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The effects of Z56822977 on biosynthesis of serotonin in the brain of rats in the conditions of MSG-induced obesity

Wpływ Z56822977 na biosyntezę serotoniny w mózgu szczurów z otyłością wywołaną przez podawanie glutaminianu sodu

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Abstract

Introduction: The present investigation aims to elucidate the effects of Z56822977 on biosynthesis of serotonin in the brain of rats in the conditions of monosodium glutamate (MSG)-induced obesity.
Material and methods: Eighteen male rats were used in the present study and divided into three groups: 1 — control group, 2 — MSG group, 3 — MSG + Z56822977 group. Newborn rats of groups 2 and 3 were administered MSG dissolved in saline at the dose 4 mg/g body weight with a volume of 8 μl/g s.c. on days 2, 4, 6, 8, and 10 of life. Group 3 received an aqueous solution of Z56822977 a in dose 25 mg/kg at volume 1 ml/kg per os. Administration was started at the end of the fourth week after birth and continued intermittently by alternating a one-week course with three-week intervals of non-treatment. The MSG-group, respectively, received 1 ml/kg of water per os. Within four months after birth, rats were on a standard diet. The content of serotonin, tryptophan, 5-hydroxytryptophan (5-HTr), and activity of tryptophan hydroxylase (TRH), amino acid decarboxylase (AADC), and monoaminoxidase (MAO) were determined in brain tissue.
Results: We have shown that administration of Z56822977 has a positive effect on the main indicators of obesity, as shown by changes in key physiological and biochemical parameters (body weight decreased by 13% vs. MSG ($p < 0.05$); body mass index (BMI), Lee index, and visceral fat mass decreased by 18%, 7%, and 55%, respectively ($p < 0.05$), in comparison with the MSG group). Content of tryptophan and serotonin significantly decreased ($p < 0.05$) in rats with MSG-induced obesity. Also, studies have shown an increase in MAO activity by 97% ($p < 0.05$) and decrease of TRH and AADC activity by 44% and 53%, respectively, ($p < 0.05$) in the brains of rats with obesity. Z56822977 increased the content of serotonin and tryptophan in rat brains and restored the activity of enzymes (MAO, TRH, AADC) to the control values.
Conclusions: It is known that under conditions of obesity there is a disruption in the functioning of the serotonin system in the brains of rats. However, the administration of Z56822977 leads to normalisation of the serotonin and tryptophan content and normalisation of the activity of enzymes involved in the pathway of biosynthesis and the breakdown of serotonin. Thus, the administration of Z56822977, which affects the serotonergic system, can be used in the treatment of MSG-induced obesity in rats. Z56822977 can be considered as a possible novel therapeutic agent, but further studies are needed to confirm its action. **(Endokrynol Pol 2018; 69 (5): 536–544)**

Key words: *serotonin, Z56822977, monosodium glutamate, brain, free fatty acid receptor GPR40*

Streszczenie

Wstęp: Badanie przeprowadzono w celu wyjaśnienia wpływu Z56822977 na biosyntezę serotoniny w mózgu szczurów z otyłością wywołaną podawaniem glutaminianu sodu (*monosodium glutamate*, MSG).
Materiał i metody: W badaniu wykorzystano 18 samców szczura. Zwierzęta podzielono na trzy grupy: 1 — grupa kontrolna, 2 — grupa MSG, 3 — grupa MSG + Z56822977. Szczurzym oseskom w grupie 2 i 3 podawano podskórnie MSG rozpuszczony w soli fizjologicznej w dawce 4 mg/g masy ciała w objętości 8 μl/g w 2., 4., 6., 8. i 10. dniu życia. Grupie 3 podawano doustnie wodny roztwór Z56822977 w dawce 25 mg/kg w objętości 1 ml/kg. Pierwszą dawkę Z56822977 podawano po ukończeniu 4 tygodni życia, a następnie kontynuowano podawanie badanej substancji cyklicznie wedlug schematu tydzień podawania substancji badanej/3 tygodnie przerwy. Zwierzętom z grupy MSG podawano odpowiednio 1 ml/kg wody doustnie. Przez pierwsze 4 miesiące życia szczury otrzymywały standardową karmę. Zmierzono zawartość serotoniny, tryptofanu i 5-hydroksytryptofanu (5-HTr) oraz aktywność hydroksylazy tryptofanowej (*tryptophan hydroxylase*, TRH), dekarboksylazy aminokwasów (*amino acid decarboxylase*, AADC) i monoaminooksydazy (MAO) w tkance mózgowej.
Wyniki: Wykazano, że podawanie Z56822977 ma pozytywny wpływ na główne wskaźniki otyłości, co odzwierciedlają zmiany podstawowych parametrów fizjologicznych i biochemicznych [zmniejszenie masy ciała o 13% vs. MSG ($p < 0,05$); zmniejszenie wskaźnika masy ciała (*body mass index*, BMI), wskaźnika Lee oraz masy tkanki tłuszczowej trzewnej odpowiednio o 18%, 7% i 55%, ($p < 0,05$) w porównaniu z grupą MSG]. Zawartość tryptofanu i serotoniny była istotnie niższa ($p < 0,05$) u szczurów z otyłością wywołaną przez MSG. W badaniach wykazano, że u otyłych szczurów aktywność MAO zwiększa się o 97% ($p < 0,05$), a aktywność TRH i AADC odpowiednio o 44% i 53% ($p < 0,05$). Podawanie Z56822977 powodowało zwiększenie zawartości serotoniny i tryptofanu w mózgach szczurów i przywracało poziom aktywności enzymów (MAO, TRH, AADC) do wartości mierzonych u zwierząt kontrolnych.
Wnioski: Wiadomo, że otyłość wiąże się z zaburzeniem syntezy serotoniny w mózgu szczurów. Jednak podawanie Z56822977 prowadzi do normalizacji stężenia serotoniny i tryptofanu oraz przywrócenia prawidłowej aktywności enzymów uczestniczących w biosyntezie i degradacji serotoniny. Podawanie Z56822977, cząsteczki wpływającej na układ serotoninergiczny, może powodować korzystne efekty w leczeniu otyłości wywołanej przez MSG u szczurów. Można rozważać zastosowanie cząsteczki Z56822977 jako nowego leku stosowanego w otyłości, jednak konieczne są dalsze badania w celu potwierdzenia jej działania. **(Endokrynol Pol 2018; 69 (5): 536–544)**

Słowa kluczowe: *serotonina, Z56822977, glutaminian sodu, mózg, receptor wolnych kwasów tłuszczowych GPR40*

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Introduction

The central nervous system has developed a meticulously interconnected circuitry to meet keeping fed and an adequate nutritional state. An energy-dense environment favours the development of obesity, whilst overcompensation may shut down the drive to feed [1]. In today's society, where evolving disease demographics and lifestyle allow for a greater diversity of metabolic phenotypes than perhaps ever before [2], disorders of both extremes of energy intake are common in health care.

Obesity is increasing at an alarming rate in industrialised and developing countries alike [3] and is associated with a wealth of conditions afflicting virtually all organ systems [4, 5]. Overweight or obesity is a condition that substantially raises the risk of morbidity from hypertension, dyslipidaemia, type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnoea and respiratory problems, and endometrial, breast, prostate, and colon cancers [6, 7]. At first sight, obesity, unlike many other common diseases, has an obvious solution: adjusting food intake and exercise until normal body weight has been restored. However, it is no great revelation that this solution is as simple as it has repeatedly proved to be elusive [8]. Experimental studies confirm the common knowledge that weight loss is almost always followed by a rapid return to initial weight once the anorexigenic regimen is terminated [9]. Available options for pharmacological therapy leave much to be desired, and compounds that have been introduced for obesity management have subsequently often been withdrawn due to intolerable side-effects [10]. Novel treatments are greatly needed.

Experimental studies [11–15] demonstrated that damage to particular regions of the hypothalamus and brainstem leads to profound alterations in feeding behaviour. It also became apparent that the signals from the peripheral energy stores [16] and gastrointestinal canal [17] provide essential cues for appetite and satiety. Metabolic state is reflected in a diverse array of signals of the brain. Recent investigations have shed light on some of the key hormones and the vagal mechanisms that shape the central response to nutritional challenges.

A link between food ingestion and brain monoamine systems may exist for substances in the blood, including such hormones as insulin and corticosterone (CORT), which are important in energy metabolism, are closely associated with feeding behaviour, and are known to influence neurotransmitter function in the brain. The release of insulin, for example, occurs in association with food intake [18] and, through its effects on tryptophan, increases the synthesis of 5-HT in the brain. Pharmacological, biochemical, and behavioural evidence has accumulated in animals in support of the idea about an inhibitory influence of brain serotonin (5-HT) on feeding behaviour [19–21]. Also, there are additional studies that indicate a potential role for brain 5-HT of being in control of human eating behaviour as well [19].

Also, previous studies have shown the involvement of serotonin in the mechanisms of obesity and diabetes [22]. In addition, disruption of serotonergic function has been described in animal and human obesity. A low activity level was indicated by the findings of diminished 5-HT turnover in the hypothalamus of genetically obese Zucker rats [23], HCD (high-calorie diet)-induced obesity rats [24, 25], and in obese humans [26]. On the other hand, there are reports suggesting that an impaired functioning of neurotransmitter systems (e.g. exacerbated 5-HT response to food intake in the ventromedial nucleus [VMH] of obese Zucker rats) may also contribute to the impaired energy balance [27–29].

Being the most widely distributed neurotransmitter in the brain, it regulates a wide range of functions including sleep, depression, anxiety, aggression, appetite, temperature, sexual behaviour, and pain sensations. Consequently, the impairment of serotonergic production or signalling leads to a large spectrum of pathologies such as anxiety, depression, schizophrenia, and obesity [29].

Hence, it was interesting to investigate the state of the serotoninergic system in the brain of animals with obesity, induced by the administration of MSG itself, which causes lesions in the hypothalamic arcuate nucleus (ARC) and impairs leptin and insulin signalling in this region, resulting in hyperleptinaemia and hyperinsulinaemia.

Obesity is associated with chronic low-grade inflammation, which drives the development of metabolic diseases, including NAFLD. That is why the search for non-toxic drugs to prevent an obesity complication, namely inflammation, is of current interest. A new potential target for treatment of type 2 diabetes (T2D) is free fatty acid receptor 1 [30–32].

In high-glucose conditions β-oxidation is inhibited by a product of the glycolytic pathway, malonyl-CoA, and fatty acids are directed towards the formation of triacylglycerol (TAG) [33, 34]. The anabolic and catabolic reactions between long-chain acyl Co-As (LC-CoA) and TAG, known as glycerolipid/free fatty acid (GL/FFA) cycle, produce lipid signalling molecules that stimulate insulin secretion [35]. In addition, FFAs serve as ligands and influence insulin secretion by interacting with G-protein-coupled receptors (GPCRs) on the plasma membrane [36, 37]. One of the GPCRs that is highly expressed in beta cells is free fatty acid receptor 1 (FFAR1 or GPR40) [36, 37]. Activation of the receptor leads to enhanced insulin secretion by activating protein kinase C (PKC) and triggering ER $Ca2+$ release [38, 39]. Recently, FFAR1 agonists have been developed as potential therapeutic agents for the treatment of type 2 diabetes [40–43].

A general problem with currently known FFA1 ligands is their relatively high lipophilicity, which is associated with numerous problems such as poor pharmacokinetic properties, metabolic instability, toxicity, and off-target effects, and correlates with attrition in clinical trials [44–47]. Therefore, it is advisable to continue the search and to research agonists for FFA1 with the focus on lowering lipophilicity [48–50].

Taking into account the aforementioned, we decided to investigate the effects of FFA1 agonist on the rat model of MSG-induced obesity in rats.

Material and methods

Research was conducted in compliance with the standards of the Convention on Bioethics of the European Convention for the Protection of Vertebrate Animals adopted by the Council of Europe, which is applicable for experimental and other scientific purposes' (1997), the general ethical principles of animal experiments, approved by the First National Congress on Bioethics in Ukraine (September 2001), and other international agreements and national legislation in this field. The animals were kept in a vivarium, which was accredited in accordance with the 'standard rules on ordering, equipment, and maintenance of experimental biological clinics (vivarium)'. All the equipment that was used for the research had been subjected to metrological control.

Animals and housing conditions

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

Synthesis procedure [51]

Monobenzyl ether of hydroquinone (5 mmol, Z1) was dissolved in 20 ml of dry acetone followed by the addition of 7.5 mmol of dry K_2CO_3. Then, Table I. Organometric parameters of glutamate-induced obesity rats and corrected parameters by Z56822977-bromoacetic acid ethyl ester (5.5 mmol) was added dropwise (Z2) and the mixture was refluxed for four hours. The solvent was removed using rotary evaporator and the residue was crystallised from ethanol with a yield of 1.35 g (96%, Z3).

Compound Z3 (1.35 g, 4.8 mmol) was dissolved by stirring in 10 ml of 30% aqueous solution of NaOH at 50°C. After the Z3 was completely dissolved, the reaction mixture was stirred for one hour then acidified with a 10% solution of HCl to pH = 2. The formed precipitate was filtered off, washed with water (3 x 10 ml), dried on air, and crystallised from ethanol. The yield was 1.15 g (93%, Z56822977 (2-[4-(benzyloxy) phenoxy]acetic acid)).

Animals and experiment design

Newborn rats of group 1 (Control) were injected with saline at a volume of 8 μl/g subcutaneously (s.c.) on days 2, 4, 6, 8, and 10 of life. Newborn rats of groups 2 and 3 were administered with MSG dissolved in saline at a dose of 4 mg/g of body weight with a volume of 8 μl/g s.c. on days 2, 4, 6, 8, and 10 of life [52–55]. The rats were on a standard diet for the entire study period. Group 3 received an aqueous solution of Z56822977 at a dose of 25 mg/kg at a volume of 1 ml/kg per os. Administration was started at the end of the fourth week after birth and continued intermittently by alternating a one-week course with three-week intervals of non-treatment. The MSG-group respectively received 1 ml/kg of water per os.

Biochemical, anthropometrical and nutritional determinations

During the four months after birth we analysed 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.

Body mass index (BMI) = body weight [g] / (nose-to-anus length)^2 [cm^2]

For each animal at the fourth month after birth we determined the obesity status using the Lee index [56].

Lee index = cube root of body weight [g] / nose-to--anus length [cm].

Adult animals were sacrificed. The rats' brains were removed and weighed. Brains were homogenised in 0.4 M perchloric acid at the rate of 5 ml buffer per half of the brain. Samples were kept at 4°C for 60 min and then centrifuged at 0°C for 5 min at 800×g. After centrifugation the supernatant was collected and the pH was adjusted to 5–6 using 2 M KOH. Samples were centrifuged for 5 min at 800×g at a temperature of 0°C.

Serotonin and tryptophan content in the brain

The tryptophan content in the brain was determined using ion-exchange chromatography and fluorescence methods, which were described previously [57].

The resulting supernatant was applied to a pre-equilibrated 0.01 M Na-phosphate buffer, pH 6.2, the column of CM-Sepharose. Elution was carried with 0.03 M sodium phosphate buffer, pH 6.2. To 1 ml fraction of serotonin was added 0.3 ml of 11.6 M HCl. Measurements were performed on a Shimadzu

spectrophotofluorometer at an excitation wavelength of 295 nm and emission wavelength of 550 nm against a blank sample that contained distilled water instead of the test sample.

Then elution was carried out with 0.01 M sodium phosphate buffer, pH 6.2. To 0.5 ml fraction of tryptophan was added 0.5 ml of a dithiothreitol mixture: ethanol (1 mg dithiothreitol/1 ml of ethanol) and 0.5 ml of ninhydrin reagent (ninhydrin dissolved in a solution of 17.4 M acetic acid and 3 M phosphoric acid in the ratio 3: 2). Samples were placed in a boiling water bath for 25 minutes and then cooled under running water. Measurements were performed on spectrophotofluorometer at an excitation wavelength of 359 nm and emission wavelength of 485 nm against the blank sample that contained distilled water instead of the test sample.

5-hydroxytryptophan content in the brain

The 5-hydroxytryptophan content in the brain was determined using the fluorescence method described previously [58].

0.5 ml of the resulting supernatant was added to 2.5 ml of 1 N perchloric acid and stirred vigorously for 5 min. Samples were centrifuged at 2000 g for 30 min. The resulting supernatant was collected and adjusted to pH 9.5–10.5 with a 5 M NaOH solution. Then 2.5 ml of n-heptane saturated with NaCl was added, and intensively stirred for 15 min. For phase separation a sample was centrifuged for 3 min at 500 g. 1 ml of the aqueous phase was collected, and 0.3 ml of 11.6 M HCl was added. Measurements were performed on a Shimadzu spectrophotofluorometer (excitation 295 nm, emission 545 nm).

Tryptophan hydroxylase activity in the brain

Determination of tryptophan hydroxylase activity was performed according to the method recommended in [59].

The complete reaction mixture contained the following components in a final volume of 0.37 ml: 50 mM Tris-acetate pH 7.5; 0.3 mM L-tryptophan; 0.1 mM GMPHa; 0.05 mM TPNH; 0.5 mM glucose-6-phosphate; 0.7 mM N-methyl-N-3.hydroxyphenyl-hydrazine; 0.4 mM dithiothreitol; catalase, glucose-6-phosphate dehydrogenase and dihydropteridine reductase in excess, and an appropriate amount of hydroxylase, usually 0.04 ml to 0.2 ml. After 30 min of incubation in a shaking water bath at 37°C, the reaction was stopped by the addition of 0.03 ml of 60% perchloric acid and the mixture was centrifuged. Precisely 0.2 ml of the supernatant fraction was added to 0.06 ml of concentrated hydrochloric acid. The amount of product, 5-hydroxytryptophan, was measured fluorometrically with a Shimadzu spectrophotofluorometer (excitation 295 nm, emission 538 nm).

Tryptophan-decarboxylase activity in the brain

Determination of tryptophan decarboxylase activity was carried out by the spectrofluorimetric method described previously [60].

Brains were homogenised in 0.4 M perchloric acid in a ratio of 1: 5 (w/v). Homogenate was kept for 60 min at 4°C and then centrifuged for 5 min at 800 g. The pH of the supernatant was adjusted to 5–6 with 2 M KOH and centrifuged for 5 min at 800 g. 100 μl of the supernatant was collected and was adjusted up to 1 ml by 100 mM Tris-HCl buffer, pH 8.0, containing 5 mM β-mercaptoethanol, 10% glycerol. Subsequently, 2 ml of 4 mM NaOH and 5 ml of ethyl acetate were added and samples were placed for 30 seconds. For phase separation samples were centrifuged for 2 min at 252 g. The determination of tryptophan-decarboxylase activity was performed in the organic phase. Measurements were performed on a Shimadzu spectrophotofluorometer (excitation 280 nm, emission 340 nm).

Monoamine oxidase activity in the brain

Monoamine oxidase activity was estimated according to the method of Ali and Bartlet, in which the production of 4-hydroxyquinoline from the oxidative deamination of kynuramine is measured [61].

The incubation mixture contained kynuramine dihydrobromide (100 mkg) 0.5 M phosphate buffer pH 7.4 (0.5 ml), homogenate (equivalent to 3.13 mg brain), and de-ionised water to a final volume of 3 ml. An incubate without a substrate served as a blank. The reaction mixtures were incubated at 37°C for 30 min under air and with shaking. The incubates were then deproteinised with perchloric acid (0.6 M) as recommended by Century and Rupp (1968) followed by centrifugation at 900 g for 10 min at 5°C. Supernatant (1 ml) was mixed with 1 N NaOH (2 ml) and placed in a quartz cuvette. The solution was activated at 315 nm, and the peak in the fluorescence scanned at 380 nm was measured on a Shimadzu spectrophotofluorometer. The presence of substances affecting the fluorescence of 4-hydroxyquinoline was sought by mixing equal volumes of tissue blank and 4-hydroxyquinoline solution. In all cases the fluorescence of the mixture was half that of the undiluted solution of 4-hydroxyquinoline, demonstrating an absence of augmentation or quenching of fluorescence.

Statistics

Statistical analysis was performed using Statistica 7 software. All data in this study were expressed as mean ± standard error (M ± SEM). Data distribution was analysed using the Kolmogorov-Smirnov normality test. Continuous variables with parametric distribution were analysed 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

Table I. *Organometric parameters of glutamate-induced obesity rats and corrected parameters by Z56822977*

Tabela I. *Parametry organometryczne zmierzone u szczurów z otyłością wywołaną przez podanie glutaminianu sodu oraz poprawa tych parametrów po podaniu Z56822977*

	Intact rats	MSG-induced obesity	
		Placebo	Z56822977
Body weight [g]	385.5 ± 28.402	339.1 ± 18.755	294.8 ± 24.383*
Body length [cm]	24.2 ± 0.935	20.8 ± 0.605*	21.4 ± 0.97*
Index Lee	0.3 ± 0.006	0.335 ± 0.006*	0.311 ± 0.01#
BMI [g/cm²]	0.655 ± 0.019	0.781 ± 0.035*	0.643 ± 0.041#
Subcutaneous adipose tissue, g/100 g body weight	9.873 ± 2.797	51.464 ± 10.471*	26.2 ± 8.549#
Gonadal adipose tissue, g/100 g body weight	6.782 ± 1.782	26.25 ± 6.627*	16.77 ± 5.47#
Visceral adipose tissue, g/100 g body weight	4.759 ± 1.011	34.05 ± 6.908*	15.326 ± 6.561#

*$p < 0.05$ differences significant compared to controls
#$p < 0.05$ differences significant compared to MSG-induced obesity

the Kruskal-Wallis and post-hoc Tukey's test were conducted for multiple comparisons. For comparisons of categorical variables, we conducted the chi-squared test. The difference between groups was defined as statistically significant when the p-value was less than 0.05.

Results

Body parameters and visceral adipose tissue mass in the conditions of Z56822977 treatment of MSG-induced obesity

Administration of MSG in the neonatal period leads to the development of obesity in four-month-old rats. Table I shows the organo-metric parameters in three groups of rats. Administration of MSG in the neonatal period caused a significant increase in index Li and BMI of rats, in comparison with the control rats. The weights of adipose tissue (subcutaneous, gonadal, and visceral) were significantly higher in the MSG group than those in the control group. A number of differences in body parameters in the MSG group treated with Z56822977 was found. Treatment with Z56822977 (25 mg/kg) significantly suppressed the increase in body weight, index Li, and BMI of MSG-obese rats. Z56822977 (25 mg/kg) treatment significantly lowered the adipose tissue when compared to the MSG group and control group (Table I).

Serotoninergic system assessment in the conditions of Z56822977 treatment of MSG--induced obesity

The level of serotonin in brain homogenate of MSG-induced rats showed a significant decrease (55.14%) compared to that of control rats. The oral administration of Z56822977 to group III rats showed a significant increase in the level of brain serotonin (63.8%) compared to MSG-induced obese rats (Figure 1). The level of tryptophan in the brain of rats with MSG-induced obesity decreased (56.25%) (Figure 2). The research of a rate-limiting enzyme of serotonin biosynthesis (tryptophan-hydroxylase) activity in the brains of rats with the experimental model of obesity showed a decrease in enzyme activity by 44.4% in comparison with the values of the control group (Figure 3). Treatment with Z56822977 (25 mg/kg) significantly increased tryptophan-hydroxylase activity of MSG--obese rats. The analysis of the brain 5-HTr content showed a decrease of this indicator in MSG-induced obese rats compared to intact animals. Z56822977 (25 mg/kg) treatment does not affect the content of 5-HTr when compared to the MSG group and control group (Figure 4). According to the data presented in Figure 5, tryptophan-decarboxylase activity was decreased by 53.2% in the rats under MSG-induced obese rats. Studies have shown an increase in MAO activity of 96.5% in the brains of rats with experimental obesity compared with the values of the control group (Figure 6).

Discussion

Hypothalamic centres have been reported to integrate information about long-term energy stores and other physiological and environmental factors to formulate appropriate feeding responses. Serotonin influences both brainstem reflex centres and hypothalamic integratory centres involved in controlling food intake.

We found that MSG-obese rats neonatally treated with MSG exhibited obesity with reduced body weight and body length and increased Lee index and BMI, compared to control rats neonatally treated with saline. An increase in adipose tissue weight was also found in the rats with obesity induced by neonatal injection of MSG. MSG-obese rats have a reduced body weight compared

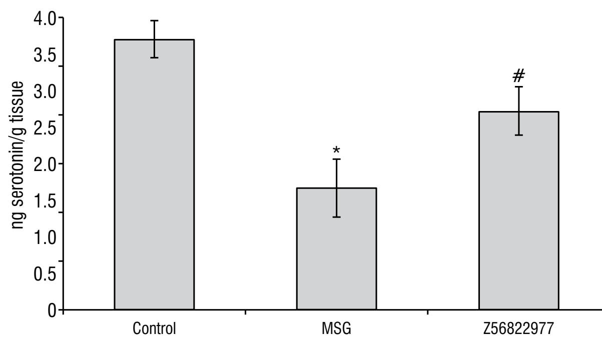

Figure 1. *Content of serotonin (ng/g tissue) in brain tissue in the condition of MSG-induced obesity and treatment with Z56822977 (M ± SEM, n = 6 in each group). 1 — intact rats, 2 — MSG--group, 3 — MSG + Z56822977 group. *p < 0.05 differences significant compared to controls; #p < 0.05 differences significant compared to MSG-induced obesity*

Rycina 1. *Stężenie serotoniny (ng/g tkanki) w tkance mózgowej szczurów z otyłością wywołaną MSG leczonych Z56822977 (M ± SEM, n = 6 w każdej grupie). 1 — szczury niepoddane interwencji, 2 — grupa MSG, 3 — grupa MSG + Z56822977*

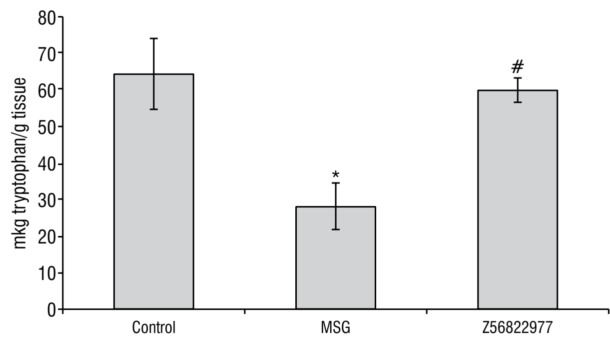

Figure 2. *Content of tryptophan (µg/g tissue) in brain tissue in the condition of MSG-induced obesity and treatment with Z56822977 (M ± SEM, n = 6 in each group). 1 — intact rats, 2 — MSG-group, 3 — MSG + Z56822977 group. *p < 0.05 differences significant compared to controls; #p < 0.05 differences significant compared to MSG-induced obesity*

Rycina 2. *Stężenie tryptofanu (µg/g tkanki) w mózgu szczurów z otyłością wywołaną MSG leczonych Z56822977 (M ± SEM, n = 6 w każdej grupie). 1 — szczury niepoddane interwencji, 2 — grupa MSG, 3 — grupa MSG + Z56822977*

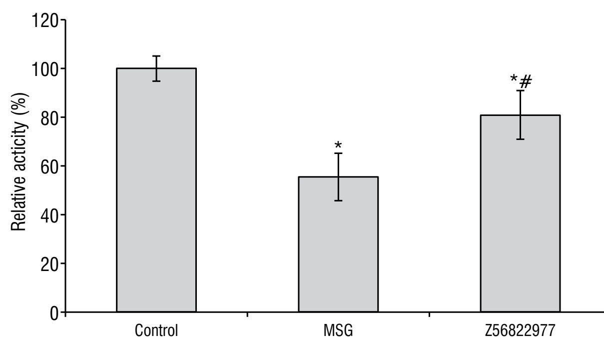

Figure 3. *Tryptophan hydroxylase (TPH) activity in brain tissue in the condition of MSG-induced obesity and treatment with Z56822977 (M ± SEM, n = 6 in each group). 1 — intact rats, 2 — MSG-group, 3 — MSG + Z56822977 group. *p < 0.05 differences significant compared to controls; #p < 0.05 differences significant compared to MSG-induced obesity*

Rycina 3. *Aktywność hydroksylazy tryptofanowej (tryptophan hydroxylase, TRH) w tkance mózgowej szczurów z otyłością wywołaną MSG leczonych Z56822977 (M ± SEM, n = 6 w każdej grupie). 1 — szczury niepoddane interwencji, 2 — grupa MSG, 3 — grupa MSG + Z56822977*

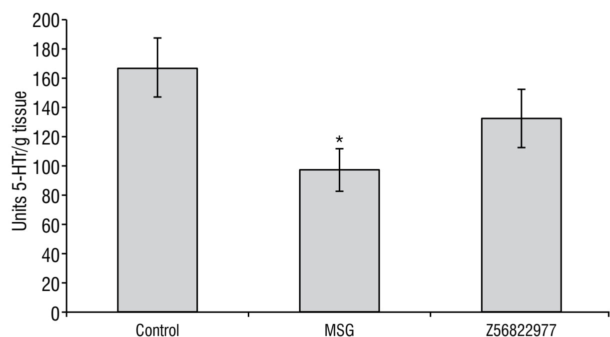

Figure 4. *Content of 5-hydroxytryptophan (units/g tissue) in brain tissue in the condition of MSG-induced obesity and treatment with Z56822977 (M ± SEM, n = 6 in each group). 1 — intact rats, 2 — MSG-group, 3 — MSG + Z56822977 group. *p < 0.05 differences significant compared to controls; #p < 0.05 differences significant compared to MSG-induced obesity*

Rycina 4. *Stężenie 5-hydroksytryptofanu (j./g tkanki) w tkance mózgowej szczurów z otyłością wywołaną MSG leczonych Z56822977 (M ± SEM, n = 6 w każdej grupie). 1 — szczury niepoddane interwencji, 2 — grupa MSG, 3 — grupa MSG + Z56822977*

to intact animals. However, most of the MSG-obese rats can be identified by their 'fatty feel' due to their greatly increased adiposity and by their changed body shape. Our results are consistent with the results of many studies [62–65].

According to literature data, obesity is characterised by increased levels of circulating proinflammatory cytokines, accumulation of leukocytes within the adipose tissue and other organs, activation of macrophages in the liver and fat, and activation of proinflammatory [66, 67]. Previous studies have been installed the increase of content of pro-inflammatory cytokines in MSG-obese rat serum [68]. Cytokines like IL-6 and TNF-α promote insulin resistance by increasing hepatic suppressors of cytokine signalling expression [69].

The activation of systemic inflammatory processes, originating from alterations in adipose tissue and gut functions, can contribute to the development of obesity-associated neuropsychiatric comorbidities. Proinflammatory cytokines released at the periphery can access the brain via several pathways (e.g. neural, humoral, and cellular routes) and induce the activation

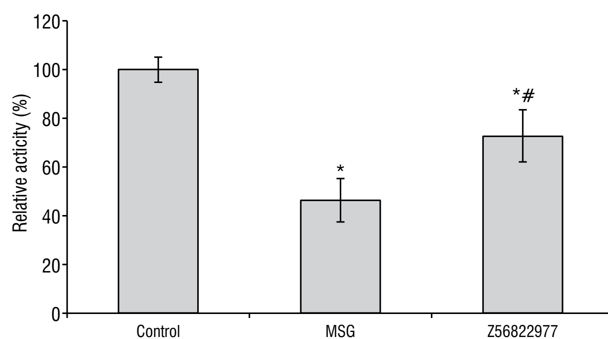

Figure 5. *Amino acid decarboxylase (AADC) activity in brain tissue in the condition of MSG-induced obesity and treatment with Z56822977 (M ± SEM, n = 6 in each group). 1 — intact rats, 2 — MSG-group, 3 — MSG + Z56822977 group. *p < 0.05 differences significant compared to controls; #p < 0.05 differences significant compared to MSG-induced obesity*

Rycina 5. *Aktywność dekarboksylazy aminokwasów (amino acid decarboxylase, AADC) w tkance mózgowej szczurów z otyłością wywołaną MSG leczonych Z56822977 (M ± SEM, n = 6 w każdej grupie). 1 — szczury niepoddane interwencji, 2 — grupa MSG, 3 — grupa MSG + Z56822977*

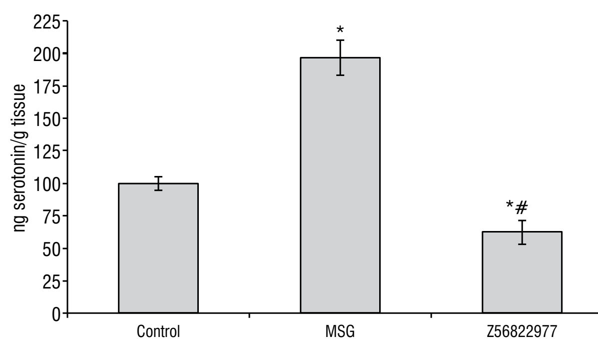

Figure 6. *Monoamine oxidase (MAO) activity in brain tissue in the condition of MSG-induced obesity and treatment with Z56822977 (M ± SEM, n = 6 in each group). 1 — intact rats, 2 — MSG-group, 3 MSG + Z56822977 group. *p < 0.05 differences significant compared to controls; #p < 0.05 differences significant compared to MSG-induced obesity*

Rycina 6. *Aktywność monoaminooksydazy (MAO) w tkance mózgowej szczurów z otyłością wywołaną MSG leczonych Z56822977 (M ± SEM, n = 6 w każdej grupie). 1 — szczury niepoddane interwencji, 2 — grupa MSG, 3 — grupa MSG + Z56822977*

of neuroinflammatory processes, primarily by activating microglia. In the brain, proinflammatory cytokines impair neuroendocrine activity, neurotransmitter function (e.g. 5HT, DA, glutamate), and neurocircuitry, involving notably the hippocampus, the hypothalamus, the basal ganglia, and the prefrontal cortex. IFN-α induces major depression, and this effect relates to inflammation-induced alterations in the hypothalamic–pituitary–adrenal (HPA) axis, neurotransmitter function, and enzymatic pathways involved in the metabolism of monoamines [70–74].

The decreases in serotonin and its metabolite concentrations in the tissue of MSG-obese rats appear to be attributable to the destruction of the VMH and ARC in the hypothalamus. Slight decreases in the concentration of serotonin and its metabolite in the brain observed after administration of Z56822977 to MSG-obese rats were assumed to be due to the serotonin uptake inhibition by Z56822977 in the hypothalamic areas that had not been destroyed, which promoted the function of the serotonin nervous system by increasing serotonin levels in synapses.

Clearly, serotonin levels in the brain are highly dependent on levels of 5-HTP and LT in the central nervous system (CNS) [75]. Synthesis of serotonin is heavily dependent upon the availability of tryptophan within the central nervous system. The production and transport of tryptophan from the bloodstream into the central nervous system can be compromised by stress, elevated cortisol levels, vitamin B6 deficiency, and high dosages of tryptophan. All these factors stimulate the conversion of tryptophan to kynurenine, lowering serum tryptophan levels [75].

Elevated serum levels of kynurenine inhibit transport of tryptophan into the central nervous system and reduce central nervous system serotonin levels. This may be one of the reasons for the lowering of tryptophan levels in the brains of rats under conditions of development of MSG-induced obesity. Another reason for lowering the content of tryptophan may be competition with other amino acids for passing the blood–brain barrier. The transport of tryptophan through the blood-brain barrier requires binding to a transport molecule, which tryptophan shares with five other amino acids (tyrosine, phenylalanine, valine, leucine, and isoleucine). Since tryptophan is present in foods in relatively small amounts in comparison to these other amino acids, as little as one per cent of dietary tryptophan may be transported into the CNS. Tryptophan is used by the body for other metabolic purposes in addition to serotonin production, including protein synthesis and the creation of niacin. The increase of serotonin in the brain of animals after administration of Z56822977 can be explained by interaction of the preparation with serotonin transporters, which stimulates the release of serotonin from the cytoplasm of the neuron into the synaptic cleft. Dedicated serotonin in the synaptic cleft is inactivated by reversible capture of serotonergic terminals using serotonin transporter and enzymatic degradation of serotonin by monoamine oxidase.

The serotonin system is relatively complex. Several receptor subtypes, anatomical projections are involved in the serotonin system. Ongoing research continues to delineate the brain pathways underlying the regulation of body weight by brain serotonin. Much remains

to be understood about the serotonin projections and about the specific roles of each of the serotonin receptor subtypes. Greater understanding of serotonergic mechanisms is likely to lead to more efficacious serotonin-based pharmacotherapies.

Conclusions

Our studies confirm the involvement of serotonin in the mechanisms of obesity. The results suggest that administration of Z56822977 inhibited serotonin uptake in the brain areas that were not destroyed in MSG-obese rats, which increased the function of the serotonin nervous system by increasing serotonin levels in synapses and thereby suppressed food intake and increased sympathetic nerve activity as well as spontaneous motor activity. In this way Z56822977 decreased the Lee index, body weight, and adipose tissue weight.

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Author contributions
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Conflicts of interest
The authors declare no conflicts of interest.

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