

# The Effects of 5-Hydroxytryptamine and Interaction With Kisspeptin in Controlling Ghrelin and Adiponectin Secretion

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## Abstract

**Introduction:** 5-Hydroxytryptamine (5HT) and kisspeptin are satiety factors, and ghrelin and adiponectin are orexigenic peptides. In this study, the mediating role of 5HT in the effects of GPR54 receptor blocking on ghrelin and adiponectin secretion was investigated.

**Methods:** Male Wistar rats weighing 220-250 g received saline, kisspeptin 10 (1 or 3 nmol), 5HT (2, 5 or 10 µg), kisspeptin plus 5HT, or peptide 234 (P234) plus 5HT via the third cerebral ventricle. Serum ghrelin and adiponectin concentrations were determined using the enzyme-linked immunosorbent assay method.

**Results:** Kisspeptin or 5HT treatment significantly reduced serum concentrations of ghrelin and adiponectin in comparison with saline. The injection of 2 nmol of P234+5HT significantly reduced serum ghrelin concentration compared to the 5HT alone, kisspeptin alone, and kisspeptin+5HT groups. Injections of either 1 or 2 nmol of P234 along with 5HT significantly declined the mean serum adiponectin level compared to rats received the kisspeptin plus 5HT.

**Conclusion:** Kisspeptin/GPR54 signaling pathway may be a putative target in serotonergic neurons to control ghrelin and adiponectin secretion.

**Keywords:** 5-Hydroxytryptamine, Ghrelin, Adiponectin, Kisspeptin, Peptide 234

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## Introduction

Central and peripheral hormones act together in hypothalamic levels to control food intake. 5-Hydroxytryptamine (5HT), known as serotonin, is produced by enterochromaffin cells of the gastrointestinal tract and brainstem. Its neural axons project to the hypothalamus.<sup>1-2</sup> The 5HT is a satiety factor, and its receptor antagonists are extensively used for controlling body weight and treatment of obesity.<sup>3</sup>

Kisspeptin is a hypothalamic neuropeptide. Its physiological is mediated by G protein-coupled receptor named GPR54.<sup>4</sup> Kisspeptin, and GPR54 is expressed in hypothalamus.<sup>5,6</sup> In addition to regulating the reproduction, kisspeptin/GPR54 signaling pathway plays a crucial role in regulating energy balance, and reduces food intakes.<sup>5,6</sup> Peptide 234 (P234) functions as antagonist of GPR54 and it blocks the physiological functions of kisspeptin.<sup>4</sup>

Adiponectin is a peptide that is mainly synthesized in adipose tissue and hypothalamus.<sup>7</sup> It regulates energy homeostasis, food intake, and reproduction.<sup>7</sup> It inhibits GnRH/LH secretion.<sup>8</sup> Also, it increases food intakes and declines energy expenditure through its central effects in the hypothalamus.<sup>7</sup>

Ghrelin is an orexigenic peptide that is synthesized in the stomach and hypothalamus.<sup>9</sup> It stimulates food intakes via binding to growth hormone secretagogues receptor (GHSR-Ia).<sup>11</sup> Ghrelin receptor antagonists are clinically used as anti-obesity drugs.<sup>11</sup> Efforts to find the involved agents in regulating ghrelin and adiponectin production could be essential for the regulation of obesity and energy homeostasis. The present study aimed to investigate the effects of 5HT and kisspeptin on ghrelin and adiponectin secretion. It aimed to determine the effects of 5HT on ghrelin and adiponectin secretion following blocking the GPR54 receptor.



## Materials and Methods

### Animals and Experimental Procedure

Male Wistar rats (n=50) weighing 230-250 g were used for this study. Food and water were provided for animals freely all the time.

Animals were anesthetized using ketamine and xylazine. The 22-gauge stainless cannulae was implanted into the third cerebral ventricle coordinates (AP=- 2.3, ML=0.0, DV=6.5).<sup>11</sup> After 1 week recovery period, 50 rats in 10 groups (n=5 in each group) received saline (3  $\mu$ L), kisspeptin (1 or 3 nmol/3  $\mu$ L), 5HT (2, 5 or 10  $\mu$ g/3  $\mu$ L), kisspeptin (1 nmol/1.5  $\mu$ L)+5HT (10  $\mu$ g/1.5  $\mu$ L), kisspeptin (3 nmol/1.5  $\mu$ L)+5HT (10  $\mu$ g/1.5  $\mu$ L), P234 (1 nmol/1.5  $\mu$ L)+5HT (10  $\mu$ g/ 1.5  $\mu$ L) or P234 (2 nmol/1.5  $\mu$ L)+5HT (10  $\mu$ g/ 1.5  $\mu$ L). The 5HT (Sigma Aldrich, USA), P234 (Phoenix Pharmaceutical Co, USA), and kisspeptin10 (AnaSpec Co, U.S.A.) were dissolved in distilled water and they were injected using Hamilton micro syringe at 8:00-9:00. P234 was injected 10 minutes before the 5HT or kisspeptin. Doses of drugs and time point of blood sampling were chosen based on previous studies which demonstrated the effective actions of kisspeptin or P234 on the neuroendocrine axis at 60 minutes following injections.<sup>11,12</sup> Also, based on previous studies, the acute injection method was chosen for the present study. The drugs were injected once via third cerebral ventricular.<sup>4,11</sup>

### Hormone Assays

After one hour blood samples were collected via tail vein. Mean serum ghrelin and adiponectin concentrations were determined by using rat ghrelin and adiponectin kit (East Biopharm Co., China) by enzyme-linked immunosorbent assay (ELISA). The sensitivity of ghrelin and adiponectin kits were 25.59 ng/L and 0.16 mg/L, respectively.

### Statistical Analysis

The data are presented as mean  $\pm$  standard error of mean (SEM). SPSS software and one-way ANOVA followed by post hoc Tukey test was used to analyze data. Significance was defined by  $P < 0.05$ .

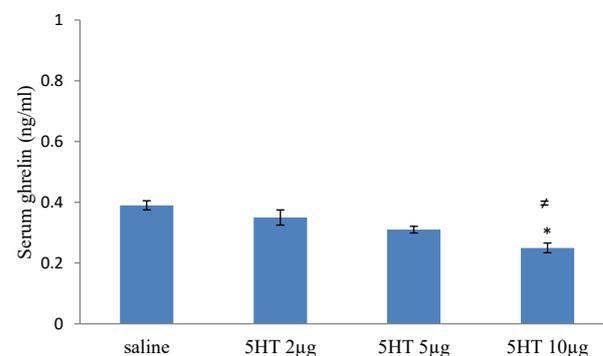
### Results

Injections of 2 or 5  $\mu$ g 5HT did not decrease serum ghrelin levels in comparison to saline. 5  $\mu$ g 5HT did not decrease the ghrelin levels compared to 2  $\mu$ g group. The injections of 10  $\mu$ g 5HT significantly decreased the serum ghrelin levels in comparison to saline or 2  $\mu$ g 5HT receiving groups ( $P < 0.05$ ). The ghrelin levels did not decrease following the injection of 10 $\mu$ g 5HT compared to 5  $\mu$ g 5HT receiving group (Figure 1).

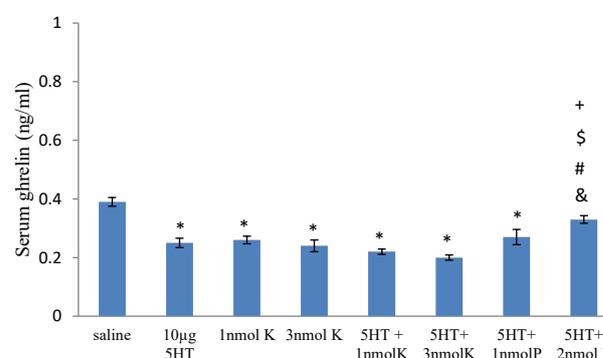
one or 3 nmol kisspeptin declined significantly serum ghrelin levels compared to saline ( $P < 0.05$ ). However, a significant decline was not occurred between the effects

of 1 nmol and 3 nmol kisspeptin groups on ghrelin secretion. One nanomole kisspeptin plus 10  $\mu$ g 5HT or 3 nmol kisspeptin plus 10  $\mu$ g 5HT significantly decreased the serum ghrelin level in comparison to saline ( $P < 0.05$ ). However, a significant decline was not observed between 1 nmol kisspeptin plus 10  $\mu$ g 5HT, 3 nmol kisspeptin plus 10  $\mu$ g 5HT, 10  $\mu$ g 5HT, 1 nmol, or 3 nmol kisspeptin groups. Ghrelin levels showed a significant decrease in rats receiving an injection of 1 nmol P234 plus 10  $\mu$ g 5HT compared to saline ( $P < 0.05$ ). In contrast, 2 nmol P234 and 10  $\mu$ g 5HT did not significantly decrease the ghrelin level compared to saline (Figure 2). The 1 nmol P234 was not able to block inhibitory effects of 5HT on ghrelin, while 2 nmol P234 blocks the inhibitory effects of 5HT on ghrelin. In rats receiving 2 nmol P234 plus 10  $\mu$ g 5HT, the ghrelin level showed a significant increase in comparison to 10  $\mu$ g 5HT, 3 nmol kisspeptin, 1 nmol kisspeptin plus 10  $\mu$ g 5HT, or 3 nmol kisspeptin plus 10  $\mu$ g 5HT groups ( $P < 0.05$ ). However, a significant increase was not occurred in ghrelin concentration in 2 nmol P234 plus 10  $\mu$ g 5HT group compared to 1 nmol P234 plus 10  $\mu$ g 5HT receiving rats (Figure 2).

Adiponectin levels did not decrease significantly in 2 or 5  $\mu$ g 5HT receiving rats in comparison to saline. A significant decrease was not observed between 5  $\mu$ g 5HT and 2  $\mu$ g 5HT groups. 10  $\mu$ g 5HT significantly decreased



**Figure 1.** The Effects of 5-Hydroxytryptamine (5HT) on Mean Serum Ghrelin Concentration. \* Compared to saline, # Compared to 2  $\mu$ g 5HT.



**Figure 2.** The Effects of 10  $\mu$ g 5-Hydroxytryptamine (5HT), 1 or 3 nmol Kisspeptin 10 (K), or Kisspeptin 10 (K) Plus 5HT or P234 (P) Plus 5HT on Mean Serum Ghrelin Concentration. \* Compared to saline; & Compared to 10  $\mu$ g 5HT, # Compared to 3 nmol kisspeptin (K), \$ Compared to 1 nmol kisspeptin (K) plus 5HT, + Compared to 3 nmol kisspeptin (K) plus 5HT.

adiponectin levels compared to saline or 2  $\mu$ g 5HT ( $P < 0.05$ ). Mean adiponectin levels did not significantly decrease following injections of 10  $\mu$ g 5HT compared to 5  $\mu$ g 5HT (Figure 3).

Mean adiponectin levels significantly decreased in 10  $\mu$ g 5HT, 3 nmol kisspeptin, 1 nmol kisspeptin plus 10  $\mu$ g 5HT or 3 nmol kisspeptin plus 10  $\mu$ g 5HT in comparison to the saline ( $P < 0.05$ ). However, the mean adiponectin levels did not show a significant decrease in rats receiving 1 nmol kisspeptin, 1 nmol P234 plus 10  $\mu$ g 5HT, or 2 nmol P234 plus 10  $\mu$ g 5HT in comparison to the saline (Figure 4). The 1 or 2 nmol P234 was able to block the inhibitory effects of 5HT on adiponectin. Adiponectin levels showed a significant increase in rats receiving 1 nmol P234 plus 10  $\mu$ g 5HT or 2 nmol P234 plus 10  $\mu$ g 5HT in comparison to 1 nmol kisspeptin plus 10  $\mu$ g 5HT or 3 nmol kisspeptin plus 10  $\mu$ g 5HT ( $P \leq 0.05$ ). However, a significant increase was not observed between 1 nmol P234 plus 10  $\mu$ g 5HT or 2 nmol P234 plus 10  $\mu$ g 5HT and 10  $\mu$ g 5HT, 1 or 3 nmol kisspeptin groups (Figure 4).

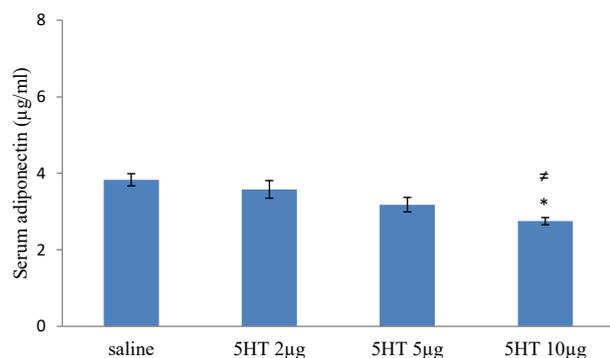
## Discussion

The present data indicated that ghrelin concentration suppressed following the central injection of 5HT. The findings are in accordance with influence of drugs that increase the 5HT secretion like fenfluramine, meta-

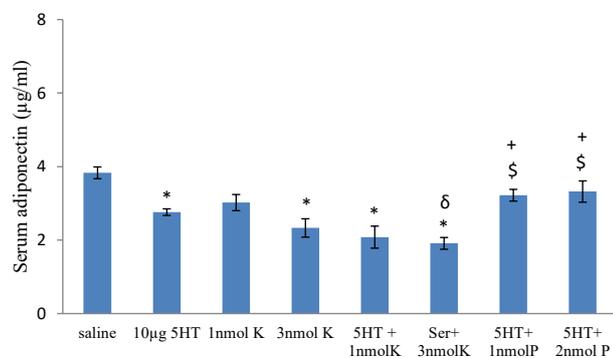
chlorophenylpiperazine or the ones which block 5HT reuptake.<sup>13-15</sup> These drugs decrease ghrelin secretion, food intakes, and body weight via activating the 5HT<sub>2C</sub> and 5HT<sub>1B</sub> receptors of 5HT which are widely expressed in hypothalamic neurons.<sup>3</sup> The 5HT increases the production of alpha melanocyte stimulating hormone ( $\alpha$ MSH, an anorexigenic hormone), via binding to 5HT<sub>2C</sub>.<sup>1-3</sup> Serotonergic drugs inhibit the synthesis of neuropeptide Y (NPY)/agouti-related peptide (AgRP) (most important orexigenic peptides of hypothalamus) via binding to 5HT<sub>1B</sub>. Using the 5HT receptor agonists such as fenfluramine suppresses the NPY induced hyperphagia.<sup>1-3</sup> There is a direct relationship between NPY/AgRP and ghrelin concentration and a reverse relationship between  $\alpha$ MSH and ghrelin.<sup>9</sup> Also, it has been established that the blocking 5-HT<sub>2C</sub> receptor potentiates the orexigenic effects of ghrelin in vivo.<sup>16</sup> So inhibiting NPY/AgRP or stimulating  $\alpha$ MSH/CART synthesis by 5HT may be involved in mediating suppressive effects of 5HT on ghrelin.

The present data demonstrated the inhibitory influences of kisspeptin on mean serum ghrelin concentrations compared to the control rats. The results are in accordance with our previous study which investigated the interaction of dopaminergic and kisspeptin to regulate ghrelin.<sup>11</sup> To find the critical mechanisms for controlling ghrelin production by kisspeptin needs further studies. However, an increase in growth hormone (GH) levels following kisspeptin injections may play a role in suppressing ghrelin signaling.<sup>17</sup> Previous studies showed that both ghrelin and kisspeptin significantly augment GH,<sup>17</sup> and high concentration of GH in turn, inhibits ghrelin via a negative feedback mechanism.<sup>14</sup> So, a decrease of ghrelin secretion may be partly due to the stimulation of GH secretion following kisspeptin injection.

Also, the effects of blocking the GPR54 were investigated on ghrelin secretion in 5HT treated rats. Blocking kisspeptin signaling system by injection of peptide 234, caused a significant increase in serum ghrelin levels compared to 5HT, kisspeptin or kisspeptin plus 5HT groups. The present data suggested that kisspeptin/GPR54 signaling pathways may be a target for the serotonergic neurons to control ghrelin secretion. There is not any previous report about the interaction of 5HT and kisspeptin on regulation of ghrelin to compare the present results with them, but a study in zebrafish confirmed a relationship between kisspeptin and the serotonergic signaling system.<sup>18</sup> The present data indicated that 5HT exerts an inhibitory influence on adiponectin secretion. However, the present data are in agreement with the previous in vitro one which showed the suppressive effects of 5HT on adiponectin secretion from CRI-D2 cell lines derived pancreatic Langerhans islets.<sup>19</sup> Also, the results are in parallel with the study in which, 5HT<sub>2</sub> receptor antagonist significantly augmented



**Figure 3.** The Effects of 5-Hydroxytryptamine (5HT) on Mean Serum Adiponectin Concentration. \* Compared to saline, # Compared to 2  $\mu$ g 5HT.



**Figure 4.** The Effects of 10  $\mu$ g 5-Hydroxytryptamine (5HT), 1, or 3 nmol Kisspeptin 10 (K), or Kisspeptine 10 (K) Plus 5HT or P234 (P) Plus 5HT on Mean Serum Adiponectin Concentration. \* Compared to saline;  $\delta$  Compared to 1 nmol kisspeptin, \$ Compared to 1 nmol kisspeptin plus 5HT, + Compared to 3 nmol kisspeptin plus 5HT.

adiponectin in type 2 diabetic patients who suffer from decreased blood concentration of adiponectin.<sup>20</sup>

The present results demonstrated that central injection of kisspeptin caused a significant decrease in serum adiponectin concentration. This data is in agreement with previous studies were done in vitro or pathological situations. There is a negative correlation between kisspeptin and adiponectin so that, adiponectin results in a significant decrease of kisspeptin gene expression level in CRI-D2 cell lines derived pancreatic Langerhans islets in male rats.<sup>20</sup> Moreover, a decrease in adiponectin concentration is accompanied with an increase in kisspeptin secretion and synthesis levels in polycystic ovary syndrome.<sup>21,22</sup>

The results related to the effects of 5HT on adiponectin secretion following blocking the GPR54 receptor showed that serum adiponectin levels in rats receiving the peptide 234 plus 5HT did not significantly increase compared to only 5HT or only kisspeptin receiving rats. There is not any previous report about the interaction of 5HT and kisspeptin in controlling the adiponectin secretion yet. The present data suggested that other intra hypothalamic pathways than hypothalamic kisspeptin/GPR54 signaling pathway may contribute to the inhibitory effects of 5HT on adiponectin secretion. However, further pharmaceutical and molecular studies are needed to conclude the precise mechanisms for interaction of kisspeptin and serotonergic pathways for regulating the ghrelin and adiponectin secretion.

### Conclusion

The 5HT, kisspeptin, or injection of 5HT plus kisspeptin significantly declined the mean serum ghrelin and adiponectin concentration compared to saline. Blocking kisspeptin receptor significantly increased ghrelin concentration compared to only 5HT, only kisspeptin or kisspeptin plus 5HT. Blocking kisspeptin receptor did not significantly increase adiponectin levels compared to only 5HT or only kisspeptin groups. Kisspeptin/GPR54 signaling pathways may be a target for the serotonergic neurons to control ghrelin secretion. Other intra hypothalamic pathways than hypothalamic kisspeptin pathway may contribute to inhibitory effects of 5HT on adiponectin secretion.

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### Authors' Contribution

FMa conceived and planned the experiments. MK and FMo carried out the experiments. FMa, HK and AB contributed to the interpretation of the results. FMa took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, data analyses, and the manuscript.

### Competing Interests

The authors have nothing to disclose. There is no conflict of interest in this article.

### Ethical Approval

All experimental procedures were done according to the guidance of the Research and Ethics Committee of the University of Mohaghegh Ardabili (IR.UMA.REC.1400:054)

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