

The Effect of Serotonin on Leptin and Ghrelin Hormones Concentrations in Female Rats

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Abstract

This study was aimed to investigate the relationship of hyperserotoninemia on the adipose tissues performance and satiety status on thirty six female rats. Randomly divided into four groups that individually housed. The first group of six females as a short-term study were given daily serotonin (25 mg/kg) for a week and six controls received saline subcutaneously. Long-term treatment study included 12 females given daily serotonin injections for 3 months vs. 12 controls given saline. The results show significantly decreased in body weights after 7 days and 3 months serotonin injection with mean (200 ± 9.31 g vs. controls 224 ± 11.9 g, $p = 0.003$ and 234 ± 7.12 g vs. controls 246 ± 14.8 g, $p = 0.025$ respectively). Plasma leptin were significantly decreased in the rats syringe with serotonin after short-term (133 ± 32.0 vs. controls 208 ± 58.4 pg/ml; $p = 0.029$) and long-term treatment (172 ± 35.6 vs. controls: 234 ± 60.5 pg/ml; $p = 0.007$), whereas ghrelin concentrations were unaffected. Consequently, hyperserotoninemia results in low concentrations of plasma leptin with unaffected ghrelin concentrations that may explains a direct serotonin influence on adipose tissues with a significant reduction in body weights of rats vs. controls.

Keywords: adipose tissue, leptin, serotonin, serotonin receptors.

تأثير السيروتونين على تراكيز هرموني اللبتين والغريلين في إناث الجرذان

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الخلاصة:

هذه الدراسة تهدف الى بحث علاقة ارتفاع تركيز السيروتونين في الدم على اداء الأنسجة الدهنية وحالة الإشباع لـ (36) من إناث الجرذان قسمت عشوائيا الى اربعة مجاميع معزولة بشكل منفرد. المجموعة الأولى تتكون من (6) إناث أعطيت (25 ملغم/كغم) من السيروتونين يوميا و(6) كمجموعة تحكمت بحقن الملح طبيعي تحت الجلد كدراسة قصيرة الأمد لمدة اسبوع . وتضمنت المعالجة الطويلة الأمد (12) انثى حقنت بالسيروتونين يوميا لمدة (3) أشهر مقابل مجموعة التحكم (12) أنثى حقنت بالملح طبيعي . بينت النتائج نقصان في وزن الحيوانات المعالجة بالسيروتونين بشكل ملحوظ بعد (7) ايام وبعد (3) اشهر ايضا ($200 \pm 9,31$ غم مقابل مجموعة التحكم $244 \pm 11,9$ غم , $p = 0,003$) و($234 \pm 7,12$ غم مقابل مجموعة التحكم $246 \pm 14,8$ غم , $p =$

بالسيروتونين لمجموعة الأمد القصير (32 ± 133 بيكوغرام/أمل مقابل مجموعة التحكم $58,4 \pm 208$ بيكوغرام/أمل, ($p = 0,029$) ومجموعة المعالجة طويلة الأمد ($35,6 \pm 172$ مقابل مجموعة التحكم $234 \pm 60,5$ بيكوغرام/أمل, ($p = 0,007$), بينما مستوى الغرلين لم يتأثر. وبالنتيجة فإن ارتفاع تركيز السيروتونين في الدم يسبب انخفاض اللبتين في البلازما وعدم تأثر مستوى الغرلين به, ربما ذلك يوضح علاقة التأثير المباشر للسيروتونين على أداء الأنسجة الشحمية مع اختزال اوزان اجسام الجرذان بشكل ملحوظ مقارنة بمجموعة التحكم.

الكلمات الإفتتاحية: الأنسجة الدهنية, اللبتين, السيروتونين, مستلمات السيروتونين.

Introduction

One of monoamines is serotonin (5-Hydroxytryptamine 5-HT) that is a unique highly distributed neurotransmitter with its receptors have been expressed in almost all organs [1]. The serotonin receptors, also known as 5-hydroxytryptamine 5-HT receptors, are a group of G protein-coupled receptors (GPCRs) and ligand-gated ion channels (LGICs) found in the central and peripheral nervous systems [2]. The 5-HT has catabolic effects on energy homeostasis, reducing appetite and body weight and prompt energy expenditure [3]. The hypophagic effect of 5-HT is evidence by, among other factors, inhibition of orexigenic system through neuropeptide Y (NPY) and hypocretins and also possibly via inducing anorexigenic melanocortin system [4,5]. The central serotonergic pathways are mainstay for medications that act on the treatment of obesity for the last three decade. Serotonin agonists and reuptake inhibitors have been investigated for influences on energy intake and satiety in both rodents and humans [5,6].

In addition to its role in central nervous system (CNS),[7] serotonin also elicits an act in peripheral nervous system (PNS), as well as in several non-neuronal tissues such as the gut,[8] cardiovascular system,[9] embryogenesis [10] and cell growth [11]. 5-HT has been implicated in the aetiology of numerous disease states,

implying depression, anxiety, social phobia, schizophrenia, obsessive-compulsive and panic disorders; in addition to migraine, hypertension, pulmonary hypertension, eating disorders, vomiting and irritable bowel syndrome [12]. Osteocytes, as well as osteoblasts, are capable of 5-HT synthesis, and express functional receptor and transporter components of the 5-HT signal transduction system [13].

Circulating 5-HT issues from the enterochromaffin cells. Another sources are parasympathetic impulsion, food-derived chemicals and some hormones set off 5-HT release to intestinal lumen as well as portal circulation. [14,15] Additionally, catecholamines in circulation are also activate enterochromaffin cells.[16] Intestinal fraction of 5-HT that enter peripheral blood circulation is metabolized in the liver and lungs.[17] Ultimately, some of the non metabolized 5-HT is got into and stored by platelet cells, small fraction remain free in plasma.[18] Total circulating 5-HT includes platelet-serotonin plus free-serotonin in the plasma increase during postprandial periods [19] and other hyperparasympathetic (physiologic or pathophysiologic) situations. [20-22]

A peak in circulatory ghrelin concentration, produced by ghrelin cells in the stomach, was seen just before a meal and a precipice of its level immediately determined

afterwards [23,24,25]. Ghrelin promote food intake and appetite behavior for food in human and rodent [26,27]. White adipose tissue produces and secretes leptin, which has been found to cross the blood–brain barrier, coordinating with ghrelin, and interacts with NPY in the hypothalamus to regulate hunger, feeding, energy expenditure and satiety [28]. Leptin is contradicted by the actions of the "hunger hormone" ghrelin. Both hormones have receptors in the hypothalamic arcuate nucleus to regulate satiety and appetite in order to achieve energy homeostasis [29].

The influence of circulating 5-HT on adipose tissues and direct effect on stomach and food intake is unclear. We aimed to investigate the relationship of hyperserotoninaemia on the adipose tissues performance and satiety status.

Materials and Methods

Animals

Thirty six female Sprague–Dawley rats were individually housed in wire-top cages. The room temperature was 25 ± 2 °C with a relative humidity of 30–40% and a 12 h light off/on cycle. They were maintained ad libitum on lab chow and tap water. Rats were randomly divided into four groups as follow:

- Short-term serotonin study: six rats were given daily serotonin injected subcutaneously (s.c.) (25 mg/kg of body weight) [5-hydroxytryptamine creatinine sulphate complex (Sigma-Aldrich)] for 7 days where higher concentration dosage results in animals dying [30]; six animal controls received saline. The serotonin was dissolved in saline (25 mg/ml) promptly before injection.
- Long-term treatment study: twelve rats were given daily serotonin injections s.c. (25 mg/kg of body weight) for 3 months; 12 control animals received saline.

For all the studies, the animals were anesthetized with 2 mL/kg body weight of a combination of fluanison (2.5 mg/mL), fentanyl (0.05 mg/mL), and midazolam (1.25 mg/mL) for blood collection [30,31]. During final anesthesia, blood samples were collected from the inferior cava vein. The samples were centrifuged at 3,000 g for 15 min, and plasma was separated for measurement of leptin and ghrelin.

Leptin And Ghrelin Measurements

A mouse/rat leptin enzyme immunoassay kit of SPI-BIO France was used to determine plasma leptin in rats according to the manufacture's protocol. The RayBio® Ghrelin Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay which used for detecting plasma ghrelin peptide based on the principle of Competitive Enzyme Immunoassay.

Statistics

Data are expressed as means \pm standard deviation as shown in the following paragraph of results and significance established for p-values was below 0.05. Normally distributed parameters were tested with an unpaired, two-tailed student's *t*-test, and diagrammatic graphs represented by a cluster bar charts as a function of variable were obtained using MINITAB 14 program.

Results

The figure (1: A&B) show the effect of hyperserotoninemia on body weight at short and long-term studies. We have a prior knowledge that rats s.c. injected with serotonin promote a durable hyperserotoninaemia quantified in whole blood [30,31]. Rats had significantly ($p = 0.003$) lesser body weights after 7 days serotonin injection (200 ± 9.31 g) confronting to controls (224 ± 11.9 g). Also found that the long-term serotonin treatment for 3 months resulted in decreased body weight (234 ± 7.12 g) collated to controls (246 ± 14.8 g, $p = 0.025$).



Figure (1): Effects of hyperserotoninemia on body weight of rats; "A" represents weights (gm) after one week of injected rats (serotonin*) with p-value = 0.003 compared to control group;"B" reveals long-term effects (serotonin*) on weights of rats with p-value = 0.025 compared to control group.

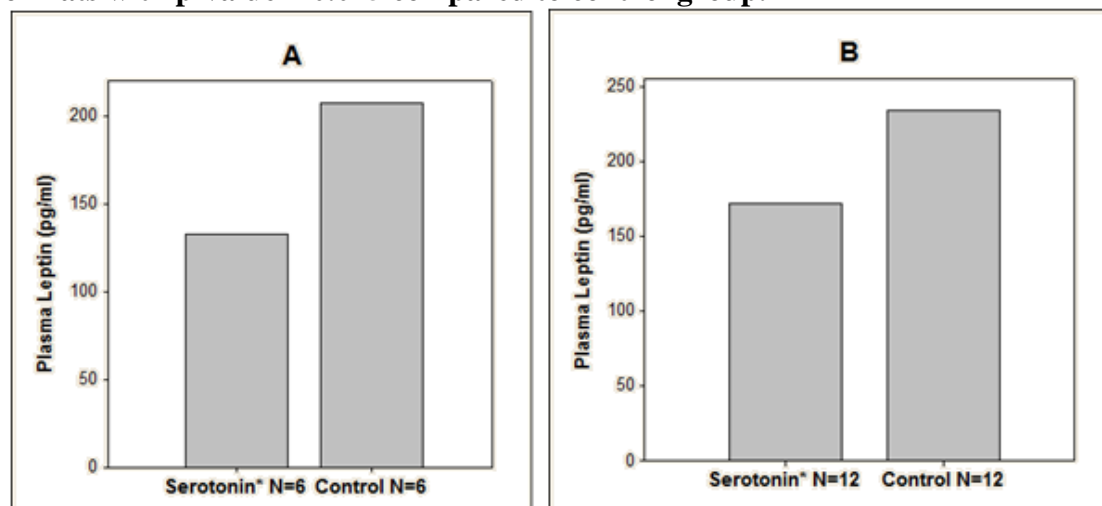


Figure (2): Effects of hyperserotoninemia on plasma leptin concentrations in rats; "A" represents plasma leptin concentrations of short-term influences of serotonin injected rats (serotonin*) with p-value = 0.029 compared to control group;"B" reveals long-term effects (serotonin*) on plasma leptin concentrations with p-value = 0.007 compared to control group.

Figure (2:A) reveals the decreased concentrations of plasma leptin (133 ± 32.0 pg/ml) in rats syringed with serotonin compared to controls after short-term of treatment (208 ± 58.4 pg/ml) significantly ($p = 0.029$) in mean. Figure (2:B) reveals that the concentrations of circulating leptin were also decreased after long-term treatment significantly (172 ± 35.6 vs. controls: 234 ± 60.5 pg/ml; $p = 0.007$).

Controversially, circulating ghrelin concentrations was stable and unaffected in serotonin treated animals

observed in short-term treatment 3.19 ± 0.0631 ng/ml vs. controls: 3.24 ± 0.0827 ng/ml and in long-term treatment: 3.62 ± 0.0219 ng/ml vs. controls 3.61 ± 0.0277 ng/ml.

Discussion

People mostly facing hard of weight diminution in spite of diets regimen and exercise courses, and arduousness of keeping weight loss.[32] The serotonergic system, is implicated in the CNS regulation of food consumption and energy homeostasis and may stipulated as anagrammatic

target, especially at adipose tissue level.

We have shown a reduction in a body weight within one week after serotonin injection subcutaneously (200 ± 9.31 g) confronting to controls (224 ± 11.9 g, $p = 0.003$). In addition, long-term serotonin treatment for 3 months resulted in decreased body weight (234 ± 7.12 g) collated to controls (246 ± 14.8 g, $p = 0.025$). This indicate a direct influence of circulating serotonin on adipocytes as well as its leptin production where significantly reduces leptin concentrations both at short and long-term administrations compared to controls (133 ± 32.0 vs. control 208 ± 58.4 pg/ml with clinical significance $p = 0.029$) and (172 ± 35.6 vs. controls: 234 ± 60.5 pg/ml; $p = 0.007$) respectively as shown in figure (1; A & B). Keep in mind that circulating serotonin couldn't cross blood brain barriers [33] this may present new prime in controlling body weight and energy metabolism.

Appetite and food intake are peripherally ordered by gastrointestinal tract and adipose tissue, and these signals are centrally coordinated mainly in the hypothalamus.[34] Circulating leptin concentrations are directly proportional to adiposity in animals and humans and correlate better with total fat mass than with body weight [35]. Concomitantly low leptin concentrations with hyperserotoninemia may reflects and explain a correlate regulation of energy homeostasis between these factors, where increases in one regulatory system performance (peripheral serotonin) driving back the other regulatory system role (plasma leptin) as shown in our results above. Evidently, these two factors work at the same direction of weight losing.

Additionally, plasma ghrelin concentrations didn't affected by

hyperserotoninemia may promote the interplay of regulatory systems of energy homeostasis. Clearly, the diarrheal effect of high plasma serotonin[36] results in constant concentrations of ghrelin as a hunger signal [37] and a potent orexigenic hormone within the central nervous system. [38] both in short and long-term studies as observed in results. Hypothalamic feeding centers including the paraventricular nucleus and the lateral hypothalamus, [39] can respond to the hunger signal and execute effectors commands via the endocrine and autonomic systems. These ghrelin-regulated mechanisms ensure the homeostatic control over feeding and energy expenditure [40]. Consequently, circulating serotonin, leptin and ghrelin with another factors interplay crucially in food intake and energy consumption.

Our results consistent with A. K. Stunes study [41] that (serotonin may regulate adipocyte function in a direct manner via the blood circulation and/or paracrine and autocrine mechanisms, and not only indirectly via the CNS as previously assumed). In contrast to our results, Justin D Crane et al,[42] show that serotonin is elevated in obesity as primary metabolite among others that inhibits the activity of brown adipose tissue in mammals. Thus, tryptophan hydroxylase-1 inhibition minimizes serotonin synthesis which may be effective in reversing obesity.

As a conflict of this study was the appearance of clinical signs in long-term treatment animals, such as flushing, loose stools and anorexia. These signs was also observed as pathologic cardiac disease as shown by Bjom, et al.[43]

In conclusion hyperserotoninemia results in low concentrations of plasma leptin with unaffected ghrelin concentrations that may exerts a direct influence of serotonin on adipose

tissues with a significant reduction in body weights of rats compared to control group.

Future studies should focus on long-term effects of serotonergic drugs on heart and heart valves. Investigations that explain whether specific serotonin receptor antagonists can prevent cardiac changes resulting from hyperserotoninemia may be of clinical importance.

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