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Research Article

Docosahexaenoic acid attenuates the rewarding property of nicotine-induced conditioned place preference in male rats

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Abstract

Objectives: Nicotine is a substance associated with rewarding and abusive effects. The rewarding effects of nicotine are thought to be due to dopamine signaling, which is negatively controlled through peroxisome proliferator-activated receptors (PPARs). Docosahexaenoic acid (DHA), also known as omega-3, can trigger the peroxisomal β-oxidation enzymes through PPARs. In this study, we planned to examine the effect of DHA on the rewarding properties of nicotine-induced conditioned place preference (CPP) in male rats.

Methods: CPP was established by giving male rats an intraperitoneal injection of nicotine (0.5 mg/kg). The effects of PPAR agonist DHA on the rewarding properties of nicotine were evaluated with the administration of DHA (150 mg/kg and 250 mg/kg, p.o.) or saline 30 min prior to nicotine injection.

Results: The present finding confirms that DHA attenuated nicotine acquisition (150 and 250 mg/kg, $p<0.01$) and failed to produce CPP or/and conditioned place aversion.

Conclusion: These findings could be a bridge from bench to bedside as DHA may be helpful as an adjuvant for smoking cessation; however, these are the preliminary results, and further research is needed to illuminate this feature completely.

Keywords: CPP, docosahexaenoic acid, nicotine, PPARs, rewarding properties

Substance abuse is a long-lasting, recurrent illness that manifests dramatic financial, social, and health burdens. Six million people die of smoking [1] every year. Largely, men consume tobacco-related products at much higher rates in comparison with women [2]. The data collected through National Health Interview Survey in the United States showed that 16.7% of adult men and 13.6% of adult women smoked in 2015 [3]. The difference in tobacco consumption in men and women can be attributed to behavioral, cultural, and physiological (hormone) factors [4].

Tobacco-enriched products such as cigarettes are responsible for quick nicotine distribution to the brain. Nicotine shows its rewarding properties through the mesolimbic dopamine system like other abusive drugs [2] in humans and animals [3]. The binding of nicotine with acetylcholine receptors in the ventral tegmental area (VTA) intensifies the firing of dopamine in the nucleus accumbens (NA) shell [4, 5]. Recently, it has been found that dopamine signaling brought by nicotine has been negatively controlled by "α-type" nuclear peroxisome proliferator-activated receptors (PPAR-α) [6].

PPAR-α are found in brain-specific areas and many other tissues [7]. These nuclear receptors play a significant role in anti-inflammatory and neuroprotective metabolism [8]. Experimental drugs that activate PPAR-α block nicotine-stimulated dopamine firing in the brain's reward center, such as VTA and NA of rats. A decrease in nicotine-seeking behavior was observed in animals [6, 9].

Limited treatments are approved and are used for nicotine addiction; however, the results are not satisfactory. Tobacco use is associated with cardiovascular disease and additional morbidities such as high levels of triglycerides and low levels of HDL [10]. Fibrates directly activate PPAR-α and have been accepted for decades to treat hypercholesterolemia [11, 12].

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Fibrate adoption as a remedy to smoking cessation will be a breakthrough as this group is already used in clinical practice.

Similarly, many studies showed the role of polyunsaturated fatty acids (n-3 PUFAs) such as docosahexaenoic acid (DHA) on the central nervous system [13]. It has been found that DHA can stimulate and induce the enzymes related to a peroxisomal β-oxidation pathway through the mediation of PPARs [14]. Sufficient supplementation of n-3 PUFAs revealed beneficial effects for treating many central nervous system-related diseases such as attention deficit hyperactivity disorder [15] and depression [16]. The n-3 PUFA supplementation also proved effective for motor deficiencies and memory dysfunction linked to oxidative stress [17, 18]. Furthermore, many clinical studies have demonstrated that low levels of n3-PUFAs could lead to mood disorders, aggressiveness, and substance abuse [19, 20].

The conditioned place preference (CPP) is a popular preclinical behavioral pavlovian model used to investigate the rewarding and aversive properties of abusive drugs or other chemical substances [21]. The effects of DHA on nicotine reward have not been assessed in human or animal models. Therefore, the principal aim of this experiment was to study the impact of DHA, a PPAR agonist, on the rewarding effects of nicotine in adult male rats.

Materials and Methods

Animals

In this study, we used male adult Wistar albino rats weighing 300-350 g. Four rats were maintained in each cage and habituated for 3 days earlier than the place preference trails. Animals were kept in a well-controlled environment, 50% humidity, 22±1°C temperature, and 12 h light-dark cycle. The rats were fed ad libitum and had free access to water. The experiments were approved by the Local Ethics Committee on Animal Experiments, Istanbul University Cerrahpasa, per the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2022/13).

Drugs

Nicotine (nicotine hydrogen tartrate salt) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The nicotine was prepared fresh every day by dissolving in 0.9% saline (distilled water). DHA was gifted by Eeose Laboratuarlari Koz. ve İlaç San. Ltd Company. Nicotine was given intraperitoneally (i.p.) and DHA was given perorally (p.o.) to the rats. The dose of nicotine and DHA was selected according to previous studies with minor modifications [22-24]. The rats of the control group received saline. Nicotine stocks were prepared fresh every morning.

Apparatus

In this study, we used a two-chambered CPP paradigm which is similar to our previous experiments [21]. The apparatus was made up of a Plexiglas box (61×31×13 cm) with opaque gray walls and a dropping tray (2.5 cm). The floor of compartment "A" was made up of 4×4 mm stainless steel mesh sheet (29×30 cm) and white walls. To offer a different texture to the rats, the floor of chamber "B" was composed of grid rods (3 mm diameter) made up of stainless steel, mounted 7 mm apart with black walls. The chamber was cleaned entirely with damp and dry clothes between the animals. The CPP apparatus was considered "biased" as the rats preferred the black chamber to the white one. The place conditioning is commonly observed in the biased paradigm when a drug is paired with the nonpreferred side. In this study, we used a biased experimental design by pairing drugs with the white compartment (mesh floor), which is in accordance with our previous study [21, 25].

Place conditioning procedure

The experiment comprised four phases: habituation, pretest, conditioning, and posttest.

Habituation phase

In the habituation phase, all the rats were put inside the apparatus with the lid open and allowed to both the compartments for 5 min. This session was intended to reduce the novelty of the experimental apparatus procedure.

Pretest phase

In the pretest, all rats were confined inside the paradigm and allowed to explore both the compartments for 15 min. The time spent (seconds) by the rats in the individual chambers was noted.

Conditioning phase

This session starts soon after the pretest. This phase lasted for 3 days and comprised three saline-paired and three drug-paired sessions of 40 min. On the first day of morning sessions of the conditioning, rats were given drugs and put inside the drug-paired side for 40 min. In the afternoon trials, rats were given saline and kept inside the saline-paired side of the apparatus for 40 min. The same (treatment) was repeated on day 3. However, on the second day, saline was given in the morning and drug in the afternoon sittings.

Posttest phase

After the conditioning session, the posttest was carried out. This phase was conducted in the same way as the pretest session. Rats were placed inside the apparatus with the lid open and with access to both chambers. The time spent by the rats in each chamber was recorded 15 min.

Effect of DHA on nicotine-induced CPP

To estimate whether DHA (150 and 250 mg/kg, p.o.) could develop CPP, rats were subjected to conditioning ses-

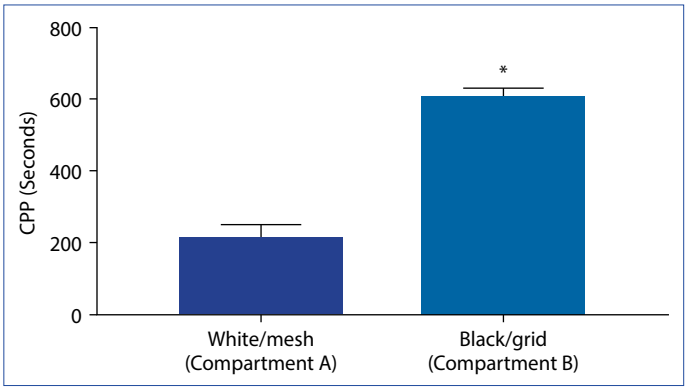

Figure 1. Pretest showing the preference of animals (*p<0.001, Student's t-test).

CPP: Conditioned place preference.

sions for 3 days. The effect of DHA on the development of nicotine-induced CPP, the nicotine (0.5 mg/kg) was given to rats during the conditioning sessions, while DHA (150 and 250 mg/kg, p.o.) was injected intraperitoneally 30 min prior to nicotine administration.

Data analysis

The results were analyzed by GraphPad Prism® (Version 5.0, GraphPad Software, Inc., La Jolla, CA, USA) software, and the data were expressed as mean±S.E.M. To evaluate the preference of animals, we used unpaired Student's t-test. The effect of DHA on the acquisition of nicotine-induced conditioned place preference was evaluated using the one-way analysis of variance followed by Tukey's posttest. The level of significance was considered p<0.05 level.

Results

The results of the pretest showed that rats significantly spent more time in the black compartment as compared with the white one (p<0.001, Fig. 1). The preference made by the animals noticeably indicated that the experiment design was biased.

Rats treated with nicotine (0.5 mg/kg, i.p.) showed a preference for the drug-paired side (p<0.001) in comparison with the control group, as shown in Figure 2, whereas DHA (150 and 250 mg/kg, p.o.) failed to induce CPP or conditioned place aversion (CPA) (p>0.05). Thus, DHA itself had no tendency for dependence (Fig. 2).

We used a biased protocol to study the acquisition of DHA on nicotine-induced CPP in male adult rats. The priming injection of DHA (150 and 250 mg/kg, p.o.) with nicotine (0.5 mg/kg, i.p.) during the conditioning trials completely abolished the acquisition of nicotine-induced CPP (Fig. 3). The mean time spent by the animals in DHA high dose group (234 s, 250 mg/kg, p.o.) was found to be slightly more significant (p<0.001) in decreasing nicotine-induced place preference (470 s) in comparison with low dose DHA (286 s, 150 mg/kg, p.o.) (p<0.001 and p<0.01, respectively).

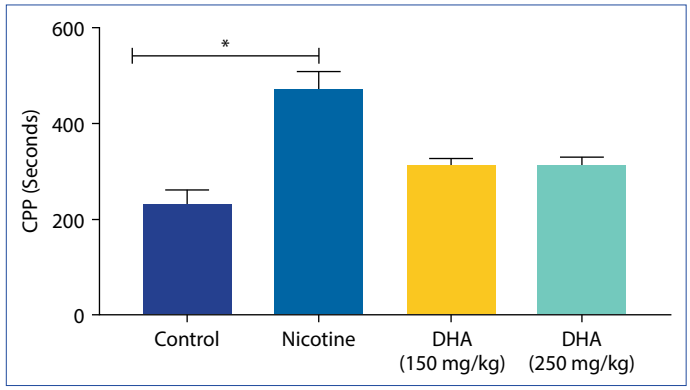

Figure 2. The effect of nicotine and DHA (150 and 250 mg/kg) on place conditioning. Conditioning sessions were conducted twice daily for 3 days and involved alternate injections of saline (1 mL/kg, i.p.), nicotine (0.5 g/kg, i.p.), and DHA (150 and 250 mg/kg, p.o.). The time spent by rats on the drug-paired side (nonpreferred side) was measured in seconds. Each value was expressed as mean±S.E.M. compared with the saline (Tukey's test).

*p<0.05. CPP: Conditioned place preference; DHA: Docosahexaenoic acid.

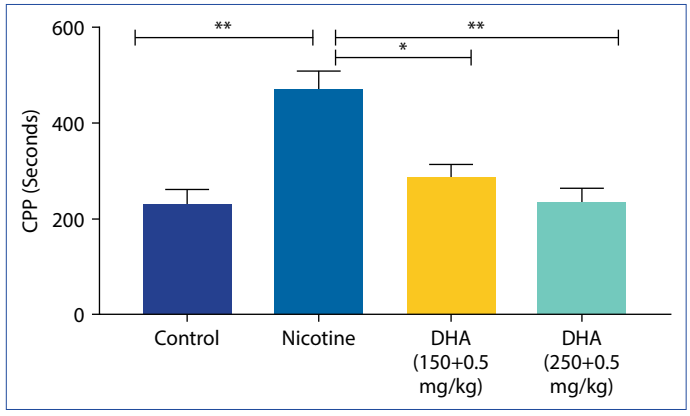

Figure 3. Effect of DHA combination treatment on nicotine-induced CPP. Conditioning sessions were conducted twice daily for 3 days and involved alternate injections of saline (1 mL/kg, i.p.), nicotine (0.5 g/kg, i.p.), and DHA plus nicotine (150 and 250 mg/kg, p.o. plus nicotine 0.5 mg/kg, i.p.). The time spent by rats on the drug-paired side (nonpreferred side) was measured in seconds. Each value was expressed as mean±S.E.M. (Tukey's test).

*p<0.01 and **p<0.001 relative to the saline and nicotine groups, respectively. CPP: Conditioned place preference; DHA: Docosahexaenoic acid.

Discussion

In the conditioned place preference paradigm, the animals are repeatedly exposed to a known environment, and the time spent in the chamber primed with rewarding drugs is compared with the other compartment [21]. In this study, we used the biased procedure to evaluate the rewarding effects of DHA alone or along with nicotine. The data revealed that DHA did not produce CPP or CPA though it attenuated the nicotine-induced CPP. This could be due to the modulation at DA neurons of VAT via PPAR-α. To the best of the authors' knowledge, there is no study that provides evidence of an important functional role of DHA,

a PPAR agonist, in nicotine-induced CPP models. It also emphasized the role of PPAR-α in the regulation of neuronal functions.

PPAR-α belongs to the nuclear steroid receptor superfamily. PPAR-α receptors are ubiquitously found in the CNS [7] and regulate numerous physiological functions, including inflammation, energy homeostasis, and metabolism of glucose and lipids [26]. PPARs received significant interest as a possible intervention to treat substance abuse because of their localization in brain reward centers [27]. It is believed that PPAR-α plays an essential role in the dopaminergic signaling of substance abuse and psychiatric disorders [28]. Amid these effects, the stimulation of dopaminergic transmission from the mesolimbic region is one of the symbols that define nicotine's addictive potential [29]. Nicotine also activates the DA neurons of VTA through nicotinic acetylcholine receptors [7].

Chronic tobacco consumption dramatically raises health and economic burdens. Among smokers, nicotine addiction is considered a primary concern related to cardiovascular disease [30]. Nicotine is the main component of tobacco-related products and is responsible for addiction and drug-seeking behavior. The literature suggests that the activation of a7 nACh receptors decreases nicotine-induced CPP in a PPAR-α-dependent manner. To assess the stimulation of PPAR-α in nicotine dependency, Jackson and colleagues [31] selected WY-14643, a potent PPAR-α agonist, and fenofibrate, a clinically known PPAR-α agonist, in nicotine-induced CPP, and the data showed a decrease in nicotine-induced CPP [31].

The results from our study revealed that the effects of DHA at different doses were consistent in attenuating the rewarding properties of nicotine accounted for acquisition phase of CPP. Rats administered nicotine (0.5 mg/kg) during the conditioning test exhibited an increase in CPP for the nicotine-paired compartment in comparison with the saline-paired side, which is in line with previously reported studies [24, 31, 32]. Our findings showed that DHA attenuates the development of nicotine-induced CPP. It has been found that n-3 PUFA-enriched diet modifies the neurobehavioral and cellular adaptations to chronic morphine exposure [33]. Similarly, in a double-blind, randomized, placebo-controlled clinical trial conducted on heavy smoker males, a high dose of n-3 fatty acid supplementation significantly decreased the cigarette desire and oxidative stress index [34]. In another study, low levels of peripheral n-3 PUFAs were found in the smoker's population, and the treatment with omega-3 fatty acids decreased the rewarding effects of nicotine [35].

The project has certain limitations. First, we performed this study only on male rats. The literature revealed that nicotine replacement pharmacotherapies are less successful in women. Thus, it is more challenging for them to quit smoking than men [36]. In nicotine addition, gender differences influence dependence, withdrawal, and reinstatement [37, 38]. Second, we could not conduct experiments on withdrawal and reinstatement. Thus, new studies should investigate the extensive role of DHA in nicotine abstinence and relapse.

Conclusion

PUFAs possibly interfere with smoking habits, and the upsurge in the consumption of DHA may become a viewpoint in smoking cessation. The findings of this study suggest that DHA, a supplemental medication, could be added to the treatment protocol for nicotine abuse.

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Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by The Istanbul University-Cerrahpasa Local Ethics Committee for Animal Experiments (No: 2022/13, Date: 08/06/2022).

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