoxy] acetic acid - treated group as compared to control and MSG-induced obesity group (Table 2). There was a significant reduction in triglycerides, LDL cholesterol and VLDL, found in 2-[4-(benzyloxy) phenoxy] acetic acid - treated group.

These results in MSG-obese rats with 2-[4-(benzyloxy) phenoxy] acetic acid treatment suggested that it has therapeutic potential for the management of obesity and its co-morbidities.

We have registered the development of MSG-induced obesity, but there were no functional changes in the liver observed. It was confirmed by determination of direct and indirect bilirubin concentration and activity of ALT and AST in blood serum (Table 3). Administration of 2-[4-(benzyloxy) phenoxy] acetic acid did not affect the concentration and activities of these parameters.

Accordingly, to our results we can assume that 2-[4-(benzyloxy) phenoxy] acetic acid was able to inhibit fat accumulation and ameliorated dyslipidemia in MSG-obese rats; which may be due to improvement in glucose and lipid metabolism, enhancement of insulin sensitivity, but further studies are necessary to confirm such hypothesis.

Table 1
Mean organ's weight (mg/g body weight) of MSG-induced obesity male rats.

	Control rats	MSG-induced obesity	
		Placebo	2-[4-(benzyloxy) phenoxy] acetic acid
Heart	3,61 ± 0,325	3,85 ± 0,583	3,82 ± 0,547
Liver	29,01 ± 4289	25,83 ± 5795	27,02 ± 5643
Kidneys	6,68 ± 0,476	5,95 ± 0,446	6,62 ± 0,612
Spleen	3,96 ± 0,911	2,44 ± 0,297*	3,44 ± 0,813
Thymus	0,61 ± 0,205	0,66 ± 0,097	0,89 ± 0,281
Adrenals	0,24 ± 0,022	0,31 ± 0,041*	0,33 ± 0,051*

Data are presented as the M ± SEM. *p < 0.05 compared with intact rats.

Table 2
Effect of 2-[4-(benzyloxy) phenoxy] acetic acid on serum lipid levels in MSG-induced obesity male rats.

	Control rats	MSG-induced obesity	
		Placebo	2-[4-(benzyloxy) phenoxy] acetic acid
Total cholesterol, mmol/L	4,31 ± 0,253	6,97 ± 0,301*	5,31 ± 0,228*/**
Triglyceridess, mmol/L	1,09 ± 0,171	3,71 ± 0,692*	2,08 ± 0,546*/**
HDL cholesterol, mmol/L	1,72 ± 0,090	0,98 ± 0,121*	1,53 ± 0,101*/**
LDL cholesterol, mmol/L	2,52 ± 0,312	4,69 ± 0,221*	4,02 ± 0,311*/**
Very low-density lipoproteins, mmol/L	0,421 ± 0082	1,36 ± 0,122*	0,99 ± 0,0201*/**

Data are presented as the M ± SEM. *p < 0.05 compared with intact rats. **p < 0.05 compared with MSG-group.

Table 3
Effect of 2-[4-(benzyloxy) phenoxy] acetic acid on serum levels of liver enzyme markers in MSG-induced obesity male rats.

	Control rats	MSG-induced obesity	
		Placebo	2-[4-(benzyloxy) phenoxy] acetic acid
Alanine aminotransferase, μkat/L	0,231 ± 0028	0,215 ± 0029	0,225 ± 0,31
Aspartate transaminase, μkat/L	0,394 ± 0031	0,386 ± 0038	0,391 ± 0043
Indirect bilirubin, μmol/L	8,1 ± 1601	7,9 ± 1102	8,3 ± 1096
Direct bilirubin, mmol/L	4,2 ± 0,892	4,4 ± 0,910	4,5 ± 0,883

Data are presented as the M ± SEM.

Conflicts of interest

All author none to declare.

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