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Levamisole: A Positive Allosteric Modulator for the $\alpha 3\beta 4$ Nicotinic Acetylcholine Receptors Prevents Weight Gain in the CD-1 Mice on a High Fat Diet

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Abstract

Neuronal nicotinic acetylcholine receptors (nAChRs) regulate the function of multiple neurotransmitter pathways throughout the central nervous system. This includes nAChRs found on the proopiomelanocortin neurons in the hypothalamus. Activation of these nAChRs by nicotine causes a decrease in the consumption of food in rodents. This study tested the effect of subtype selective allosteric modulators for nAChRs on the body weight of CD-1 mice. Levamisole, an allosteric modulator for the $\alpha 3\beta 4$ subtype of nAChRs, prevented weight gain in mice that were fed a high fat diet. PNU-120596 and desformylflustrabromine were observed to be selective PAMs for the $\alpha 7$ and $\alpha 4\beta 2$ nAChR, respectively. Both of these compounds failed to prevent weight gain in the CD-1 mice. These results suggest that the modulation of hypothalamic $\alpha 3\beta 4$ nAChRs is an important factor in regulating food intake, and the PAMs for these receptors need further investigation as potential therapeutic agents for controlling weight gain.

Keywords

Allosteric modulation; Body weight; High calorie diet; Levamisole; Nicotinic acetylcholine receptors; Positive allosteric modulators

1. INTRODUCTION

The growing rate of obesity in children and adults is a public health concern throughout the world [1]. Obesity is now considered to be the leading cause of preventable death worldwide [2]. While diet control and increase in physical activity are considered to be the main ways of controlling obesity [3], pharmacological treatments are being sought to control and mitigate this problem [4]. The hypothalamus is the central regulator of energy homeostasis in the human body [5]. Neurons originating in the arcuate nucleus of the hypothalamus that

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

secrete pro-opiomelanocortin (POMC) are involved in the regulation of key components of energy homeostasis [6]. This includes functions like regulating caloric intake, satiety and expenditure of energy [6]. In the brain slices obtained from the arcuate nucleus of the hypothalamus in mice, it was found that nicotine increases the rate of firing of the POMC-secreting neurons [6]. POMC is a large polypeptide that is cleaved into multiple components, including various melanotropins [7]. The nicotine-induced release of POMC is followed by the activation of melanocortin 4 receptors in the paraventricular nucleus of the hypothalamus, and this process is crucial in the reduction of appetite [6].

The endogenous receptors for nicotine are the nicotinic acetylcholine receptors (nAChRs) that are membrane bound ion channels in the cys-loop receptor family [8, 9]. Nicotine mimics the actions of the endogenous neurotransmitter acetylcholine by acting on a variety of different nAChR subtypes that are widely distributed in the central nervous system (CNS) [9, 10]. In the CNS, nine different nAChR subunits are known to exist, which include α (2 to 10) and β (2, 3 and 4) subunits [11]. The various nAChR subunits combine in different combinations to express as either homo- or heteromeric receptors [12–14]. While homomeric $\alpha 7$ and heteromeric $\alpha 4\beta 2$ receptors are thought to be the predominant subtypes of nAChRs in the brain [15–19], it has been shown that nicotine acts on the hypothalamic $\alpha 3\beta 4$ nAChRs to activate POMC-secreting neurons [20]. Another study, however, has indicated that it is the activation of $\alpha 7$ and $\alpha 4\beta 2$ receptors by nicotine that leads to the activation of POMC cells [21]. While nicotine lacks selectivity in its action on nAChRs, a group of positive allosteric modulators (PAMs) have been discovered or developed that demonstrate selectivity for different subtypes of nAChRs [14, 22]. PAMs are the compounds that bind to the nAChRs at an allosteric site that is distinct from the orthosteric site where agonists bind [22, 23]. For example, desformylflustrabromine (dFBr), a secondary metabolite obtained from the marine bryozoan *Flustra foliacea*, acts as a PAM for the $\alpha 4\beta 2$ subtype of nAChRs [23–25]. Similarly, PNU-120596 acts as a selective PAM for the $\alpha 7$ receptors and levamisole, which is an anthelmintic agent, acts as a PAM for the $\alpha 3\beta 2$ and $\alpha 3\beta 4$ subtype of nAChRs [26–28] (Table 1).

This study tested the effects of selective allosteric modulators for various nAChRs on the body weight in CD-1 mice that were fed a diet high in fat content. For this purpose selective allosteric modulators, levamisole, dFBr and PNU-120596 were utilized; of these, only levamisole prevented weight gain in CD-1 mice, thus indicating the involvement of the $\alpha 3\beta 4$ receptors in the nicotine-induced weight control.

2. METHODS AND MATERIAL

2.1. Study Design

To assess the effects of various nAChR PAMs, three different compounds were used; dFBr for $\alpha 4\beta 2$ receptors, levamisole for $\alpha 3\beta 4$ receptors, and PNU-120596 for $\alpha 7$ receptors. These drugs were administered to CD-1 mice in their drinking water in 2 different doses of 50 μ g/ml and 200 μ g/ml. To assess the effect of these nAChR PAMs on body weight, the weight of the mice over a period of 30 days was recorded that were receiving a diet with a high proportion of calories from fats. Correspondingly, the drug treatments were carried out over a 30 day period. The 1st weight recording was carried out before starting the drug

treatments (day-0). This was followed by 10 additional weighing's of the mice every 3 days for the 30 day testing period when they were receiving the drug treatments and eating a diet high in fat content.

2.2. Animals

112 CD-1 mice over the age of 32 days were purchased from Charles River Lab, Inc. (Wilmington, MA, USA). After a quarantine period of 7 days, the mice were divided into 8 groups with 14 mice in each group. Each group contained 7 male and 7 female CD-1 mice. Of the 8 groups of mice, 2 groups received dFBr, 2 groups received levamisole, and 2 groups received PNU-120596 (in both doses); the final 2 groups of mice received the vehicle (drinking water). During the study period the mice had access to drinking water and high calorie diet ad libitum. The high calorie diet that was fed to the mice during the study contained 60% of calories from fat, and it was obtained from Research Diets, Inc. (New Brunswick, NJ, USA). Procedures pertaining to CD-1 mice were reviewed and approved by the Animal Care and Use Committee (IACUC) of the National Institute of Environmental Health Sciences (NIEHS/NIH) and the University of Alaska Fairbanks (UAF).

2.3. Chemicals and Solutions

PNU-120596 and dFBr was purchased from Tocris, Inc. (Bristol, UK), and levamisole was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). These drugs were dissolved directly into the regular drinking water and administered to the CD-1 mice orally.

2.4. Data analysis and Statistical Procedures

The values of the weight of the mice receiving vehicle and drug treatments were compared using one-way ANOVA, or with a two-tailed Student's *t*-test, where the mean values of difference doses of drug treatments were compared with the vehicle and with each other. All statistical differences were deemed significant at the level of $p < 0.05$. The mice weights are expressed as mean $\pm$ se (standard error). Statistical analysis was carried out using GraphPad Prism 6.05 version software (San Diego, CA, USA).

3. RESULTS

3.1. Action of Levamisole on the Weight of CD-1 Mice

Initially, it was tested whether the selective PAM for $\alpha 3\beta 4$ receptors, levamisole, had any effect on the weight of CD-1 mice that were fed a diet with high fat content. The weight of the mice receiving vehicle treatment before being fed the high fat diet was 29.4 ± 0.75 grams, which increased to 34.8 ± 0.98 grams 30 days later ($p = 0.004$). The mice receiving vehicle treatment showed weight gain from day-3 that continued up to day-30. On the other hand, the mean weight of the mice that were treated with $50 \mu\text{g/ml}$ levamisole was initially 29.2 ± 0.93 grams on day-0, and did not change significantly after 30 days. The weight after 30 days was 30.1 ± 0.83 ; (Fig. 1). Similarly, the mean weight of the mice treated with $200 \mu\text{g/ml}$ levamisole did not change (it was initially 29.4 ± 1.03 grams on day-0 and was 29.6 ± 1.03 grams after 30 days; Fig. 1). Thus, the weight of the two groups of mice on high fat diet that received different doses of levamisole showed no weight gain. A significant difference in the weight was seen from day-15 onwards between the vehicle group and the

mice in the levamisole group ($F_{2, 39} = 3.796$, $p = 0.0312$) (Fig. 1). Interestingly, the mean weight of the mice in the 50 μ g/ml and 200 μ g/ml levamisole dose group was similar to each other, with no significant differences during the entire 30 day study ($P=0.444$; day-30). This indicates that the effective oral dose of levamisole for preventing weight gain was lower than 50 μ g/ml. Moreover, no treatment related to adverse clinical signs was observed in the mice at these doses.

3.2. Action of PNU-120596 and dFBr on the Weight of CD-1 Mice

Following this, the effects of selective PAMs for $\alpha 7$ (PNU-120596) and $\alpha 4\beta 2$ nAChRs (dFBr) on the body weight of mice fed a diet high in fat content were studied. Both PNU-120596 and dFBr failed to prevent weight gain in the mice treated with two doses (50 μ g/ml and 200 μ g/ml) of these drugs. At the end of day-30 of the test period, no significant difference was observed in the mean weight of the mice receiving vehicle treatment, and those treated with PNU-120596 ($F_{2, 39} = 0.05729$, $p = 0.9444$) (Fig. 2) and dFBr ($F_{2, 39} = 0.07752$, $p = 0.9256$) (Fig. 3). In addition, no drug treatment related to adverse clinical signs was observed in the mice.

4. DISCUSSION

Levamisole (L-[-]-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1b]-thiazole hydrochloride) is a drug that is routinely used in veterinary medicine to treat parasitic worm infections [29]. Additionally, levamisole acts as an allosteric modulator of human neuronal $\alpha 3\beta 2$ and $\alpha 3\beta 4$ nAChRs [27]. A previous study has reported that the activation of $\alpha 3\beta 4$ receptors in the arcuate nucleus of the hypothalamus by nicotine causes an activation of POMC neurons [20]. This leads to an activation of melanocortin 4 receptors that ultimately cause a reduction in the food intake and the body weight in mice [20]. Another study found that nicotine induces excitation of POMC cells by increasing spike frequency along with the depolarization of membrane potential, and opening of ion channels which is mediated by the activation of the both $\alpha 7$ and $\alpha 4\beta 2$ nAChRs [21].

While the $\alpha 7$, $\alpha 4\beta 2$ and $\alpha 3\beta 4$ receptors are reported to be present on the POMC neurons [6, 21], results obtained in this study show that PAMs selective for $\alpha 7$ and $\alpha 4\beta 2$ receptors, PNU-120596 and dFBr respectively, failed to prevent weight gain in the CD-1 mice that were fed a diet high in fat content. In contrast, levamisole, which is a positive allosteric modulator of $\alpha 3\beta 4$ receptors, was able to prevent weight gain in the CD-1 mice. It is possible that the administered doses of dFBr and PNU-120596 (50 and 200 μ g/ml) might not be sufficiently high to produce effects on $\alpha 7$ and $\alpha 4\beta 2$ receptors respectively, while levamisole at the same doses was able to prevent weight gain in the CD-1 mice. The effective concentration of dFBr and PNU-120596 on both $\alpha 7$ and $\alpha 4\beta 2$ receptors expressed in heterologous systems such as *Xenopus* oocytes was about 1 μ M [24, 26]. While higher doses of both dFBr and PNU-120596 that may produce an anorexigenic effect could be tested, this may also lead to non-specific interactions within and outside the CNS. Previously, levamisole has been reported to induce significant weight loss with a daily dose of 40 mg/kg body weight/day in beagle dogs [30]. This observation in dogs, combined with the results obtained in this study, implies that levamisole can prevent weight gain by the

functional modulation of the hypothalamic $\alpha 3\beta 4$ receptors, leading to the activation of POMC secreting neurons.

5. CONCLUSION

This study demonstrated that a positive allosteric modulator of the $\alpha 3\beta 4$ receptor (levamisole) can prevent weight gain in the mice fed a diet high in fat content. This study indicates that nAChR-specific PAMs need further investigation since they have clear potential to become clinically useful drugs in the prevention of weight gain and fighting the global epidemic of obesity.

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LIST OF ABBREVIATION

POMC	Pro-opiomelanocortin
nAChRs	Nicotinic acetylcholine receptors
dFBr	Desformylflustrabromine
PAMs	Positive allosteric modulators

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Fig. (1).

Effect of Levamisole on mean body weight of CD-1 mice. *Indicates significant differences in mean weight between the vehicle treatment and 2 doses of Levamisole, 50µg/ml and 200µg/ml. Levamisole in 2 test doses prevents weight gain in the mice during the 30 day study period. Significant differences ($F_{2, 39} = 3.796$, $p = 0.0312$) in the mean weights between vehicle treated mice and Levamisole treated mice was first seen on day-15 of the study that continued up to day-30 ($F_{2, 39} = 9.267$, $p = 0.0005$). Body weight is expressed as mean $\pm$ se; $n=14$ for each data point.

Fig. (2).

Effect of PNU-120596 on body weight of CD-1 mice. Differences in the mean weight of mice receiving vehicle treatment and PNU-120596 treatments in 50µg/ml and 200µg/ml doses were not significant during the 30 day study period ($F_{2, 39} = 0.05729$, $p = 0.9444$). Body weight is expressed as mean $\pm$ se; $n=14$ for each data point.

Fig. (3).

Effect of dFBr on body weight of CD-1 mice. Differences in the mean weight of mice receiving vehicle treatment and dFBr treatments in 50µg/ml and 200µg/ml doses were not significant during the 30 day study period ($F_{2, 39} = 0.07752$, $p = 0.9256$). Body weight is expressed as mean $\pm$ se; $n=14$ for each data point.

Chemical structure and potentiating effects of dFBr, PNU-120596 and levamisole on $\alpha 4\beta 2$, $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 7$ subtypes of nAChRs.

Table 1

PAM	Chemical Structure	$\alpha 4\beta 2$ receptors	$\alpha 3\beta 2$ receptors	$\alpha 3\beta 4$ receptors	$\alpha 7$ receptors
dFBr		120nM <i>d</i> [24]	ND	NP [25]	NP [24]
PNU-120596		NP [26]	ND	NP [26]	216nM <i>d</i> [26]
Levamisole		NP [27]	300µM <i>c</i> [27]	100µM <i>c</i> [27]	NP [27]

ND = Not determined, NP = No potentiation,

c – concentration of maximal potentiation at EC50 of ACh for the receptor,

d – half maximal potentiating concentration (pEC50)