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ORIGINAL ARTICLE

Effect of cannabinoid-serotonin interactions in the regulation of neuropeptide Y1 receptors expression in rats: the role of CB1 and 5-HT_{2C} receptor

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Abstract

Neuropeptide Y (NPY) is involved in a diversity of critical functions such as circadian rhythms, energy homeostasis, and appetite regulation in the hypothalamus. It has identified as a crucial participant in adjusting energy intake and energy storage as fat via central neuropeptide Y1 receptor (NPY1R), leading to obesity and metabolic disorders. The present study was expected to investigate the interaction between 2-AG (CB1R agonist), m-CPP ($5HT_{2C}$R agonist), SB-242084 ($5HT_{2C}$R antagonist), and SR-141716A (CB1R antagonist) by mediating through the NPY1R for treating or preventing obesity, metabolic disorders, and other abnormalities. The expression level of NPY1R mRNA has studied on the rat brain by real-time quantitative PCR assay. Based on our findings, intracerebroventricular (ICV) injection of combined 2-AG (1 μg) + m-CPP (2.5 μg) has antagonistic interaction in the expression of the NPY1R gene ($P < 0.001$). Moreover, the ICV co-injection of SB-242084 (3 μg) + SR-141716A (1 μg) has antagonistic interaction in the NPY1R gene expression ($P < 0.001$). Co-administration of 2-AG (1 μg) + SB-242084 (3 μg) amplified NPY1R gene expression ($P < 0.001$), while the ICV co-injection of m-CPP (2.5 μg) + SR-141716A (1 μg) decreased NPY1R gene expression in the hypothalamus ($P < 0.001$). These results revealed the interference in cannabinoid and serotonergic systems via CB1 and $5HT_{2C}$ receptors in the expression of NPY1R mRNA in the hypothalamic area of rats.

Keywords Hypothalamic NPY1 receptor · SB-242084 · M-CPP · 2-AG · SR-141716A · Obesity · Metabolic syndrome

Introduction

Neuropeptide Y (NPY) with a 36 amino-acid peptide shares a significant structural homology with pancreatic peptides, which is involved in a variety of critical functions such as anxiety, blood pressure, circadian rhythms (Sindelar et al. 2005), energy homeostasis, and appetite regulation through binding to a family of G protein-coupled Y receptors (GPCR) and classified as Y1R, Y2R, Y4R, Y5R, and Y6R subtypes (Blomqvist and Herzog 1997). NPY is one of the most potent hyperphagic neuropeptides produced at the different locations in the brain, particularly the hypothalamus with the highest concentration in the arcuate nucleus (ARC) (Bell et al. 2005; Lin et al. 2004). It has been identified as a critical participant in adjusting the energy expenditure and pathophysiology of obesity (Hokfelt et al. 2008). NPY administration in a particular hypothalamic region or the cerebral ventricle leads to a rapid increase in food intake and suppresses energy consumption, which ultimately leads to the storage of energy as fat and consequently results in obesity and metabolic disturbances over time (Loh et al. 2015). Evidence from other studies suggests that neuropathic signals are associated with obesity and metabolic disorders through the central system. The NPY1 receptor is one of the highest numbers of recipients in obesity research due to its initial diagnosis as a "nutrition receptor" for NPY's overeating mediation (Lundberg et al. 2007).

Cannabinoids are found basically in two forms including the exogenous form as psychoactive components found in marijuana (Δ_9–tetrahydrocannabinol, THC) (Novoseletsky et al. 2011) and endogenous form as the arachidonic acid

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derivative compounds formed through binding to the two cannabinoid receptors including CB1R and CB2R belonging to the GPCRs in the brain and the peripheral areas (Devane et al. 1988; Matsuda et al. 1990; Munro et al. 1993). CB1R is predominantly expressed in the hypothalamus (Wittmann et al. 2007), and activation of CB1R results in producing distinct locomotion, cognitive analgesia, stimulation of appetite, and metabolism of lipid and glucose for maintaining the energy homeostasis (Kirkham 2005). Previous studies showed the stimulated food consumption following the intracerebroventricular (ICV) administration of CB1R agonists in rats. However, SR-141716A (as a potent, selective CB1 receptor antagonist, rimonabant) adversely caused a decrease in food intake in rats (Chen et al. 2006). The orexigenic function of the hypothalamic CB1 receptors and an increased expression level of NPY in the ARC are also well documented in rats (Akabayashi et al. 1993; Cota et al. 2003; Sergeyev et al. 2000).

A few significant roles of 5-HT in influencing the appetite are facilitated mainly by $5HT_{2C}R$ through GPCR (Gq, phospholipase C). There are several vital functions of serotonin, including energy balance mediators of the hypothalamus, regulation of mood, locomotion, feeding, and reproductive behavior (Berglund et al. 2013; Burke et al. 2014). The administration of m-CPP as a $5HT_{2C}R$ agonist caused a decrease in central food intake and hunger (Sargent et al. 1997). In this experiment, SB-242084 was used to assess the consequence of blockade in the central $5HT_{2C}R$ (Sant'Ana et al. 2019).

The route of interaction between serotonin and other anorexigenic agents is possible through the $5\text{-}HT_{2C}$ receptor, for instance, the anorexic function of urocortin (Harada et al. 2014), LPS (lipopolysaccharide) (Kopf et al. 2010), CCK (cholecystokinin), and GLP (glucagon-like peptide) (Asarian 2009).

The orexigenic and anorexigenic signals are taking part in controlling hypothalamic NPY/AgRP (agouti-related protein) and POMC/CART systems (proopiomelanocortin/cocaine and amphetamine-regulated transcript) to coordinate the balance between food intake and energy consumption (Bell et al. 2005). The inhibition of central NPY function is induced by anorexigenic factors including leptin and alpha-melanocyte-stimulating hormone (a-MSH) (King et al. 2000), whereas the expression of c-fos, as the NPY neuron, is caused by orexigenic factors such as orexin (Horvath et al. 1999; Yamanaka et al. 2000). The effect of synergistic interaction between cannabinoids and serotonergic systems on food intake has investigated before (Wierucka-Rybak et al. 2016); however, such cooperation was approved further according to their similar function and localization in the same areas of the nervous system. The orexigenic and anorexigenic nature of cannabinoids and serotonin, and their roles in the enhancing neurotransmitter release, has suggested that they control the release of NPY. Many studies indicated that the increase in the hypothalamic NPY level mediates anabolic effects through hypothalamic NPY1Rs.

Therefore, there is a need for researching medical science and the pharmaceutical fields to design and develop drugs for modulating central NPY signaling in combating obesity, metabolic disorders, and other abnormalities that resulted from these hormonal changes. Employing these drugs (m-CPP, SB-242084, 2-AG, SR-141716A) for inhibiting obesity or provoking appetite and smoking session has revealed several side effects. In the current study, the sub-effective doses are used to minimize those side effects in the drugs compound.

Thus, this study intended to investigate the possible interactions between CB1R plus $5HT_{2C}R$ agonists and CB1R plus $5HT_{2C}R$ antagonists and examine the interactions between CB1R agonists plus $5HT_{2C}R$ antagonist and CB1R antagonist plus $5HT_{2C}R$ agonist on the expression of NPY1R in the hypothalamic area of rats.

Materials and methods

Experimental drug

2-Arachidonylglycerol (2-AG), as the selective endogenous cannabinoid-1 (CB1) receptor agonist; rimonabant hydrochloride (SR-141716A), as the selective CB1R cannabinoid antagonist; m-chlorophenylpiperazine (m-CPP), as $5HT_{2C}R$ agonist; and SB-242084, as a selective $5HT_{2C}R$ antagonist (6-Chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl) oxy]-5-pyridyl] carbamoyl]-indole), were purchased from Sigma Aldrich Co (UK). SB-242084 was dissolved in the pure dimethyl sulfoxide and then diluted with 0.9% saline until the dilution of DMSO reaches 0.05%. 2-AG, m-CCP, and SR-141716A directly diluted in 0.9% saline.

Animals, experiments, and injection procedures

About 54-male Wistar rats (6 weeks of age) weighted 150–180 g, were obtained, and are housed in standard cages with food and water available (ad libitum). All experimental procedures were performed on the animals according to the guidelines of the Care and Use of Laboratory Animals by the National Institutes of Health (USA) and approved by the Animals' Cares and Use Committee of the ethical committee of the university. On the first day of the study, animals were cannulated by a rat stereotaxic apparatus (Stoelting, USA) with a gauge 22 stainless steel cannula into the lateral ventricle [according to the Paxinos and Watson: ML = 1.6 mm; AP = − 0.8 mm; DV = 3 mm] under ketamine (60 mg/kg, IP) and xylazine (6 mg/kg, IP) anesthesia intraperitoneally, before being transferred to an individual cage. After 1 week postsurgical recovery period, rats were divided into nine treatment groups with six animals in each group, and different concentrations of

intracerebroventricular (ICV) injections of 0.5 μl of 2-AG, SR-141716A, m-CPP, and SB-242084 were administered. Group 1 was the control group that was injected with 0.5 μl normal saline, 0.9% intracerebroventricularly (ICV). Groups 2 and 3 were injected with co-administration of 2-AG (1 μg) + m-CPP (2.5 μg) after 60 (group 2) and 120 (group 3) min. Groups 4 and 5 were ICV injected with co-administration of SB-242084 (3 μg) + SR-141716A (1 μg) after 60 (group 4) and 120 (group 5) min. Groups 6 and 7 received co-administration of 2-AG (1 μg) + SB-242084 (3 μg) after 60 (group 6) and 120 (group 7) min. Groups 8 and 9 received co-administration of m-CPP (2.5 μg) + SR-141716A (1 μg) after 60 (group 8) and 120 (group 9) min. These doses of drugs were calculated based on pilot experiments (unpublished data). In pilot experiments, three doses of each substance were injected into three groups of six rats (n = 18), after which the lowest amount of substance that caused a significant difference in the expression of the NPY1R mRNA was selected for the main experiments (Figs. 1, 2, 3, and 4). At the end of the ICV administration of drugs, the rats were given food and water available (ad libitum) for 60 min or 120 min, and they were euthanized for brain processing under CO_2 inhalation anesthesia and decapitation.

Brain processing

The hypothalamic portion of the brain (as a whole) was collected from all the animals immediately before being frozen in the liquid nitrogen for any further analysis. The samples were kept at – 80 C until they were finally used.

RNA isolation

The total RNA was isolated from the hypothalamic portion of the brain using YTA total RNA purification mini kit according to the manufacturer's instruction. The final concentration of RNA was determined using ND-1000 spectrophotometer, Nanodrop®. The purity of RNA was confirmed by the ratio of absorbance in 260 and 280 nm.

cDNA synthesis

About 1 μg of the total RNA was used to synthesize the first-strand cDNA using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit, k1622. The RNase-free DNase1 enzyme is used before the reverse transcription reaction.

Evaluation of relative expression level of NPY1R gene by qRT-PCR assay

Real-time PCR was performed using qPCR Green Master with a low Rox system Rotor-Gene Q (QIAGEN) to quantify NPY1R and beta-actin mRNA levels. Details of primer sequences used for evaluating gene expression in this study are provided in Table 1. The PCR amplification was performed in a 20 μl volume using the qRT-PCR master mix. Each reaction included 0.6 mM of each primer, 10 μl qRT-PCR Green Master Mix, and 1 μl of cDNA preparation. Then, the samples were incubated at 95 °C for 2 min, followed by 35 cycles of amplification (95 °C for 30 s, 60 °C for 1 min). For each set, the control reaction is performed by omitting all the

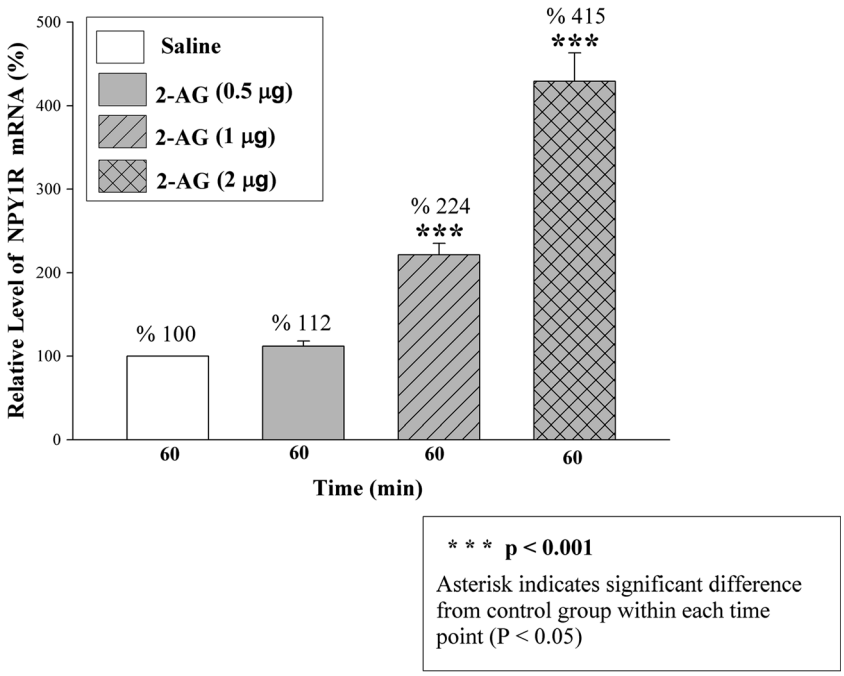

Fig. 1 Effect of ICV injections of 2-AG (0, 0.5, 1, 2 μg/rat) on the percent of NPY1R mRNA expression values in male Wistar rats (n = 24). 2-AG, 2-arachidonylglycerol, selective CB1 receptor agonist. Data are mean ± SEM. F and P value for within and between subject factors are as follows: time = 60 min; 2-AG, F (3, 20) = 210.2, $P \leq 0.001$; 2-AG $_{(0.5)}$, $P \geq 0.05$; 2-AG $_{(1)}$, $P \leq 0.001$; 2-AG $_{(2)}$, $P \leq 0.001$

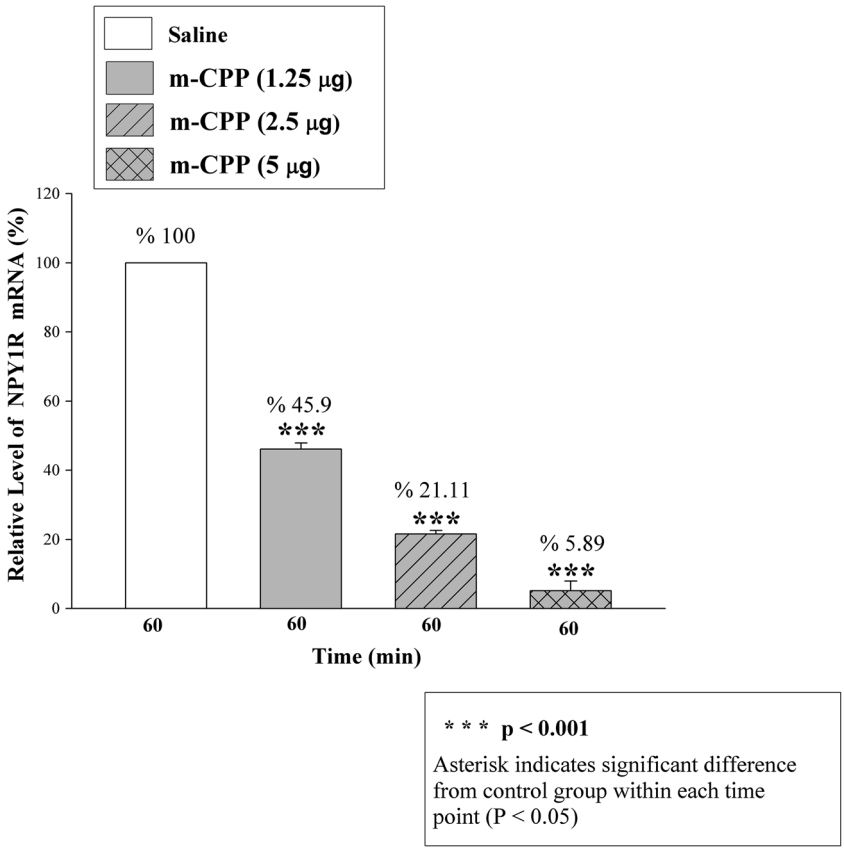

Fig. 2 Effect of ICV injections of m-CPP (0, 1.25, 2.5, 5 μg/rat) on the percent of NPY1R mRNA expression values in male Wistar rats ($n = 24$). m-CPP, meta-chlorophenylpiperazine $5HT_{2c}$ receptor agonist. Data are mean ± SEM. F and P value for within and between subject factors are as follows: time = 60; m-CPP, F (3, 20) = 4179.6; $P \leq 0.05$; m-CPP $_{(1.25)}$, $P \leq 0.001$; m-CPP $_{(2.5)}$, $P \leq 0.001$; m-CPP $_{(5)}$, $P \leq 0.001$

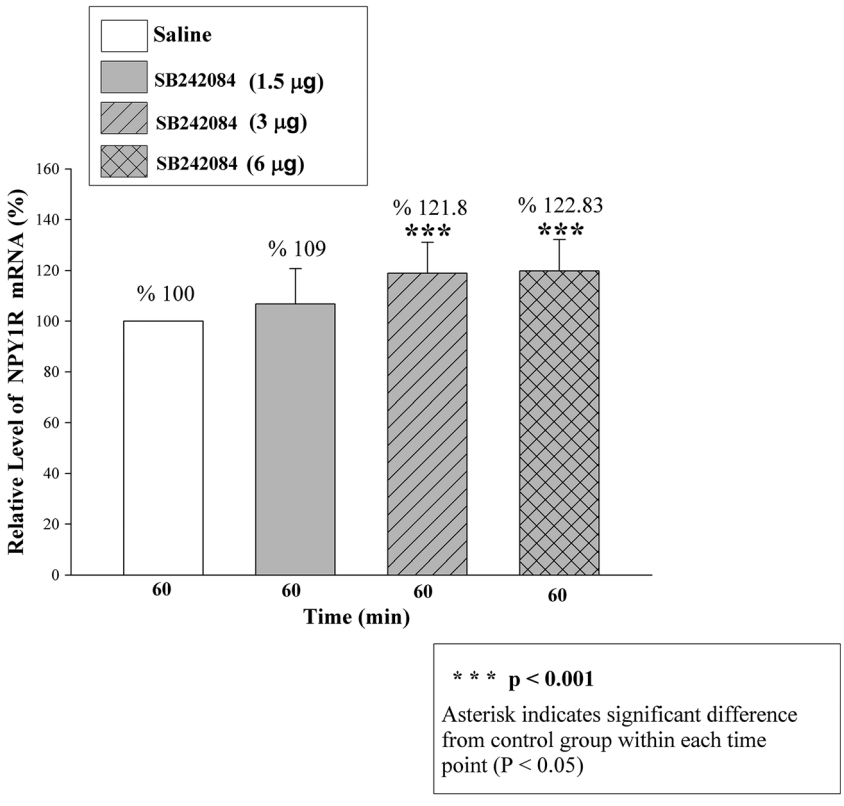

Fig. 3 Effect of ICV injections of SB-242084 (0, 1.5, 3, 6 μg/rat) on the percent of NPY1R mRNA expression values in male Wistar rats ($n = 24$). SB242084, $5HT_{2C}R$ antagonist. Data are mean ± SEM. F and P value for within and between subject factors are as follows: time = 60 min F (3, 20) = 28.8, $P \leq 0.001$; SB-242084 $_{(1.5)}$, $P > 0.05$; SB-242084 $_{(3)}$, $P \leq 0.001$; SB-242084 $_{(6)}$, $P \leq 0.001$

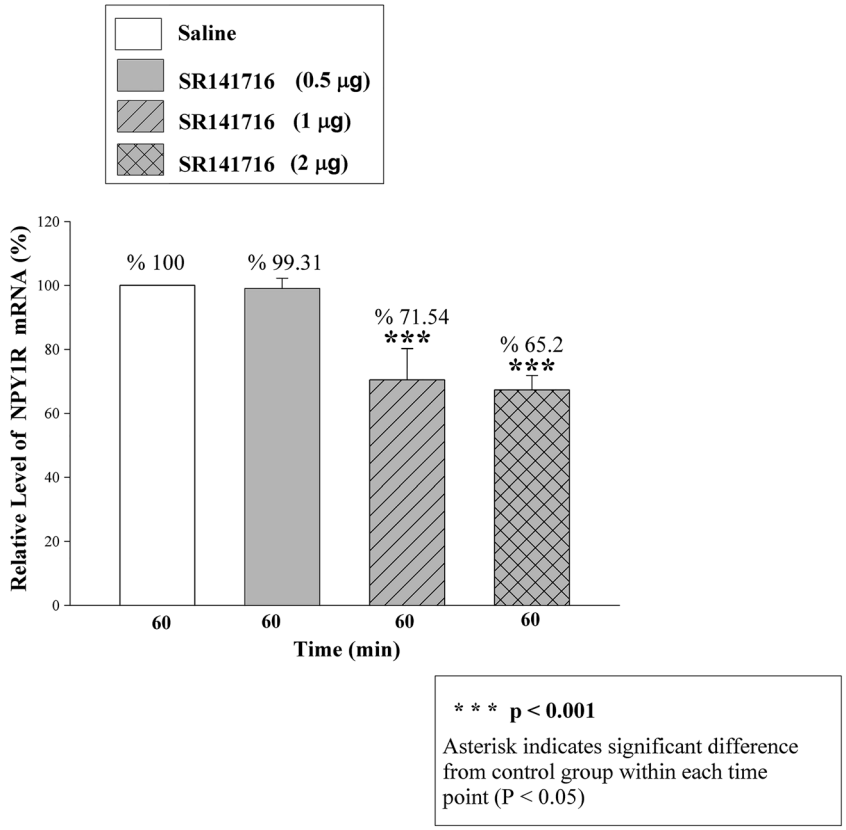

Fig. 4 Effect of ICV injections of SR-141716A (0, 0.5, 1, 2 µg/rat) on NPY1R mRNA expression in male Wistar rats ($n = 24$). SR-141716A: rimonabant hydrochloride, selective CB1 receptor antagonist. Data are mean ± SEM. F and P value for within and between subject factors are as follows: time = 60 min SR-141716A F (3, 20) = 28.82, $P \leq 0.001$; SR-141716A $_{(0.5)}$, $P > 0.05$; SR-141716A $_{(1)}$, $P \leq 0.001$; SR-141716A $_{(2)}$, $P \leq 0.001$

steps with no template cDNA; moreover, beta-actin mRNA is used as the internal control to evaluate the relative expression level of the NPY1R gene. The relative quantities of the transcripts are recorded $2^{-\Delta\Delta Ct}$. Final results showed relative expression levels. The melting curve analysis is carried for further confirmation of the primers' specificity.

Statistical analysis

Data analysis was calculated using IBM statistics SPSS 23. Data were expressed as mean values ± SEM. One-way ANOVA and Tukey's posttest are used to compare the mean values among the experimental groups. P values which were less than 0.05 are considered as significant.

Results

Antagonistic interactions between m-CPP plus 2-AG and SR-141716 and SB-242084 on the level of NPY1R mRNA expression in rats are shown in Figs. 5 and 6. In experiment 1, ICV injections of 2-AG (1 µg) caused a significant increase in the level of NPY1R expression compared to the control group [F (3, 20) = 210.219, $P < 0.001$] (Fig. 1). ICV injections of m-CPP (2.5 µg) caused a significant decrease in the level of NPY1R mRNA expression compared to the control group [F (3, 20) = 4179.6, $P < 0.001$] (Fig. 2). Co-administration of 2-AG (1 µg) + m-CPP (2.5 µg) caused a significant decrease in NPY1R expression compared to the 2-AG-induced overexpression group [time = 60 min and 120 min F (4, 25) = 760.7, $P < 0.001$] (Fig. 5). In experiment 2, ICV injections of

Table 1 The primers used for the evaluation of gene expression

Gene	Oligonucleotides (5′ - 3′); F: forward; R: reverse	Reference
β–actin	F: GAAATCGTGGACATTAAG R: GCTAGAAGCATTTGCGGTGGA	Fonseca et al. (2009)
Neuropeptide 1 receptor (NPY1R)	F: ACGTTCGCTTGAAAGGAGA R: CATGACGTTGATTCGTTTGG	Page et al. (2009)

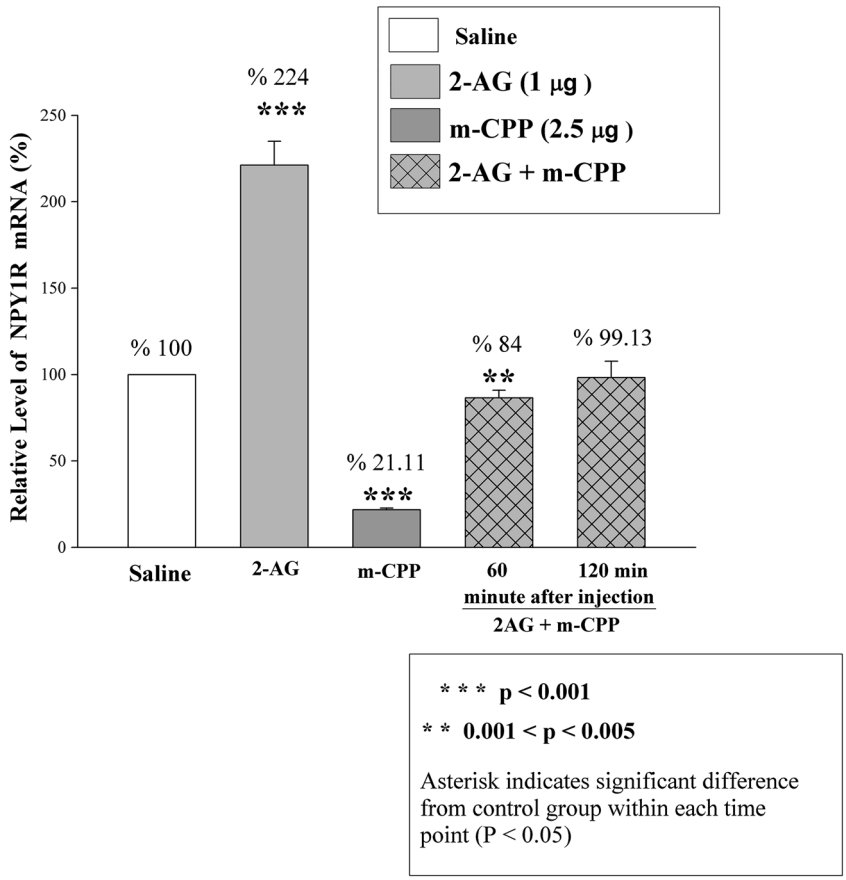

Fig. 5 Effect of ICV injections of m-CPP (2.5 μg/rat), 2-AG (1 μg/rat), m-CPP (2.5 μg/rat) + 2-AG (1 μg/rat) on NPY1R mRNA expression in male Wistar rats (n = 30). m-CPP: meta-chlorophenylpiperazine, $5HT_{2C}$ receptor agonist; 2-AG, 2-arachidonylglycerol; selective CB1 receptor agonist. Data are mean ± SEM. F and P value for within and between subject factors are as follows: 2-AG $_{(1)}$, F (3, 20) = 210.219, P < 0.001; m-CPP $_{(2.5)}$, F (3, 20) = 4179.6, P < 0.001; time = 60 min, m-CPP $_{(2.5)}$ + 2-AG $_{(1)}$, F (4, 25) = 760.76, $P \leq 0.01$; time = 120 min, m-CPP $_{(2.5)}$ + 2-AG $_{(1)}$, F (4, 25) = 760.76, $P \leq 0.001$

$5HT_{2C}$R antagonist increased NPY1R mRNA expression compared to the control group [SB-242084 (3 μg), F (3, 20) = 9.816, P < 0.001] (Fig. 3). ICV injections of SR-141716A (1 μg) decreased the level of NPY1R gene expression compared to the control group [F (3, 20) = 28.822, P < 0.05] (Fig. 4). Co-injection of SB-242084 (3 μg) + SR-141716A (1 μg) caused a significant decrease in NPY1R mRNA expression after 60-min postinjection compared to the SR-141716A and SB-242084 groups and after 120 min compared to the SB-242084 (3 μg/rat) group [time = 60 F (4, 25) = 17.33, P < 0.001, time = 120 F (4, 25) = 17.334, P < 0.001] (Fig. 6).

Synergistic interactions between CB1R agonist plus $5HT_{2C}$R antagonist on the level of NPY1R mRNA expression in rats are shown in Figs. 7 and 8. In experiment 3, ICV injections of 2-AG (1 μg) increased the level of NPY1R mRNA expression significantly compared to the control group [F (3, 20) = 210.219, P < 0.001] (Fig. 1). ICV injections of SB-242084 (3 μg) had NPY1R mRNA overexpression effect compared to the control group [F (3, 20) = 9.816, P < 0.001] (Fig. 3). Co-administration of 2-AG (1 μg) + SB-242084 (3 μg) significantly amplified 2-AG-induced NPY1R mRNA overexpression after 60- and 120-min postinjection [time = 60 min; SB-242084 $_{(3)}$ + 2-AG $_{(1)}$; F (4, 25) = 177.7; $P \leq 0.001$] [time = 120 min; SB-242084 $_{(3)}$ + 2-AG $_{(1)}$; F (4, 25) = 177.7; $P \leq 0.001$] (Fig. 7). In experiment 4, ICV injections of m-CPP (2.5 μg) significantly decreased the level of NPY1R mRNA expression compared to the control group [F (3, 20) = 4179.6, P < 0.001] (Fig. 2). ICV injections of SR-141716A (1 μg) decreased the level of NPY1R mRNA expression significantly compared to the control group [F (3, 20) = 28.822, P < 0.05] (Fig. 4). Co-injection of m-CPP (2.5 μg) + SR-141716A (1 μg) decreased the reduction of NPY1R mRNA expression of $5HT_{2C}$ agonist effect significantly after 60- and 120-min postinjection [time = 60 min; m-CPP $_{(2.5)}$ + SR-141716A $_{(1)}$; F (4, 25) = 423.748; $P \leq 0.001$ compared to control, m-CPP $_{(2.5)}$, and SR-141716A $_{(1)}$] [time = 120 min; m-CPP $_{(2.5)}$ + SR-141716A $_{(1)}$; F (4, 25) = 423.78; $P \leq 0.001$ compared to control, m-CPP $_{(2.5)}$, and SR-141716A $_{(1)}$] (Fig. 8).

Discussion and conclusion

These findings revealed that NPY1R activated by SB-242084 and 2-AG and inhibited by m-CPP and SR-141716A. Deafferentation of hypothalamic neurons leads to various consequences including (1) hyperphagia and obesity due to the

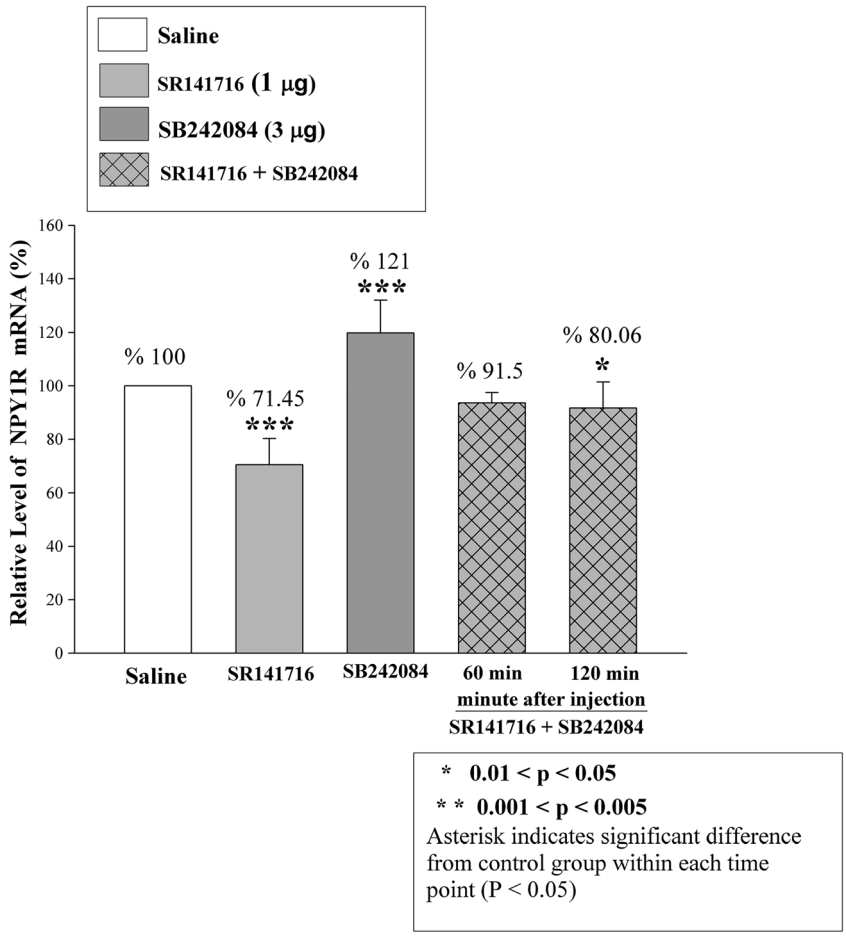

Fig. 6 Effect of ICV injections of SB-242084 (3 μg/rat) + SR-141716A (1 μg/rat) interaction on NPY1R mRNA expression in male Wistar rats ($n = 30$). SB-242084: $5HT_{2C}R$ antagonist; SR-141716A, rimonabant hydrochloride; selective CB1 receptor antagonist. Data are mean ± SEM. F and P value for within and between subject factors are as follows: SB-242084 (3 μg/rat), F (3, 20) = 9.816, $P < 0.001$; SR-141716A (1 μg/rat), F (3, 20) = 9.816, $P < 0.05$; time = 60 min, SB-242084 $_{(3)}$ + SR-1041716A $_{(1)}$, F (4, 25) = 17.334, $P \leq 0.05$ compared to SB-242084 $_{(3)}$ and SR-141716A $_{(1)}$; time = 120 min, SB-242084 $_{(3)}$ + SR-141716A $_{(1)}$, F (4, 25) = 17.334, $P \leq 0.001$ compared to SB-242084 $_{(3)}$

ARC NPY neuron and (2) blunted responses of counter-regulatory hormones to hypoglycemia as a result of the POMC neuronal association in the ARC, the CRH neuronal contribution in the paraventricular nucleus (PVN) and those in ventromedial hypothalamic nucleus (VMH). These interruptions eventually develop to enhance central food intake, suppression of energy expenditure, and dysregulation of the release of glucagon, epinephrine, and norepinephrine in rats (Schwartz et al. 2000; Watanabe et al. 2008).

There was a dense interconnection between NPY and POMC neurons in the ARC, which releases NPY to influence postsynaptic POMC neurons through NPY1R and NPY2R, causing membrane hyperpolarization (Roseberry et al. 2004). The high-fat diet increased the NPY1R mRNA expression as a hyperphagic NPY receptor, causing insulin-sensitive obesity in rats, signifying that the NPY gene expression level was related to the susceptibility of obesity-mediated through the NPY1R (Wang et al. 2007). The current study focused on an essential factor to take into consideration in developing NPY1R target therapeutically. These findings indicated that NPY1Rs antagonism might be useful in the treatment of obesity, bone homeostasis, and metabolic disorders which are associated with NPY levels such as central food intake, energy consumption, and pancreatic insulin secretion (Baldock et al. 2007; Lee et al. 2010; Lee et al. 2011; Lundberg et al. 2007). Therefore, the current study focused on an essential factor takes into consideration in developing NPY1R as a therapeutic target.

The homeostatic and hedonic roles of the hypothalamus in the orexigenic activity of cannabinoids were demonstrated by inhibiting POMC neurons and inducing NPY neurons in the ARC (D'Addario et al. 2014; Verty et al. 2004). The high level of CB1 cannabinoid receptors in the hypothalamic area is demonstrated by Herkenham et al. (1991) and Fernandez-Ruiz et al. (1999). The decrease in D_2 receptor mRNA levels would be mediated by stimulation of CB1Rs in rats' brain (Franklin and Carrasco 2012).

These findings showed that the ICV injection of a sub-effective dose of 2-AG (selective CB1R agonist; 1 μg/rat) induced overexpression of hypothalamic NPY1R mRNA, and the ICV injection sub-effective dose of SR-141716A (selective CB1R antagonist; 1 μg/rat) inhibited the expression of the hypothalamic NPY1R mRNA. Gamber et al. (2005) verified the direct influence of alterations in NPY level on the

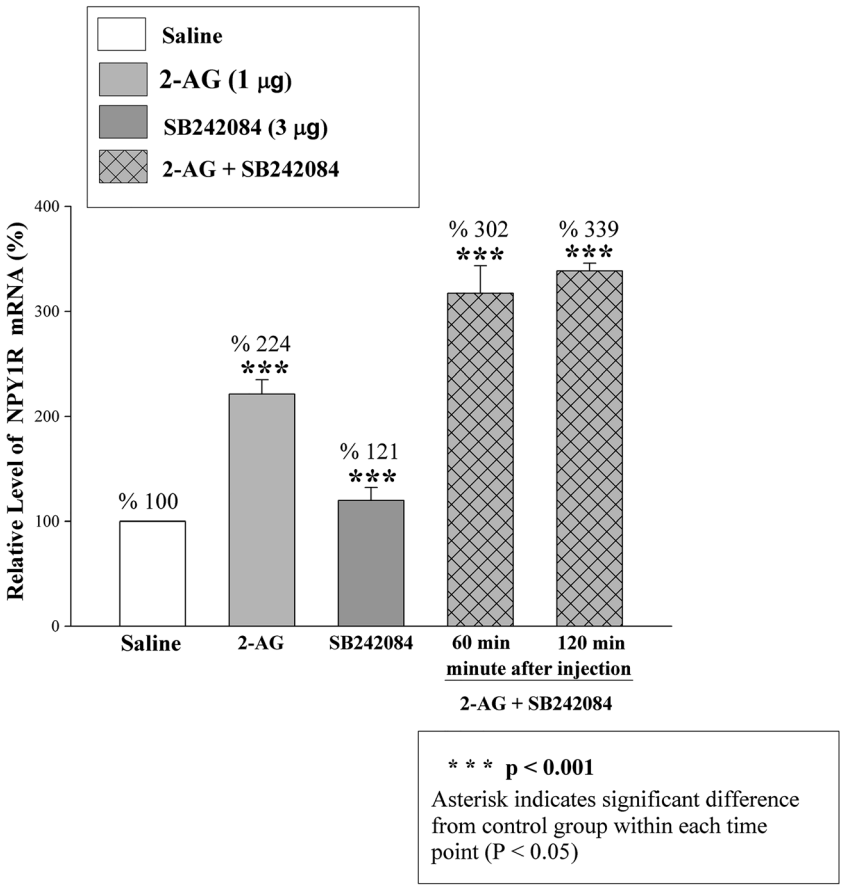

Fig. 7 Effect of ICV injections of 2-AG (1 μg/rat), SB-242084 (3 μg/rat), and SB-242084 (3 μg/rat) + 2-AG (1 μg/rat) on NPY1R mRNA expression values in male Wistar rats ($n = 30$). SB-242084, $5HT_{2C}$ receptor antagonist; 2-AG, 2-arachidonylglycerol, selective CB1 receptor agonist. Data are mean ± SEM. F and P value for within and between subject factors are as follows: 2-AG $_{(1)}$, $F\,(3, 20) = 210.219$, $P < 0.001$; SB-242084 $_{(3)}$, $F\,(3, 20) = 9.816$, $P < 0.001$; time = 60 min, SB-242084 $_{(3)}$ + 2-AG $_{(1)}$, $F\,(4, 25) = 177.753$, $P \leq 0.001$; time = 120 min, SB-242084 $_{(3)}$ + 2-AG $_{(1)}$, $F\,(4, 25) = 177.753$, $P \leq 0.001$

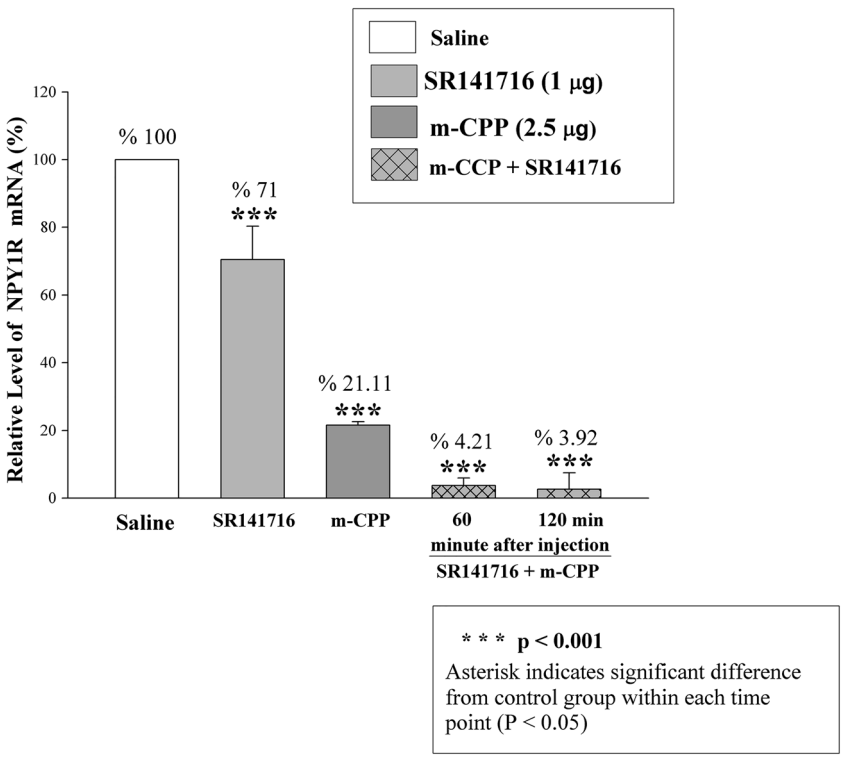

Fig. 8 Effect of ICV injections of m-CPP (2.5 μg/rat), SR-141716A (1 μg/rat), co-injections of m-CPP (2.5 μg/rat) + SR-141716A (1 μg/rat) on NPY1R mRNA expression values in male Wistar rats ($n = 30$). m-CPP, meta-chlorophenylpiperazine, $5HT_{2C}$ receptor agonist; SR-141716A, rimonabant hydrochloride, selective CB1 receptor antagonist. Data are mean ± SEM. F and P value for within and between subject factors are as follows: m-CPP $_{(2.5)}$, $F\,(3, 20) = 4179.6$, $P < 0.001$; SR-141716A $_{(1)}$, $F\,(3, 20) = 9.816$, $P < 0.05$; time = 60 min, m-CPP $_{(2.5)}$ + SR-141716A $_{(1)}$, $F\,(4, 25) = 423.748$, $P \leq 0.001$ compared to control, m-CPP $_{(2.5)}$ + SR-141716A $_{(1)}$; Time = 120 min, m-CPP $_{(2.5)}$ + SR-141716A $_{(1)}$, $F\,(4, 25) = 423.78$, $P \leq 0.001$ compared to control, m-CPP $_{(2.5)}$, and SR-141716A $_{(1)}$

food intake using cannabinoid and through the enzyme immunoassay technique. However, they did not study the specific action of CB1-selective agonists directly. Alternatively, 2-AG as the endogenous CB1 receptor agonist was used in this study by the qRT-PCR assay.

The significant roles of NPY and cannabinoids enhanced the consumption of sucrose solution and the inhibition of sucrose consumption by SR-141716A (Watanabe et al. 2008). Poncelet et al. (2003) showed the inhibition of the NPY release in CB1-knockout mice, and they found that there was an association between CB1 receptors and the orexigenic effects of NPY.

The result of the present study showed a decrease in NPY1R mRNA expression level induced by ICV injection of $5HT_{2C}R$ agonist (m-CPP) with sub-effective dose 2.5 μg/rat as well as the overexpression of NPY1R mRNA induced by ICV injection of a selective $5HT_{2C}R$ antagonist, SB-242084, with sub-effective dose 3 μg/rat. Earlier studies have shown that pharmacological motivation of $5HT_{2C}R$ leads to the development of anxiety, stress, and irregularities of feeding behavior (Higgins and Fletcher 2003), sexual dysfunction, and urinary incontinence (Cryan and Lucki 2000; Wacker and Miller 2008).

The sturdy serotonergic innervation of pre-somatic areas signifies the direct influence of serotonergic innervation on the NPY neurons in the soma position, influencing the neuronal function. In a large population of NYP-producing neurons, both $5\text{-}HT_{1A}$ and $5\text{-}HT_{2C}$ receptors are co-expressing (Fernandez and Gaspar 2012). The despair of $5HT_{2C}R$ RNA editing may be associated with overexpression of NPY mRNA in the nucleus accumbens (NAc) of INI mice leading to metabolic disorders, aggressive manners, and mood abnormalities (Aoki et al. 2016). The present study on the role of $5\text{-}HT_{2C}R$ was supported by previous investigations (Currie and Coscina 1997; Heisler et al. 2007), which have focused on both CRF- and NPY-related genes due to their close association with $5\text{-}HT_{2C}R$. Pyramidal cell inhibition is enhanced through the expression of NPY neurons resulted from serotonergic induction in the $5\text{-}HT_{2C}R$.

Temporary arrangement of 5-HT/NPY interaction through the various receptors likely caused by desensitization and changes in the receptor functions (Snoeren et al. 2011). The process might also provide the expression of both $HT_{1A}R$ and $5\text{-}HT_{2C}R$. However, the anxiogenic properties of $5\text{-}HT_{2C}R$ agonists in the basolateral do not entirely reconcile with the adverse effects of $5\text{-}HT_{2C}R$, which mediate the induction of NPY-producing neurons (Christianson et al. 2010; Li et al. 2012). In the basolateral amygdala, the interaction of 5-HT/NPY through different receptors contributes to the complex sets of inhibitory routs. The basolateral amygdala is associated with emotional learning and memory formation (Lowry et al. 2005; Roozendaal et al. 2009). Serotonergic transmission for mental functions for adapting with the environmental anxieties is related to receptor expression of the inhibitory NPY-producing neurons suggesting the different actions of 5-HT. The rat's lateral and basolateral amygdaloidal nucleus considered as a serotonergic afferent innervation is related closely to the NPY-producing neurons (Bonn et al. 2013). Advanced concerns related to central hyperserotonemia are strongly associated with the decline in the level of $5\text{-}HT_{2C}R$ and pharmacological stimulation of $5HT_{2C}R$, leading to decrease appetite. However, inhibition of the $5HT_{2C}R$ function causes the induction in food intake and obesity. The expression of $5\text{-}HT_{2C}R$ is associated with POMC to mediate $5HT_{2C}R$ agonist (m-CPP), which is appropriate for the suppression of the appetite (Xu et al. 2008). The effect of interaction between cannabinoid and serotonergic systems on food intake in mice has been documented previously (Ward et al. 2008).

The finding revealed that the ICV injections of a combination of CB1 plus $5HT_{2C}R$ agonists have antagonistic interaction in the NPY1R gene expression, as well as the co-injection of CB1 plus $5HT_{2C}R$ antagonists has antagonistic interaction significantly in the NPY1R gene expression in the hypothalamus, while the combination of CB1 agonist plus $5HT_{2C}R$ antagonist amplified NPY1R gene expression. Moreover, the co-administration of CB1 antagonists and $5HT_{2C}R$ agonists has significantly decreased NPY1R gene expression, and the use of CB1 antagonists and $5HT_{2C}R$ agonists is the best way to design a drug to counteract the neuropeptide Y1 receptor gene expression in the brain and deal with obesity. However, advance studies need to be conducted to identify the role of other receptors, such as NPY5R, in the drug development of metabolic diseases.

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Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest.

Ethical approval The article does not contain any studies involving human participants performed by any of the authors. All applicable institutional guidelines for the care and use of animals were approved by the ethical committee of the Science and Research Branch, Islamic Azad University(IR. IAU. SRB. REC. 1397. 12). All experimental procedures were performed on the animals according to the guidelines of the Care and Use of Laboratory Animals by the National Institutes of Health (USA).

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