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Influence of betahistine repeated administration on a weight gain and selected metabolic parameters in the model of excessive eating in rats

Kamil Mika [a], Małgorzata Szafarz [b], Jacek Sapa [a], Magdalena Kotańska [a,*]

[a] Department of Pharmacological Screening, Jagiellonian University Medical College, Medyczna 9, PL 30-688 Cracow, Poland
[b] Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Krakow, Poland

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ABSTRACT

It is important to search for a promising therapeutic target or small molecules that can control excessive eating since limiting the intake of foods, especially tasty ones, could be effective in the treatment or prevention of obesity. Some studies indicate betahistine as an unique drug having the ability to ameliorate, for example, antipsychotic-induced weight gain. This study aimed to determine whether repeated administration of betahistine (histamine H1R agonist and H3R antagonist) could be beneficial in reducing the intake of tasty foods or the body's response to overeating via mechanisms such as by influencing the levels of hormones involved in the regulation of food intake or the levels of selected metabolic parameters. Studies were performed in the excessive eating model in rats, which perfectly illustrates the harmful high-caloric intake from freely available tasty products rich in sugar and fat. Our results indicated that repeated administration of betahistine to rats caused lower gain of body mass compared to the control rats fed palatable feed. Interestingly, betahistine treatment increased the consumption of cheese, which is a source of histamine. Although betahistine did not prevent the development of metabolic disorders, such as reduced glucose tolerance, in test animals, it significantly increased the level of high-density lipoprotein cholesterol, which could certainly be considered beneficial. Further studies should be conducted to investigate the effect of repeated administration of betahistine on satiety, gastrointestinal disorders, and the preference for histamine-containing foods.

1. Introduction

According to the first law of thermodynamics, an imbalance between energy intake and expenditure can lead to the development of weight disorders such as obesity or excessive thinness. Under the conditions of increase in energy intake or reduction in energy expenditure or a combination of both, the body fat stores increase followed by the development of obesity [1]. Although it is well known that obesity results from the consumption of high-caloric food or very large portions, some people do not refrain from excessive eating, and as a result, develop obesity, which is considered an epidemic of the 21st century [2]. This condition is also recognized as a major risk factor for many chronic diseases such as ischemic heart disease and hypertension, type 2 diabetes, atherosclerosis, hyperlipidemia, and even some types of cancer [2,3]. Therefore, preventing the development of obesity is important at individual, population, social, and economic levels.

Simple changes in body weight alone can be related to physical factors. Changes in body composition and metabolic responses to food are additionally influenced by physiology and genetics including individual differences in genetic susceptibility to environmental factors such as diet [4–7]. Studies on humans have shown that a high-fat diet can easily induce obesity [1] which can develop faster in the case of susceptible individuals. Unlike protein and carbohydrate, fat stimulates the excessive intake of energy due to its high palatability and lack of satiating power [8]. People tend to overconsume foods with a sweet taste and a high amount of fat because the ability to reduce food intake after a recently eaten meal is impaired when the subsequently taken food is high in fat [9,10] and especially high in both fat and sugar [11]. The choice of nutrient composition is also influenced by different social factors such as easy availability of inexpensive and unhealthy foods, commercials, or stressful situations that lead to compensatory overfeeding. This is part of the reasons that the strict pharmacotherapy of obesity has failed so far.

It is important to search for a promising therapeutic target or small molecules that can control excessive eating since limiting the intake of foods, especially tasty ones, could be effective in the treatment or

* Corresponding author.
E-mail address: magda.dudek@uj.edu.pl (M. Kotańska).

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prevention of obesity. In this study, we investigated the effect of repeated administration of betahistine on feeding behavior in rats, as well as on the induction of metabolic changes that usually develop during the unlimited availability of a palatable diet. Betahistine (N-methyl-N-β-[2-pyridyl]ethylamine dihydrochloride), originally synthesized by Löffler in 1904, is a centrally acting histamine H1 receptor agonist which can also bind to histamine H3 receptor but has no affinity for histamine H2 receptor [12–14]. Brain histamine performs several functions, including the regulation of the neuroendocrine system [15], feeding and drinking behaviors [16,17], and sleep–wake cycles [18]. As a drug, betahistine has two particular advantages: an excellent safety profile and unique pharmacological properties. Currently, betahistine is the only drug in use that is both a histamine H1 agonist and a histamine H3 antagonist. This compound and its metabolites can exert direct histamine-like action(s), or by blocking histamine H3 receptors, increase the release of histamine and other heterogeneous neurotransmitters, including dopamine, acetylcholine, noradrenaline, gamma-aminobutyric acid, and serotonin [19]. Additionally, betahistine capacity to directly activate postsynaptic H1 receptors gives it a key advantage over selective H3 antagonists as an anorectic and antiobesity agent [12].

Weight gain caused by treatment with second-generation antipsychotics was shown to be related to increased caloric intake. Since increase in appetite resulted from the blockade of H1 receptor, therefore some studies indicated betahistine as a unique drug having the ability to ameliorate antipsychotic-induced weight gain [20–23]. However, only a few studies have analyzed the effect of this compound on food intake in nonobese individuals [24,25], and there are no reports showing its effect (especially after repeated administration) in preventing the development of obesity or metabolic changes under conditions of excessive consumption of tasty foods in nonobese individuals or animals.

Why do we see the potential of betahistine here? In earlier studies, it was noted that histamine release was decreased by sucrose and saccharin solutions, which may suggest that palatable food reduces the release of histamine resulting in overeating [26]. Dietary interventions (e.g. self-imposed) that modify food intake or diet composition can affect the response of the histaminergic system [27]. Brain histamine serves as a relay station integrating peripheral signals and central functions to control energy expenditure and appetite, as well as influencing the emotional value of different experiences [28], such as the hedonic value of food [27]. Therefore, an increase in histamine release or enhancement of histaminergic signal by betahistine by directly stimulating histamine receptors may inhibit the consumption of tasty foods and thus the development of obesity. Hence, treatment with this drug could be beneficial to people with obesity related to overeating. Moreover, search for new therapeutic indications for already registered drugs can be economically advantageous.

The model of excessive eating in rats, proposed in our study, perfectly illustrates the harmful high-caloric intake associated with overeating of freely available tasty products rich in sugar and fat [29,30]. The model demonstrates that unlimited availability of tasty foods prompts the body to consume them even when extra calories are not needed, which leads to a significant increase in body weight and the development of metabolic disorders over a short period of time. In the model of excessive eating, animals are given access to high-caloric foods such as peanuts, cheese, milk with increased fat content, and chocolate, as well as standard feed. Most importantly, feeding is not forced in any way, as in other models where animals are fed only a high-fat diet [31–33] or temporarily deprived of food (binge eating models) [34]. In the case of our model, rats are allowed to decide when, what, and how much to eat. Obesity develops fairly quickly, which shows that these animals want to consume palatable feed containing more calories than they need. It is therefore an ideal model to test whether the administration of betahistine could have a beneficial effect on reducing tasty food consumption or its effect on the body's response to overeating via mechanisms, such as by influencing the levels of hormones involved in the regulation of food intake or the levels of selected metabolic parameters.

2. Methods

Scheme of the experiment is shown in the Fig. 1.

2.1. Animals

Experiments were carried out on female Wistar rats with the initial body weight from 170 to 180 g. Animals were housed in pairs in plastic cages in constant temperature facilities exposed to a light-dark cycle; water and food were available ad libitum. Control and experimental groups consisted of six animals each. All experiments were conducted according to the guidelines of the Animal Use and Care Committee of the Jagiellonian University and were approved for realization (Permissions No: 185/2017, 220/2019 and 223A/2019).

2.2. Drugs

Betahistine was purchased from FluoroChem (Derbyshire, United Kingdom), heparin was from Polfa Warszawa S.A. (Warsaw, Poland) and thiopental sodium from Sandoz GmbH (Kundl, Austria).

2.3. The effect of betahistine on body weight and food intake by rats fed palatable diet (model of excessive eating)

Female Wistar rats were housed in pairs. Two groups of 6 rats were fed diet consisting of milk chocolate with nuts, cheese, salted peanuts, and 7% condensed milk for 4 weeks. Rats also had access to standard feed (Labofeed B, Morawski Manufacturer Feed, Poland) and water ad libitum. Palatable test group (palatable diet + betahistine) was injected intraperitonealy (i.p.) betahistine suspended in 1% Tween 80 at the dose of 8 mg/kg body weight/day (mg/kg b.w./day). Dose of betahistine was selected based on the studies that showed a reduction in food intake by rats after administration of this drug [25]. Palatable control group (palatable diet + vehicle; control obese group) received vehicle (1% Tween 80, i.p.) and the third group received only standard feed (control group). Intake of food was evaluated daily.

Palatable diet contained: 100 g peanuts – 614 kcal; 100 ml condensed milk – 131 kcal; 100 g milk chocolate with hazelnuts – 195 kcal; 100 g cheese (Greek type) – 270 kcal.

Standard diet (fats 8%, carbohydrates 67%, proteins 25%) contained 100 g feed – 280 kcal.

2.4. The effect of betahistine on rats spontaneous activity

The spontaneous activity of rats was measured on the 1st and 27th day of treatment with a special RFID-system – TraffiCage (TSE-Systems, Germany [32,33]. The animals were subcutaneously implanted with transmitter identification (RFID), which enabled the presence and time spent in different areas of the cage to be counted and then the data was grouped in a special computer program.

2.5. Biochemical analysis

On the 31st day of experiment, 20 min after i.p. administration of heparin (5000 units/rat) and thiopental (70 mg/kg b.w.), blood was collected from the left carotid artery and then centrifuged at 600×*g* (15 min, 4 °C) in order to obtain the plasma. At the same time an intraperitoneal fat pads and the selected organs such as liver, kidneys, heart and brain were dissected out and weighted. To determine glucose, total cholesterol and HDL-cholesterol levels in plasma, standard enzymatic and spectrophotometric tests (Biomaxima S.A., Lublin, Poland) were used. For determination of grehline, leptin or C-reactive proteine (CRP) levels in plasma, standard ELISA Kits (Bioassay Technology

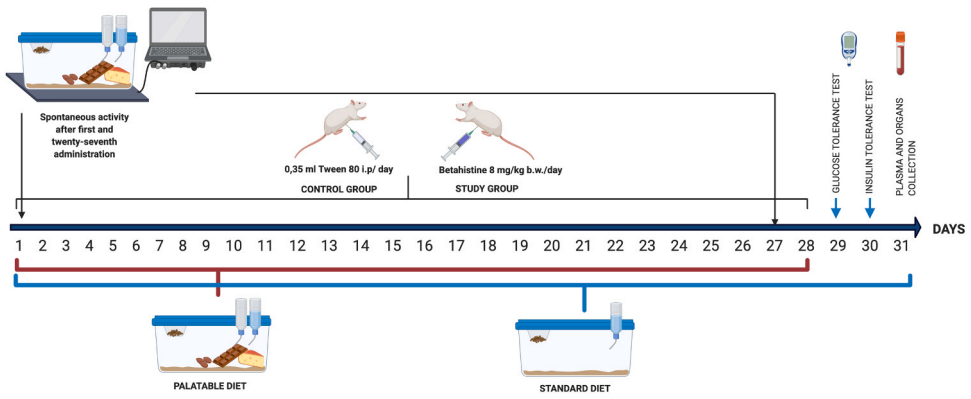

Fig. 1. Scheme of experiment.

Laboratory, China) were used.

2.6. Glucose tolerance test

The test was performed on a 29th day of an experiment. After twenty eight administrations of the tested compound, food was discontinued for 20 h and then glucose (1 g/kg b.w.) was administrated i.p. [35,36]. Blood samples were taken from the tail vein at the time points: 0 (before glucose administration), 30, 60 and 120 min after administration. Glucose levels were measured by glucometer (ContourTS, Bayer, Germany, test stripes: ContourTS, Ascensia Diabetes care Poland Sp. z o.o., Poland, REF: 84239666). The area under the curve (AUC) was calculated using the trapezoidal rule.

2.7. Statistical analysis

Statistical calculations were performed using GraphPad Prism 6 program (GraphPad Software, USA). Results are given as arithmetic means with a standard error of the mean. The normality of data sets was determined using Shapiro–Wilk test. Statistical significance was calculated using one-way ANOVA or two-way ANOVA multiple comparisons tests with Tuckey or Bonferroni post hoc test. Differences were considered statistically significant at: $*p \leq 0.05$, $**p \leq 0.01$ and $***p \leq 0.001$.

3. Results

3.1. Influence of betahistine on body weight and caloric intake of rats

A significant increase in body weight (by 46%) was observed in rats fed palatable feed (obese control group) compared to the control group fed only the standard feed. After repeated i.p. administration of betahistine, at the dose of 8 mg/kg b.w., the body weight of animals slightly reduced (by about 19.5%) compared to the control animals fed palatable feed. A significant difference was noticed within several days of observation. At the end of the experiment, no significant differences were found in the body weight of the test animals and the control rats that only had access to the standard feed (Fig. 2a, b).

Animals consuming palatable feed had a significantly increased amount of fat in peritonea vs those consuming standard feed. The weight of peritoneal fat in the betahistine-treated group was comparable to that of the control group fed palatable feed (Fig. 2c), while the weight of other tissues did not differ significantly between the groups (Fig. 3).

Betahistine administered i.p. for 28 days did not significantly influence the amount of calories consumed by animals in the test group compared to the control group fed palatable feed (Fig. 2d). It was observed that betahistine-treated rats consumed a similar amount of standard feed, in the individual weeks of the experiment, as control rats that also had access to tasty food (control group fed palatable feed; Fig. 2e). Furthermore, no statistically significant differences were found in the amount of eaten chocolate, nuts, and milk (Fig. 2g–i). However, animals receiving betahistine consumed statistically significantly more amount of cheese compared to the control group fed palatable feed during the first, second, and third weeks of the study (Fig. 2f).

3.2. Influence of betahistine on plasma levels of glucose, total cholesterol, and high-density lipoprotein cholesterol (HDL-cholesterol)

In animals fed palatable feed, the plasma levels of both glucose and total cholesterol were significantly higher compared to the control group fed only standard feed. In contrast, the levels of HDL-cholesterol were comparable in these groups, which indicates that the percentage of this cholesterol was lower in the control obese group. Administration of betahistine for 28 days, at the dose of 8 mg/kg b.w. (i.p.), did not decrease the levels of glucose (Fig. 4a), slightly decreased the levels of total cholesterol (Fig. 4b), and significantly increased the levels of HDL-cholesterol in the plasma of test animals in comparison to the control obese group (Fig. 4c). No significant differences were noted in the levels of total cholesterol between the control group fed standard feed and the animals treated with betahistine and fed palatable feed.

3.3. Influence of betahistine on plasma levels of leptin and grehlin

No significant differences were observed in the plasma levels of leptin between the studied groups (Fig. 5a), although the animals in the groups fed palatable feed consumed more calories. Betahistine-treated group had significantly higher plasma levels of ghrelin compared to both control groups (Fig. 5b).

3.4. Changes in plasma glucose levels in a glucose loading test

After i.p. administration of glucose at a dose of 1 g/kg b.w., the glucose levels did not differ significantly at individual time points between the rats from all studied groups. One exception was that after 30 min of glucose administration, the levels were significantly higher in rats fed palatable diet (regardless of betahistine treatment) than in the control group (Fig. 6).

3.5. C-reactive protein (CRP) levels in plasma

Plasma CRP levels were significantly higher in rats in the control obese group compared to the control group having access only to the standard feed. On the other hand, the levels in animals fed palatable feed and treated with betahistine were not significantly different from those determined in the control group (Fig. 7).

3.6. Influence of betahistine on spontaneous activity

Intraperitoneal administration of betahistine at the dose of 8 mg/kg

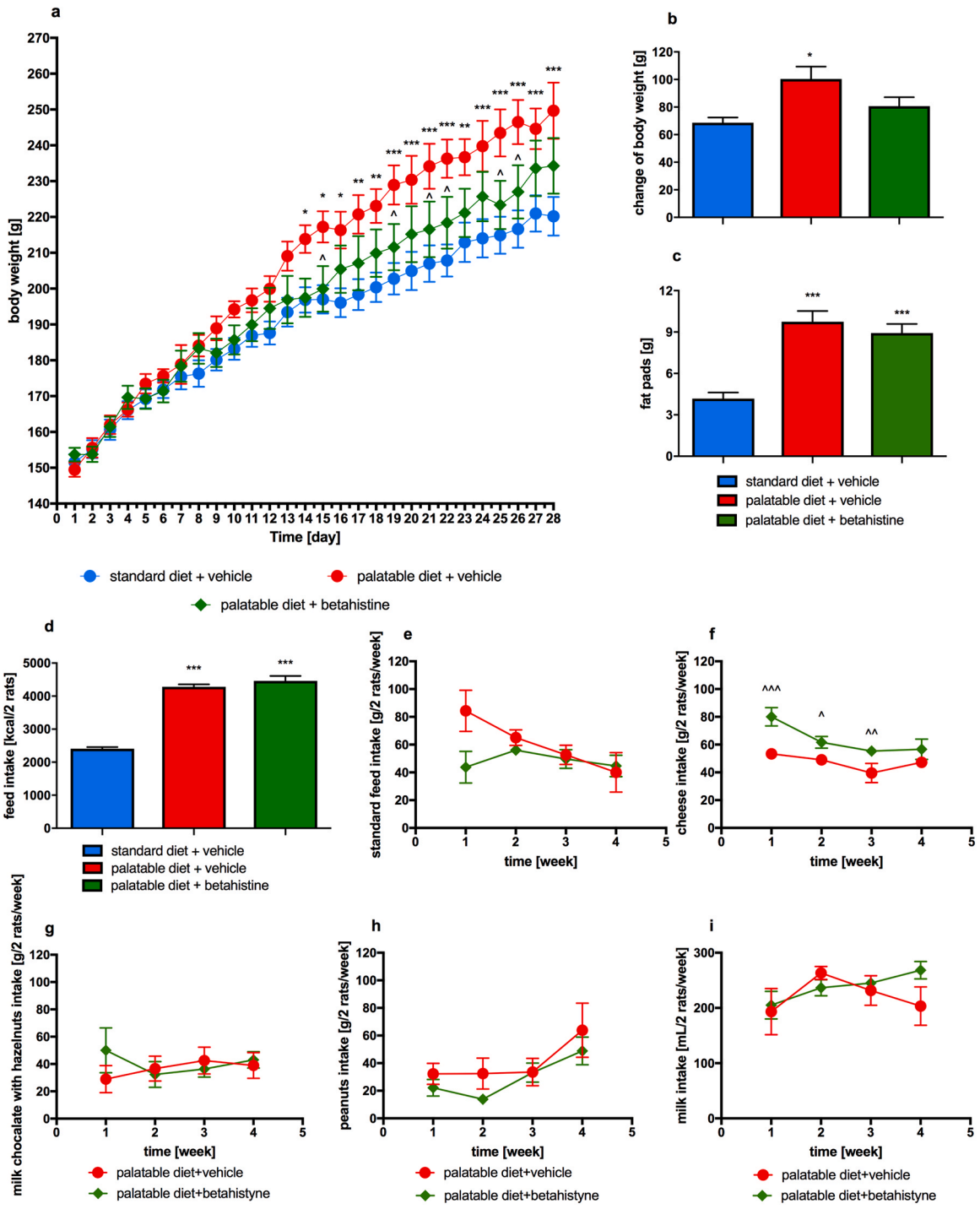

Fig. 2. Effect of diet or long-term administration of betahistine on body weight (a,b), amount of intraperitoneal fat (c), feed intake (d), standard feed intake in individual weeks (e), cheese intake in individual weeks (f), chocolate intake in individual weeks (g), peanuts intake in individual weeks (h), milk intake in individual weeks (i), in female Wistar rats. Results are means ± SEM, n = 6 (or n = 3 - feed intake). *Comparisons versus the vehicle-treated control group fed standard diet, ^Comparisons versus the vehicle-treated control group fed palatable diet; (a) – two-way ANOVA, Tukey's post hoc; (b, c, d) – one-way ANOVA, Tukey's post hoc, (e, f, g, h, i) two-way ANOVA with repeated measure, Bonferroni's post hoc; *,^p < 0.05, **,^^p < 0.01, ***,^^^p < 0.001.

b.w. did not significantly affect the spontaneous activity of rats. No significant differences were noted in activity in the test animals following both single and chronic administration compared to the control obese group (Fig. 8).

4. Discussion

Our results indicate that repeated administration of betahistine caused lower gain of body mass in rats having free access to tasty and high-caloric products rich in fat and sugar (model of excessive eating) in comparison to the control group fed palatable feed. At the end of the experiment, no difference was observed in the body weights between the rats from the test group and the control group fed palatable feed or between the rats from the test group and the control group fed only standard feed. Based on this finding, it can be concluded that the weight of the test animals did not increase to the same extent as the weight of

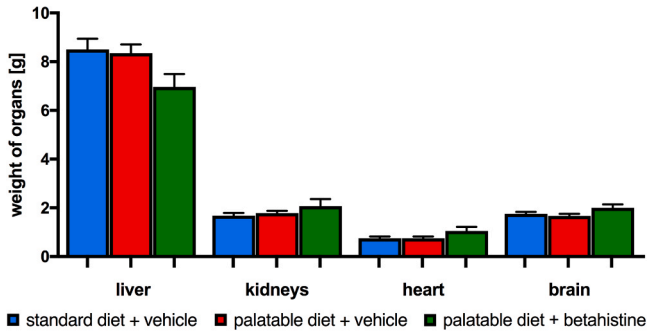

Fig. 3. Weight of organs. Results are means ± SEM, n = 6. One-way ANOVA, Tukey's *post hoc*.

the control animals fed palatable feed. Such an effect (less weight gain) was expected, and the available literature data [25] suggest that it could be due to the reduction in caloric intake by the test animals. However, surprisingly, the results of this study proved that this is not the case. In fact, betahistine-treated rats consumed a similar amount of calories as the control rats fed palatable feed and the weight of peritoneal adipose tissue in rats from the test group was only slightly, but nonsignificantly, lower compared to the control group fed palatable feed.

Interestingly, when analyzing the consumption of particular ingredients present in the palatable food, it was found that the rats treated with betahistine consumed more amount of cheese in the first three weeks of the experiment compared to the animals from the control group. The kcal intake was similar in both groups, which indicates that the animals consumed slightly less of the remaining products at that time. Cheese is a highly nutritious and palatable food and has a significant value in the diet, since it contains a high amount of protein, fat, and essential minerals as well as vitamins and other nutrients [37]. Therefore, it can be concluded that betahistine-treated animals consumed more fat and protein. The advantage of eating animal protein-containing foods (e.g. cheese) is that, besides being a source of protein, they usually provide a good amount of calcium, which improves the functions of β-pancreatic cells, reduces lipogenesis, increases lipolysis, and contributes to reducing body adiposity [38]. Moreover, fermented food products such as cheese may offer ideal conditions for the formation of biogenic amines, which influence health due to their physiological and pharmacological effects [39]. The biogenic amines found in food are histamine, tyramine, phenylethylamine, putrescine, agmatine, cadaverine, spermine, spermidine, and tryptamine [40], but fermented foods contain mostly histamine [41,42]. Additionally, cheese is most commonly associated with histamine poisoning [37,42]. The reason why betahistine-treated animals preferred cheese above other foods, especially in the context of betahistine as a drug that increases the release of endogenous histamine by blocking histamine H3 receptors, still remains open. Another question is whether the toxicity related to the increase in both endogenous and exogenous histamine could be responsible for the lower body weight gain in rats receiving betahistine. We do not know if cheese also serves as the source of histamine in rats as in humans. In addition, it is not clear whether treatment with betahistine is safe in individuals whose diet contains large amounts of cheese, as betahistine could increase the tendency to overconsume it. All these require further research especially due to the fact that the number of animals used in this study was small, which is undoubtedly one of its limitations.

Reduction of weight gain and/or food consumption in rats and mice can result not only from enhancing the satiety or metabolic adjustments

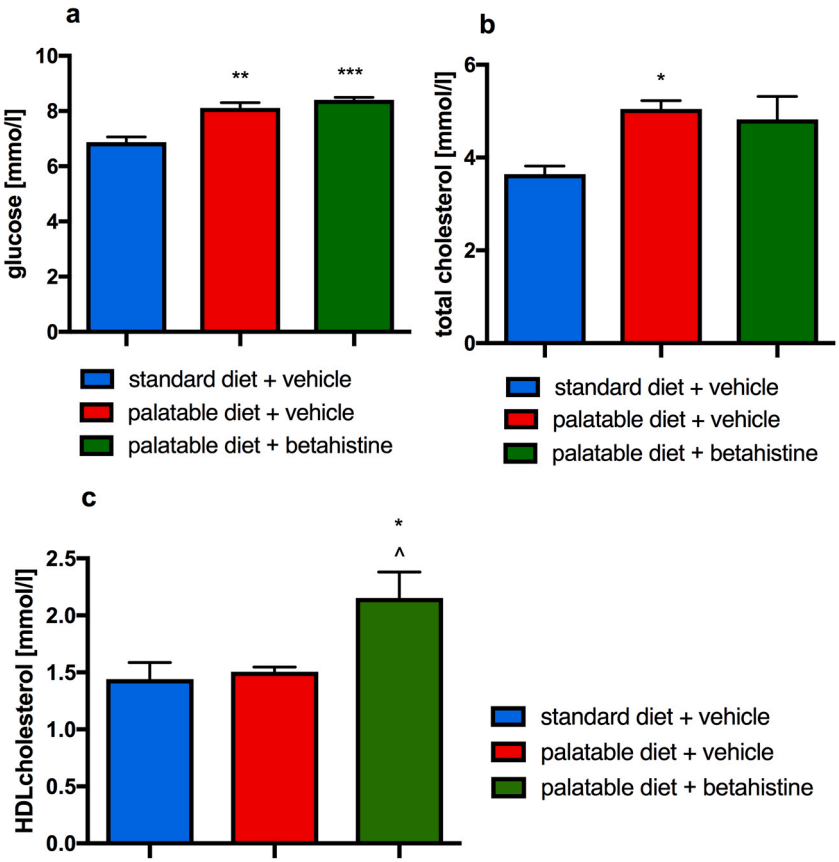

Fig. 4. Effects of diet or of long-term administration of betahistine on blood glucose level (a) or blood total cholesterol level (b) or blood HDL cholesterol level (c) in female Wistar rats. Results are means ± SEM, n = 6. *Comparisons versus the vehicle-treated control group fed standard diet, ^Comparisons versus the vehicle-treated control group fed palatable diet; One-way ANOVA, Tukey's *post hoc*; *,^p < 0.05, **p < 0.01, ***p < 0.001.

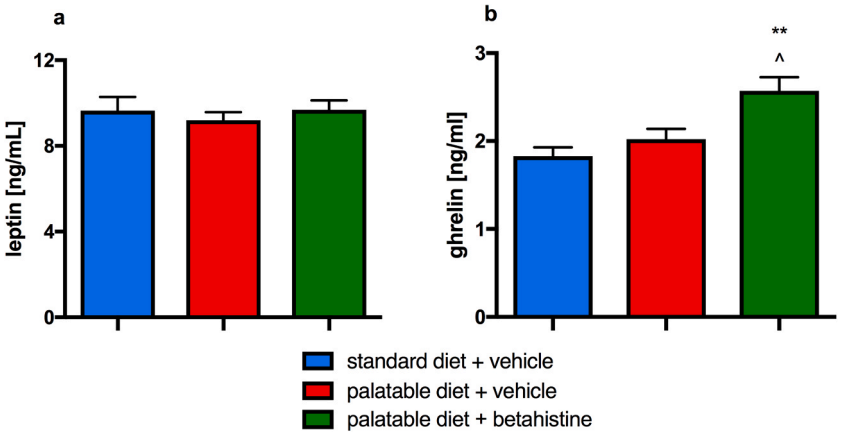

Fig. 5. Effects of diet or of long-term administration of betahistine on blood leptin level (a) or blood ghrelin level (b) in female Wistar rats. Results are means ± SEM, n = 6. *Comparisons versus the vehicle-treated control group fed standard diet, ˆComparisons versus the vehicle-treated control group fed palatable diet; One-way ANOVA, Tukey's *post hoc*; ˆp < 0.05, **p < 0.01.

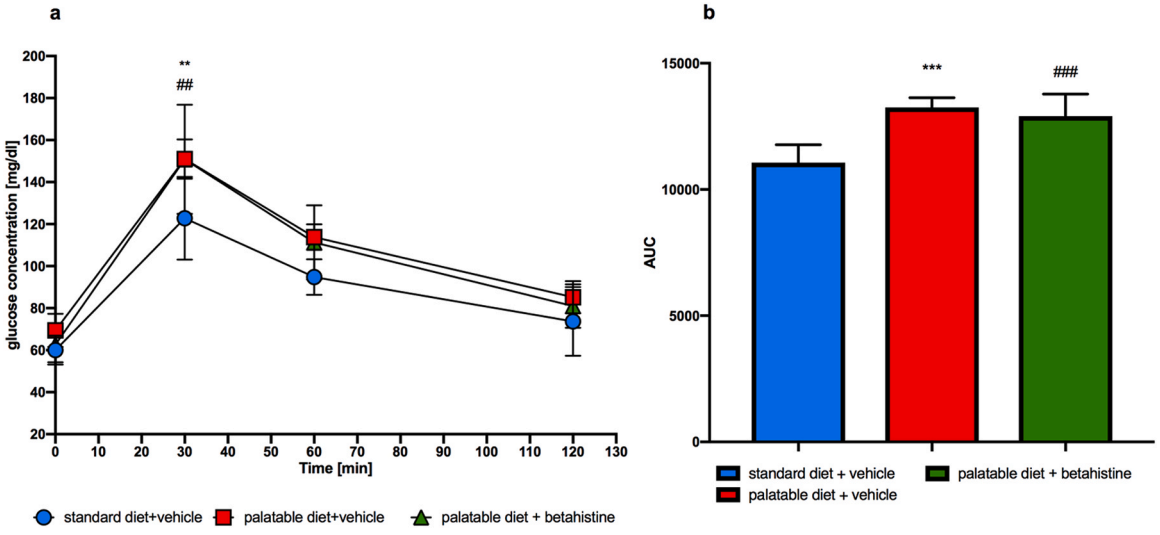

Fig. 6. Glucose tolerance test. Intraperitoneal glucose tolerance test (IPGTT) (a) or area under the curve of IPGTT (b). Results are means ± SEM, n = 6. *Comparisons the vehicle-treated control group fed standard diet and the vehicle-treated control group fed palatable diet, #Comparisons the betahistine-treated group fed palatable diet and the vehicle-treated control group fed standard diet; (a) – two-way ANOVA, Tukey's *post hoc*; (b) – one-way ANOVA, Tukey's post hoc; **,##p < 0.01, ***,###p < 0.001.

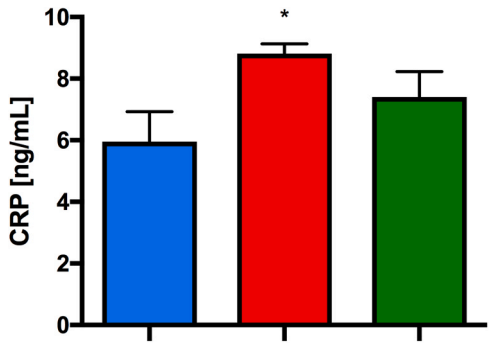

Fig. 7. Effects of diet or of long-term administration of betahistine on blood C-reactive protein level in female Wistar rats. Results are means ± SEM, n = 6. *Comparisons versus the vehicle-treated control group fed standard diet; One-way ANOVA, Tukey's post hoc; *p < 0.05.

but also can be caused by other factors including stress, sickness, sedation, or drug-induced toxicity [43]. During this study, we continuously monitored the effect of betahistine on the spontaneous activity of experimental animals because sedation or overstimulation may reflect the occurrence of the above-mentioned disorders. Interestingly, we did not find any significant difference in the hourly spontaneous activity between the studied groups, and therefore, it can be concluded that the lower weight gain observed after betahistine administration was not due to sedation. Besides, in order to determine if the animals from the test group were under stress or felt unwell, we observed for disturbances in spontaneous activity. However, our results did not indicate such an effect.

Due to an unexpected outcome of our study, we started considering other possible reasons for lower weight gain in rats that were chronically treated with betahistine other than a reduction in caloric intake. We analyzed why the body weight of the rats that were chronically treated with betahistine was lower, even though the peritoneal steatosis was similar to that observed in the control rats fed the palatable feed. As a result, a list of additional factors, besides the ones already measured, which could have a significant influence was identified. For example, we

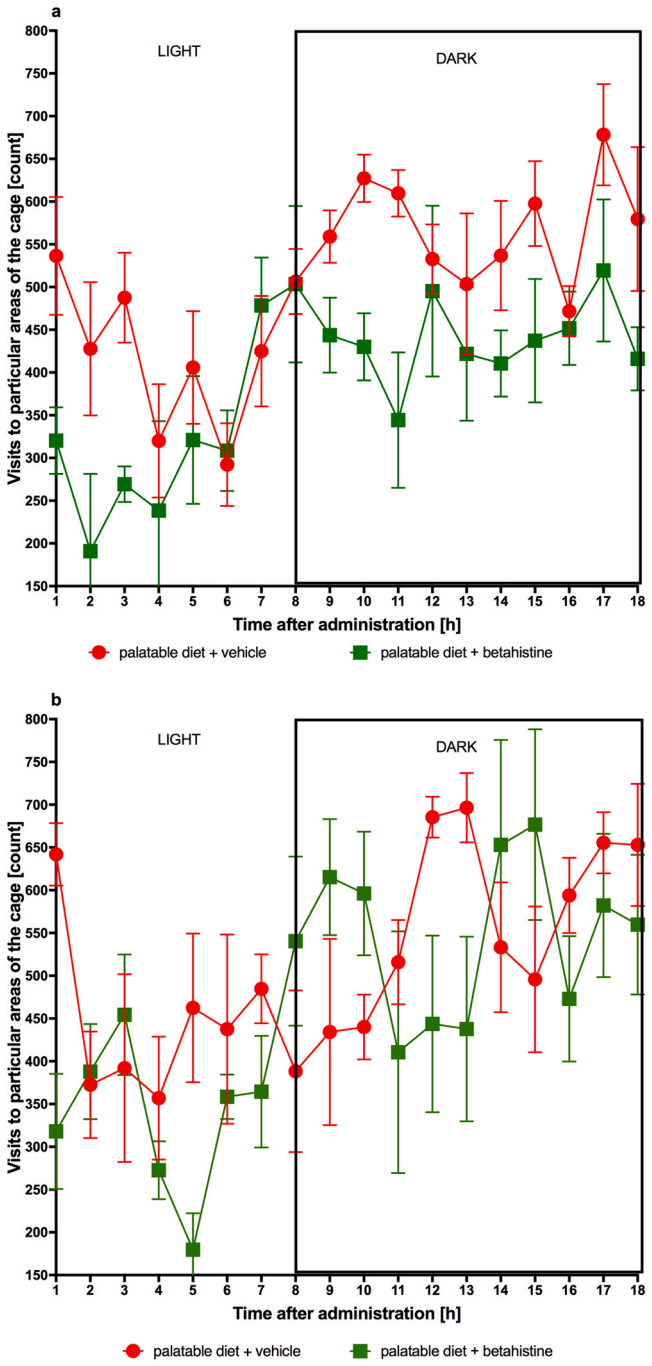

Fig. 8. Changes in the spontaneous activity after single (a) and twenty seven (b) administration of betahistine to rats fed palatable diet. Results are mean ± SEM; n = 6, two-way ANOVA with repeated measure, Bonferroni's *post hoc*.

did not measure the subcutaneous fat mass, as well as bladder or intestinal filling, in the studied rats. The effect of betahistine on intestinal transit seems to be the most likely answer. Betahistine-treated animals could theoretically have had less food remaining in their intestines than the control animals fed palatable feed and therefore they could have a lower weight. We know from our experience that fasting even for 18 h does not completely empty the intestines. Body weight measurement cannot be fully standardized like, for example, the determination of biochemical parameters, when animals in the studied groups are deprived of food for the same period of time before the actual measurements to obtain reliable and comparable results, since for example, the glucose level increases significantly immediately after a meal. It is known that histamine can cause intestinal contractions and accelerate intestinal transit [44,45]. Therefore, histamine-releasing compounds, such as betahistine, may reduce the amount of residual food in the intestines, and by accelerating the intestinal passage, also reduce the time needed for nutrient absorption. Thus, further studies seem to be warranted for analyzing the influence of chronic betahistine administration on intestinal transit in an excessive eating model. Literature data indicate that betahistine can cause diarrhea [20], nausea, and vomiting [46]. The latter effect cannot be observed in rats as they do not have a gag reflex. Therefore, we could not investigate this effect in our excessive eating model. Additionally, it is not clear whether the increase in the intestinal passage and consequently the reduction in nutrient absorption do not trigger beneficial metabolic changes, which in turn can slow down the development of obesity and diabetes.

We analyzed a panel of basic biochemical parameters, such as the levels of glucose and cholesterol, as well as that of leptin and ghrelin, in plasma collected from the test animals, to obtain information on certain metabolic changes that occur after repeated administration of betahistine.

According to the available literature, we expected increased plasma levels of leptin and decreased plasma levels of ghrelin (fasting state) in animals that consumed a tasty diet compared to the animals eating standard feed [47–50]. However, surprisingly, we did not observe such a response. Considering that the level of leptin increases in the state of satiety and is proportional to the amount of adipose tissue, while the level of ghrelin decreases in such state [51], we were surprised by the lack of differences in the levels of these hormones between the groups consuming standard and tasty feed. Although overeating (increase in caloric intake) was observed in animals fed tasty food, the level of ghrelin was not significantly different between these two control groups – the satiety state probably did not last longer in animals consuming more calories (these animals also had continuous access to standard diet and could choose what, how much, and when to eat). Rats fed tasty diet developed obesity, and had an increased level of body fat, but their leptin levels did not change. However, a longer experiment could possibly show changes, especially due to the fact that the level of leptin in the body is proportional to the amount of adipose tissue [51]. Moreover, rats that had access to tasty food products and were treated with betahistine had significantly higher levels of ghrelin than the animals from both control groups (consuming standard or tasty feed). This finding suggests that in these animals the level of hunger could be higher. Betahistine-treated animals indeed consumed more calories than rats eating standard feed only and a comparable amount of calories to the control rats fed palatable diet. Additional studies investigating the levels of ghrelin at various time points during repeated administration of betahistine, in both fasting and satiety state, would probably be helpful in further explaining our results. Based on theoretical assumptions that betahistine could affect the rate of intestinal transit, and thus the extent of food absorption, as well as cause gastrointestinal disturbances such as nausea, a significant increase in ghrelin levels might confirm that these animals feel hunger, at least at certain time points, despite constant access to food. It should be noted that in all the studied groups food was withdrawn 18 h before blood was collected for biochemical examination; however, additional administration of betahistine could have led to the greater release of ghrelin in these rats compared to those from both control groups. All these concerns indicated by the results of our study need further clarification.

We observed higher glucose levels in rats treated with betahistine than in the control rats fed standard diet, which may be related to the levels of ghrelin, a hormone that increases blood glucose levels by inhibiting the release of insulin [52,53]. However, further research is required to confirm the real cause, as the glucose level in betahistine-treated group did not differ significantly from the control group fed palatable feed. A downside of our study is that insulin levels were not simultaneously measured which probably could have explained a couple of the unknowns.

The level of total cholesterol was significantly higher in the control group fed palatable feed than in the control group fed standard feed. However, no significant difference was found in the plasma levels of total cholesterol between animals from the control group fed standard feed and the ones from betahistine-treated group. The fact that total cholesterol level in the test group was only slightly reduced compared to the control group fed palatable feed could probably be explained by an analysis of the levels of HDL-cholesterol in these rats, as the levels in the group treated with betahistine were significantly higher compared to both control groups. Increase in the level of HDL-cholesterol should be considered a beneficial effect of betahistine treatment in individuals who are capable of overeating. HDL-cholesterol, which is also called "good" cholesterol, consists of lipoproteins involved in the transport of cholesterol from tissues, blood, and blood vessels to the liver, where it is eliminated from the body either in an unchanged form or after conversion to bile acids. Thus, HDL-cholesterol reduces the risk of atherosclerosis. and consequently, heart attack or stroke caused by an artery blockage.

Development of obesity, associated with adipocyte hypertrophy and hyperplasia, is linked not only to the secretory function disorders of the fat tissue but also to increased inflammatory responses in adipocytes. It is understood that in a large number of patients obesity is associated with weak inflammation of the white adipose tissue [54–56]. Therefore, in our experiment, we measured the plasma levels of CRP, a specific inflammatory marker [57], to determine if betahistine could be beneficial in, for example, inhibiting the development of inflammation, and consequently the development of obesity. Rats from the group consuming too many calories (which developed obesity) had significantly higher levels of CRP than the control group consuming only the standard feed, and treatment of these rats with betahistine led to a smaller increase in CRP compared to the control group fed palatable feed. Although it should be admitted that this study was only preliminary and cannot confirm that the possible reduction of inflammation in adipose tissue after repeated betahistine administration is due to its anti-inflammatory activity, we consider these results as a starting point for further studies. We intend to perform additional analyses such as immunohistochemical assays investigating the putative anti-inflammatory effect of multiple betahistine administration on white adipose tissue using saved fat pads. It is most likely, however, that betahistine has no direct anti-inflammatory effect but by reducing weight gain and the development of obesity, it inhibits the inflammation to the same extent as in the control animals fed palatable feed.

Our study only examined the effect of repeated betahistine administrations (for 28 days) at one specific dose on food intake, body weight, and selected metabolic parameters. It is possible that the administration of higher doses for a longer period of time could induce more significant changes. A dose of 8 mg/kg was chosen as the available published data show that betahistine, in this particular dose, exerts an anorectic effect in the experimental animals [25,58]. However, these previously published studies were conducted in animals that did not have free access to palatable diet and did not develop obesity. Therefore, the undeniable strength of our study is the fact that it was conducted using an excessive eating model which reflects the nutritional behavior of people who tend to consume excessive amounts of calories when they have free access to tasty products.

In conclusion, it has to be admitted that despite causing a slight reduction in body weight gain, betahistine did not prevent the development of metabolic disorders, such as reduced glucose tolerance, in rats that had constant and unlimited access to tasty products. However, this interesting drug significantly increased the level of HDL-cholesterol, which could certainly be considered beneficial. The issue of a significant increase in ghrelin levels remains to be clarified. We carried out this study because currently there is much published data on the beneficial effects of repeated betahistine administration on neuroleptic-induced obesity [20–23]; however, there are no data describing the effect of chronic administration of this drug on the development of obesity resulting from excessive eating of tasty food. Unfortunately, although our study showed some beneficial changes (i.e. the weight of the test animals did not increase to the same extent as that of the control animals), the mechanism underlying this reduced weight gain effect is still unknown. Repeated administration of betahistine did not inhibit the excessive consumption of tasty foods, and therefore excessive caloric intake, which we speculate, based on the literature data, as its main mechanism of action in slowing down the weight gain. Therefore, further studies should be conducted to determine the effect of repeated administration of betahistine on satiety, gastrointestinal disorders, and the preference for histamine-containing foods.

Ethical approval

All applicable international laws for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

CRediT authorship contribution statement

Magdalena Kotańska, **Kamil Mika** conceived and designed research. **Kamil Mika**, **Magdalena Kotańska** conducted experiments. **Magdalena Kotańska**, **Kamil Mika**, **Małgorzata Szafarz**, **Jacek Sapa** analysed data. **Magdalena Kotańska**, **Małgorzata Szafarz** and **Kamil Mika** wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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