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Serotonin receptor 5-HT$_{5A}$ in rat hippocampus decrease by leptin treatment

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ABSTRACT

5-Hydroxytryptamine (5-HT) is involved in a variety of different physiological processes and behaviors through the activation of equally diverse receptors subtypes. In this work we studied the changes on the expression of 5-HT$_{5A}$ receptors in rat hippocampus induced by leptin, an adipocyte-derived hormone that has been reported to participate in the modulation of food intake and in adult hippocampal neurogenesis. To study the effect of leptin on the 5-HT$_{5A}$ receptor gene expression a qRT-PCR was used and the distribution of those receptors in the hippocampus was visualized by immunohistochemistry. Rats were separated in four groups: control (untreated rats), leptin-treated, serotonin-treated and leptin + serotonin treated. The results showed that even though the 5-HT$_{5A}$ gene expression did not change in the hippocampus of any of the treated groups, in the rats treated with leptin and serotonin, the specific immunostaining for the 5-HT$_{5A}$ serotonin receptor decreased significantly in the dentate gyrus.

Serotonin (5-hydroxytryptamine; 5-HT) is involved in many physiological processes and behaviors such as appetite [9,17,18], sleep, vascular contraction, sexual activity, locomotion, pain perception [19] and mood depression [23,5]. It also participates in the late neurogenesis that has been observed in the hippocampus of adult rats [26]. A dysfunction of serotonergic neurotransmission has been associated with neurological disorders such as depression, anxiety and schizophrenia [7]. Serotonin action is mediated by seven receptor families, including the G protein-coupled receptor 5-HT$_{5A}$ [8,16,21], which is widely distributed in different hippocampal layers during brain development and in the mature brain [11,22].

Leptin is a 16 kDa peptide secreted from white adipocytes [25,14] and has been implicated in several functions such as the regulation of food intake, energy expenditure, modulation of body mass, insulin sensitivity, reproductive capacity [13,24] as well as serotonin turnover [2] and has a neuroprotective effect in cases of neural injuries [20]. Moreover, recent findings have shown that leptin facilitates spatial learning and is also involved in hippocampal neurogenesis [12]. Leptin receptors are located in several parts of the central nervous system, including the hippocampus where they coexist with serotonin receptors [15,10]. In view of the close association between leptin and serotonergic receptors in the hippocampus, leptin was examined to see if it modifies the expression and distribution of 5-HT$_{5A}$ receptors in this area of the brain.

Adult Sprague–Dawley male rats were treated at age 40 ± 1 days (147 ± 13.6 g). The rats were housed at 22 ± 2 °C, humidity 55 ± 8% with free access to food and water and 12 h light/dark cycle. To explore the effect of leptin on expression of the 5-HT$_{5A}$ receptor gene, the rats were separated into four groups with three animals in each one. Rats were injected intraperitoneally (IP) with: (1) the vehicle (control); (2) leptin, 75 μg/rat; (3) serotonin, 7.5 mg/rat; and (4) leptin + serotonin. After 1 h the rats were anesthetized with an IP injection of sodium pentobarbital (40 mg/kg), decapitated and then the brain was rapidly removed and divided in two halves.

One half of the brains were used for qRT-PCR experiments; therefore, the hippocampus was dissected and quickly frozen in liquid nitrogen. The RNA was extracted with Trizol® reagent (Invitrogen, USA), purified with RNeasy Mini Kit (Qiagen, Germany), and then treated with Deoxyribonuclease I (Invitrogen, USA). The cDNAs were obtained by Omniscript (Qiagen, Germany). The qRT-PCR was performed by duplicate in a Roche Light Cycler with LightCycler® FastStart DNA master SYBR green I (Roche, Germany) using the following primers: 5-HT$_{5A}$ (F) 5′-ctg tgc tgg tta tgc ctc t-3′

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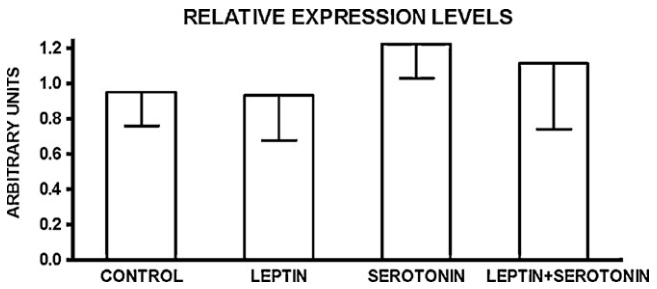

Fig. 1. Expression levels of 5-HT_{5A} receptor mRNA in hippocampus tissue. Quantitative analysis was performed by qRT-PCR and normalized to the expression of 18s. Data are presented as a mean ± SD of the ratio of activation times of the 5-HT_{5A}/18s gene (three experiments by duplicate), in arbitrary units. The results did not show statistical differences among the groups.

(R) 5′-gag aca cgc ttg cgg gc-3′; 18s gene (F) 5′-gta acc cgt tga acc cca tt-3′ (R) 5′-cca tcc aat cgg tag tag cg-3′.

The second half of the brains were used for immunohistochemistry and was fixed with 4% paraformaldehyde overnight. The fixed brains were cryoprotected with 30% sucrose and frozen in Jung tissue freezing medium (Leica, Germany). Brains were sliced with a cryostat into 12 μm coronal sections and stored until processing for immunohistochemistry as described previously [10]: briefly, endogenous peroxidase and unspecific sites were blocked by pretreatment with 1% H_2O_2 and 3% milk and then the tissues were incubated overnight with anti-5HT_{5A} (1:100) (Santa Cruz, USA). The second day the tissues were incubated with the goat anti-rabbit biotin conjugated antibody (1:750) (Invitrogen, USA) for 2 h, and then treated with the ABC-horsedish peroxidase conjugates (Vector Laboratories, USA). The color was obtained with diaminobenzidine (Sigma–Aldrich, USA) and H_2O_2. The quantification for the intensity values was computed on a gray scale with KS300 software (Carl Zeiss), using the controls without the primary antibodies to normalize density values.

The results were expressed as mean ± SD. Statistical analyses were performed with a one-factor ANOVA followed by Tukey *post hoc* comparisons and $P \leq 0.05$ was considered statistically significant.

The internationally accepted recommendations for the care and use of experimental animals were followed in this work.

On examining the gene expression of the 5-HT_{5A} receptor in the hippocampus by qRT-PCR, no significant differences were found in any of the treated groups as compared with the control (Fig. 1). The results shown here are the mean ± SD of three different experiments in duplicate and normalized to the *housekeeping* gene 18 s with constant expression labels.

In the immunohistochemical experiments, the slides treated with the secondary antibody alone, and those with no antibody, did not present a detectable signal. In the group of control animals, the 5-HT_{5A} receptors in rat hippocampus were widely distributed over the four hippocampal layers (Fig. 2A).

The 5-HT_{5A} receptor in the CA (Ammon's horn) 1 hippocampal layer showed intense immunostaining in the control rats and was significantly decreased in the leptin treated rats (Fig. 2). In the serotonin and the leptin + serotonin treated groups the signal decreased, but the difference was not statistically significant compared with the control.

In the dentate gyrus a significant decrease of immunostaining in the leptin was observed compared with the control and also in the serotonin-treated group compared with the leptin treated group (Figs. 2 and 3); indicating that the injected leptin as well as serotonin induced a reduction of the 5-HT_{5A} receptors in this region. The immunostaining of the CA2 and CA3 regions were not different compared with the control (Fig. 3).

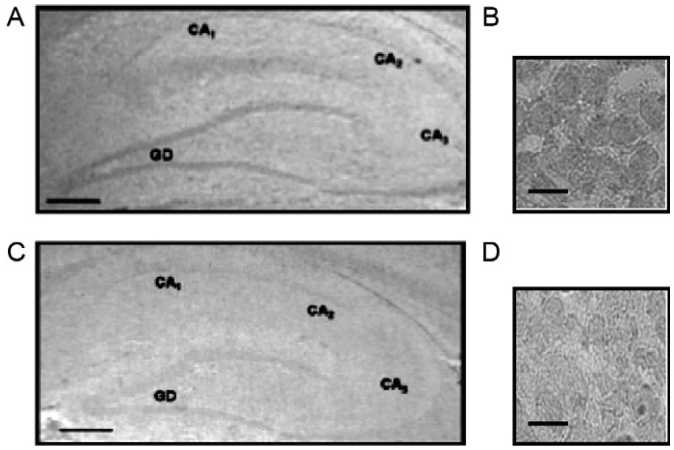

Fig. 2. Distribution of 5-Ht_{5A} receptors in rat hippocampus. (A) Control immunostaining of hippocampus coronal sections, (B) dentate gyrus cells in control rats, (C) in the leptin treated rats the signal significantly decreased in dentate gyrus and (D) dentate gyrus cells in leptin treated rats. Bar: 200 μm (A and C). Bar: 10 μm (B and D).

This study is the first to use qRT-PCR in order to evaluate the changes induced by leptin on the 5-HT_{5A} receptors in the hippocampus. In each qRT-PCR, the negative control was amplified with the samples, in order to confirm that neither genomic DNA contamination nor non-specific amplification occurred.

These results suggest that the treatments used in these experiments did not increase the expression of the 5-HT_{5A} receptor gene during the first H after initiated the treatment with leptin, serotonin or both. It is therefore speculated that this lack of effect may be due to leptin loss by absorption or may be associated with the time necessary to activate the leptin signal transduction [14], as well as the 5-HT_{5A} receptor transcription. On the other hand the results could indicate that the leptin pathways do not regulate the transcription of that particular gene.

The distribution of 5-HT_{5A} receptors in the hippocampus of the control rats was consistent with other reports [10,25]; and the decrease of 5-HT_{5A} receptor immunostaining in the rats treated with leptin suggests that leptin participates in regulating the number of 5-HT_{5A} receptors present in the membrane of neurons in

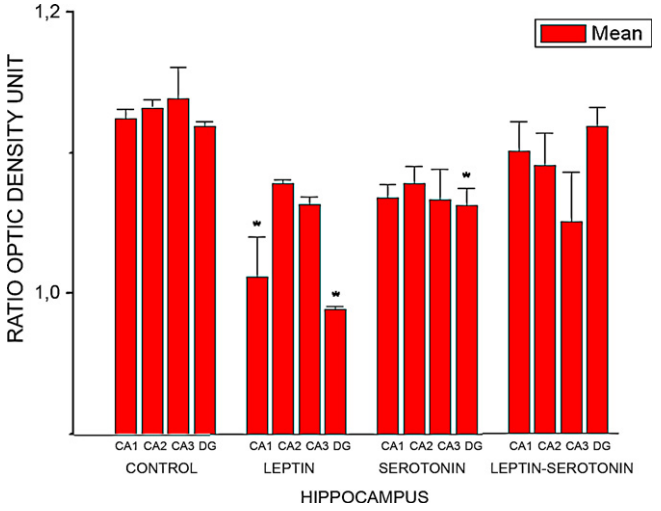

Fig. 3. Densitometry of the 5-HT_{5A} receptors in the hippocampus. Densitometry of CA (Ammon's horn)1, CA2, CA3 and dentate gyrus in the rats: control, and the leptin, serotonin and leptin–serotonin treated rats. The bars indicate the mean ± SD (standard deviation), and asterisks on the bars stand for significant difference compared with the same layer in the control group. $P \leq 0.05$.

the CA1 and dentate gyrus hippocampal regions. These results are important because the decrease of serotonin 5-HT_{5A} receptors in the hippocampus could be associated with emotional disorders [23], as well as with changes on the neurogenesis in the dentate gyrus in Alzheimer disease [1,4]. It will be interesting to extend this study, in order to determine if the mechanisms of such interaction are associated with receptor internalization [2]. In addition, it is also important to extend the study of leptin effects to receptors and ion channels that could modulate hippocampal functions [6,3].

In summary, this study provides an initial comparative analysis of the abundance of 5-HT_{5A} receptors in rats treated with either leptin or serotonin, or with both. It was found that leptin reduces the number of 5-HT_{5A} receptors in the plasma membrane of neurons of the CA1 and dentate gyrus. However, further investigation is necessary to determine the way in which leptin induces these changes and also to explore its possible participation on the function of the receptors.

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