

Mutual assistance of nucleus accumbens cannabinoid receptor-1 and orexin receptor-2 in response to nicotine: a single-unit study

Reza Fartootzadeh, Hojjatallah Alaei, and Parham Reisi*

Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Background and purpose: The nucleus accumbens (NAc) express both orexin-2 receptor (OX2R) and cannabinoid receptor type 1 (CB1R). Orexin and cannabinoid regulate the addictive properties of nicotine. In this study, the effect of the CB1R blockade on the electrical activity of NAc neurons in response to nicotine, and its probable interaction with the OX2R in this event, within this area, were examined *via* the single-unit recording.

Experimental approach: The spontaneous firing rate of NAc was initially recorded for 15 min, and then 5 min before subcutaneous injection of nicotine (0.5 mg/kg)/saline, AM251 and TCS-OX2-29 were injected into the NAc. Neuronal responses were recorded for 70 min, after nicotine administration.

Findings/Results: Nicotine excited the NAc neurons significantly and intra-NAc microinjection of AM251 (25 and 125 ng/rat), as a selective CB1R antagonist, prevented the nicotine-induced increases of NAc neuronal responses. Moreover, microinjection of AM251 (125 ng/rat), before saline injection, could not affect the percentage of change of the neuronal response. Finally, simultaneous intra-NAc administration of the effective or ineffective doses of AM251 and TCS-OX2-29 (a selective antagonist of OX2R) prevented the nicotine-induced increases of NAc neuronal responses, so that there was a significant difference between the group received ineffective doses of both antagonists and the AM251 ineffective dose.

Conclusion and implications: The results suggest that the CB1R can modulate the NAc reaction to the nicotine, and it can be concluded that there is a potential interplay between the OX2R and CB1R in the NAc, in relation to nicotine.

Keywords: AM251; Cannabinoid system; Nicotine; Nucleus accumbens; Orexin system; Single-unit recording.

INTRODUCTION

Nicotine, the key psychoactive element of tobacco, is the cause of smoking addiction (1). Nicotine affects nicotinic acetylcholine receptors of mesolimbic dopaminergic neurons (2), with subsequent releasing of dopamine from the ventral tegmental area (VTA) to the prefrontal cortex, and the nucleus accumbens (NAc) (3,4). This pathway has a major role in affecting the properties of drug abuse (5).

Cannabinoid receptor type 1 (CB1R) is the most abundant G-protein coupled receptor in the CNS (6), which spreads in the mesocorticolimbic pathway, including VTA, NAc, and prefrontal cortex (7).

Endocannabinoids regulate reward in the VTA and the NAc, through the CB1R (8,9).

There are physiological and anatomical interactions between the nicotine and cannabinoid receptors (10,11), and several studies have shown the effect of their co-abuse on physiological and behavioral responses (12-14).

Access this article online



Website: <http://rps.mui.ac.ir>

DOI: 10.4103/1735-5362.310524

*Corresponding author: P. Reisi

Tel: +98-3137929033; Fax: +98-3136688597

Email: p_reisi@med.mui.ac.ir

One of the orexins/hypocretins corresponding receptors is orexin-2 receptor (OX2R) (15). Orexin neurons send projections to the regions, involved in the drug addiction and reward processing, such as the NAc (16,17). Similar to CB1R, the OX2R is a G-protein coupled receptor (18), which is expressed in the NAc (17). Recent studies have shown that the orexin system can modulate the NAc response to nicotine (9,19).

The CB1R and OX2R have presented an overlapping distribution area in some regions of the CNS, based on anatomical studies (20-22); thus could be said that they can mutually regulate several physiological functions such as reward, nociception, food intake and energy balance, as reviewed by Berrendero *et al.* (23). Moreover, bioluminescence energy transfer assay showed that both OX1R and OX2R are capable of forming homo- and heteromeric complexes with one another and with the CB1R (24). Additionally, recent studies have shown that there is a functional interplay between the cannabinoid and orexin systems within the VTA/NAc (9,16,25).

With this in mind, we have tried to figure out possible crosstalk at the electrophysiological level between the OX2R and CB1R of the NAc, in response to the nicotine.

MATERIALS AND METHODS

Animals and surgical method

The tests were performed on 94 adult male Wistar rats weighing 240-290 g. The Ethics Committee of Animal Use of Isfahan University of Medical Sciences approved the study (Ethics No. IR.MUI.REC.1395.3.810), and all tests were executed in line with the guidelines for Animal Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23), revised in 2011. The rats were anesthetized with urethane (1.2 g/kg with extra doses as required) and positioned in a stereotaxic device (Stoelting, USA). Body temperature was maintained at 37 °C, using an electrically controlled heating pad. A hole was made for the insertion of a two-barreled micropipette into the NAc (AP: +2.16 mm; ML: 1.4 mm; DV: 6-7.4 mm) (26). Besides, all efforts were made to minimize the number of animals used and their suffering.

Experimental designs

Effects of intra-NAc administration of AM251 on the neurons discharge rate

In the first step, we examined dose responses to AM251 (5, 25 and 125 ng/rat in 0.5 µL of 10% DMSO; Sigma-Aldrich, USA), as a CB1R antagonist on the NAc neurons discharge rate, in response to subcutaneous injection of 0.5 mg/kg nicotine hydrogen tartrate (1 mL/kg, Sigma-Aldrich, Germany). In addition, we evaluated the response of NAc neurons to the maximum dose of AM251 (125 ng/rat), before the administration of saline. The extracellular recordings were performed on the NAc neurons, *via* a single unit recording procedure.

Also, the dose-response effect of TCS-OX2-29 (as an OX2R antagonist) was examined in our previous work, and the effective (3 ng/rat) and ineffective (1 ng/rat) doses were used in the following experiments (19).

Effects of intra-NAc concurrent administration of effective and ineffective doses of TCS-OX2-29 with AM251 on the neurons discharge rate

In the second step, we combined the effective (3 and 25 ng/rat) or ineffective (1 and 5 ng/rat) doses of TCS-OX2-29 and AM251, respectively, and microinjected into the NAc before the nicotine injection, to assess the firing activity.

All doses used in this study were selected based on our previous behavioral and electrophysiological studies (9,19), and administered in a total volume of 0.5 µL/rat.

Drug microinjection

A two-barreled glass micropipette (one for drug administration and the other for spike recording) was lightly conducted into the NAc, using a manual micromanipulator, till the best action potential recording isolated from the background noise with a signal-to-noise ratio of more than two. The drug was injected into the nucleus, *via* a manual pressure injector, and the recording was executed, using a fine tip (1-3 µm) microelectrode, filled with 2 M sodium chloride. Recorded signals were filtered at 0.3-3 kHz, and then digitized by a commercial analog to the digital data acquisition system. Data analysis was executed, using the related software, eLab (Science Beam institute, Iran).

After a steady firing rate and a 15-min baseline recording, the antagonist was injected into the nucleus. Subsequently, 5 min later, nicotine was injected and the recording was done for 70 min.

Histological verifications

The animals were perfused transcardially with normal saline followed by 10% buffered formalin, then brains were removed, and immersed in 10% formalin for 48 h. Finally, the fixed brain tissues were cut into 55 μ m-thick coronal slices, and the sites of the recording were verified, according to the atlas of Paxinos and Watson (26) (Fig. 1A).

Data analysis

the average firing rate (in spikes per second) was defined as 15-min of spontaneous firing rate before nicotine injection. Excitatory/inhibitory response was measured as an increase/decrease of firing rates, respectively, beyond the mean \pm two-fold of the SD of the baseline firing activity, for five successive minutes (19).

The percentage of increase/decrease in firing rate, multiplied by the duration of excitation/inhibition (percent \times duration in minutes) was used in analyses (19). For multiple comparisons, we used one-way ANOVA, followed by Tukey's post-hoc test, and for comparing the inhibitory or excitatory responses, the χ^2 test was used. Data were expressed as mean \pm SEM. P values < 0.05 were considered statistically significant.

RESULTS

Nicotine excited the majority of NAc neurons

In the NAc neurons of seven rats, nicotine increased the spike frequency per seconds, in 8/11 (72.72%) neurons, decreased just 1/11 (9.09%) neuron firing activity, and 2/11 (18.18%) neurons remained unaffected (Fig. 2A₂). Also, saline in six rats, without affecting 6/12 neurons (50%), reduced the neuronal firing of 4/12 (33.33%) neurons and excited just 2/12 (16.16%) neurons (Fig. 2A₁).

Moreover, χ^2 test showed significant difference in the proportion of excited neurons between the nicotine and saline group [χ^2 (1) = 7.34, $n = 23$, $P = 0.01$].

Blockade of the NAc CB1R prevented the neurons excitement induced by nicotine

The neurons response in various interventions were as follows. in nicotine + AM251 (5 ng) group (10 rats): 6/18 (33.33%) neuron inhibition, 10/18 (55.55%) neuron excitation, and 2/18 (11.11%) neuron without response (Fig. 2A₃); in nicotine + AM251 (25 ng) group (10 rats): 7/16 (43.75%) neuron inhibition, 3/16 (18.75%) neuron excitation, and 6/16 (37.5%) neuron without response (Fig. 2A₄); in nicotine + AM251 (125 ng) group (10 rats): 9/12 (75%) neuron inhibition, 2/12 (16.66%) neuron excitation, and 1/12 (8.33%) neuron with no response (Fig. 2A₅). Furthermore, the maximum dose (125 ng/rat) of AM251 + saline (10 rats) reduced the firing rate of 4/13 (30.76%) neurons, excited just 5/13 (38.46%) neurons, and made no response in 4/13 (30.76%) responses (Fig. 2A₆).

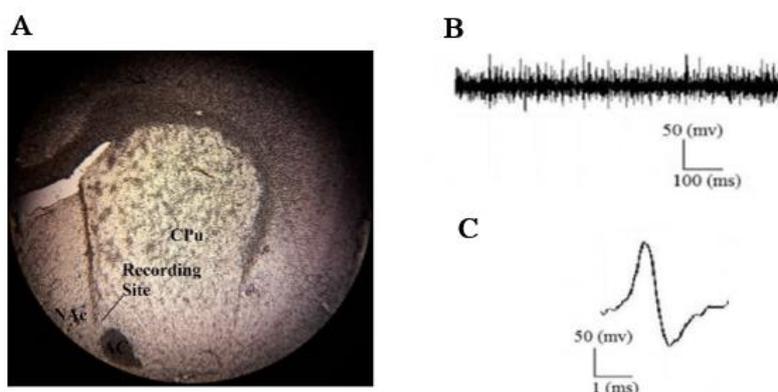


Fig. 1. (A) A representative image, showing the recording site in the NAc; (B) a representative pattern of baseline neuronal electrical activity recorded from the NAc; (C) an expanded waveform of a spike recorded from a NAc single neuron. AC, Anterior commissure; CPu, caudate putamen; NAc, nucleus accumbens.

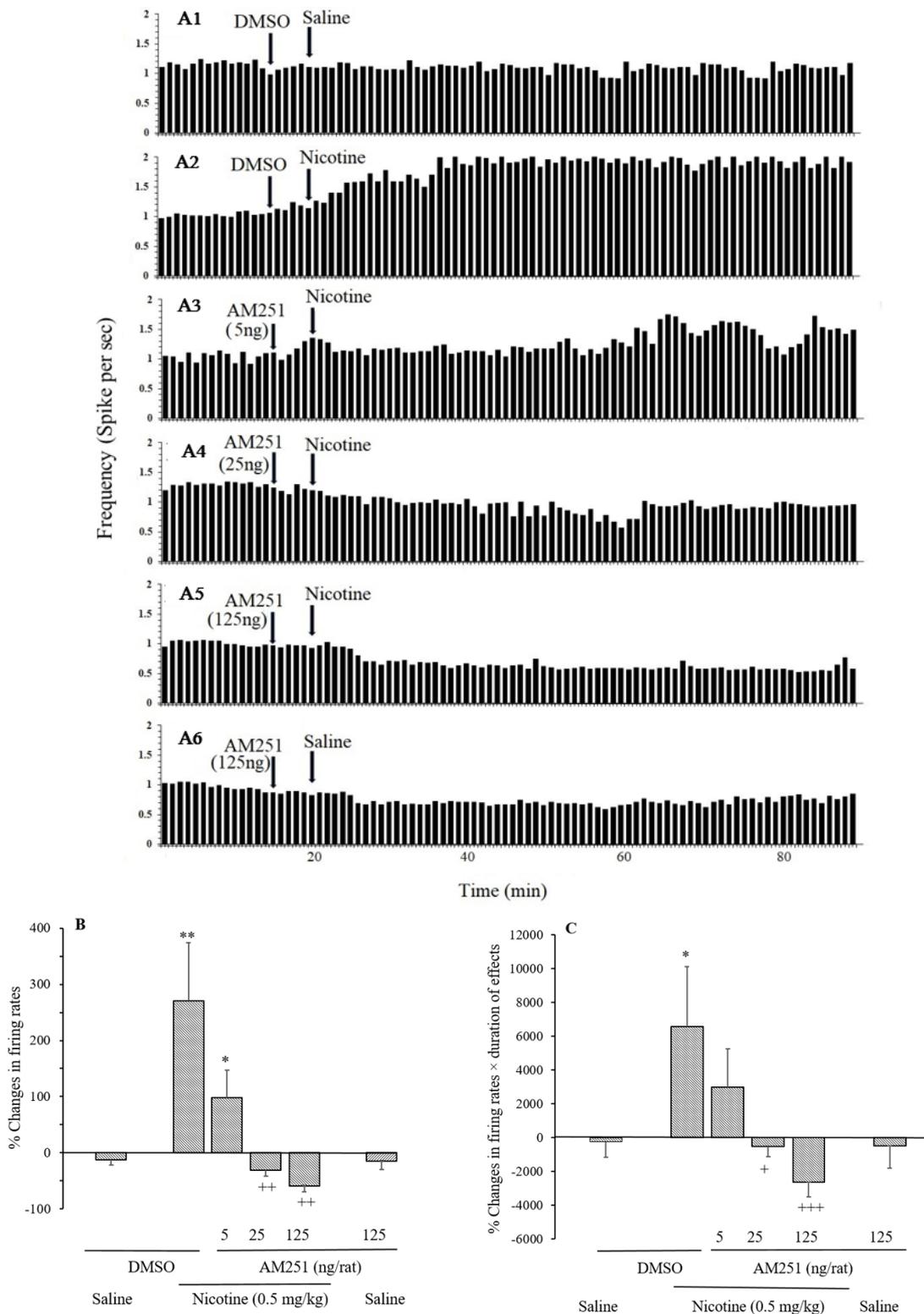


Fig. 2. (A) Histograms represent the spike frequency of the entire recording (90 min) of all neurons. (A₁) Saline could not affect the firing rate of the NAc neurons; but (A₂) nicotine increased the firing frequency; (A₃) also, AM251 at 5 ng could not inhibit the nicotine-induced excitation; (A₄ and A₅) but 25 ng and 125 ng doses of AM251 could inhibit the nicotine-induced excitation; (A₆) the microinjection of the maximum dose of AM251 (125 ng), before the subcutaneous administration of saline could change the neuronal response, compared to the saline control group. (B) The effect of the CB1R blockade on the percentage of decrease/increase activity of the NAc neurons in response to nicotine. (C) The effect of CB1R blockade on the NAc neurons in response to nicotine, when the duration of the inhibition was taken into account. Data is expressed as mean ± SEM. **P* < 0.05 and ***P* < 0.01 indicate significant differences compared to the saline control group; +*P* < 0.05 and ++*P* < 0.01, and +++*P* < 0.001 different from the nicotine control group. Data are expressed as mean ± SEM. CB1R, Cannabinoid receptor type 1; NAc, nucleus accumbens.

The neurons proportion (excitatory to inhibitory/no response), between the nicotine and AM251 (5 ng) groups was not significant [$\chi^2(1) = 0.855$, $N = 29$, $P = 0.301$]; but, it was significant between the nicotine and AM251 (25 ng) groups [$\chi^2(1) = 7.767$, $N = 27$, $P = 0.008$], and also between the nicotine and AM251 (125 ng) groups [$\chi^2(1) = 7.34$, $N = 23$, $P = 0.01$]. Neurons proportion (inhibitory to excitatory/no response) was not significantly different between the DMSO + saline and AM251 (125 ng) + saline groups [$\chi^2(1) = 0.019$, $N = 25$, $P = 0.613$].

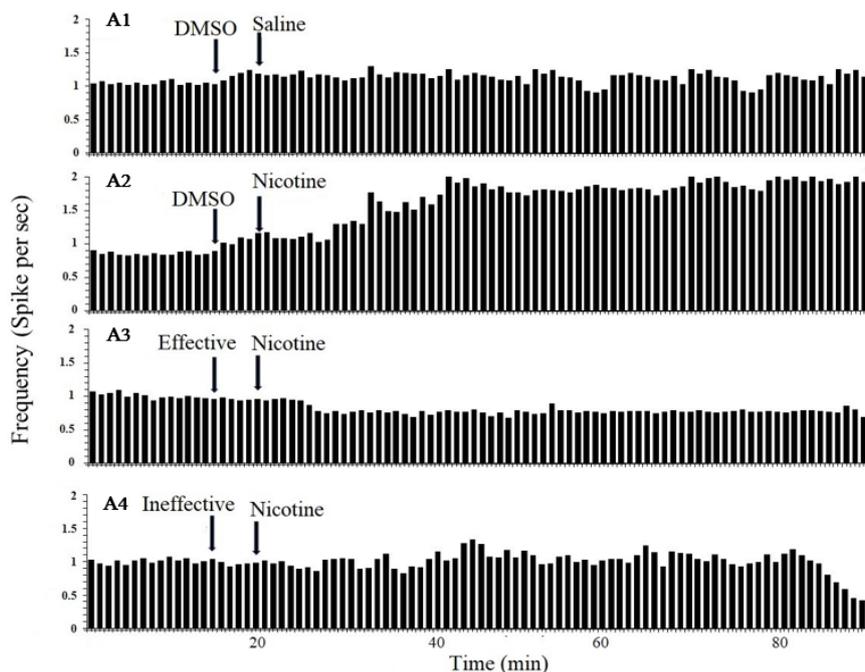
The analysis of the percentage of change of the neurons firing rates indicated that nicotine excited the neurons and AM251 adjusted the nicotine's effect [$F(5, 81) = 2.328$; $P < 0.05$] (Fig. 2B). Additionally, microinjection of the maximum dose of AM251 (125 ng) into the NAc before the subcutaneous administration of saline did not alter the percentage of change of the neuronal response, in comparison with the DMSO + saline group.

Furthermore, when the duration of responses (just excitatory/inhibitory responses) was considered, nicotine increased the neurons firing rate. In addition, AM251 modified the nicotine effect [The one-way ANOVA ($F(5, 60) = 2.471$; $P < 0.05$) (Fig 2C). Moreover, saline + the maximum dose of AM251 could not affect the neuronal firing rate, compared to the DMSO + saline group.

Effect of concurrent CB1R and OX2R blockade on the excitation of the NAc neurons induced by nicotine

Intra-NAc concurrent microinjection of the effective (25 and 3 ng/rat) or ineffective (5 and 1 ng/rat) doses of AM251 and TCS-OX2-29, respectively, were done, prior to the nicotine administration. The neurons' responses, in the group given effective doses (14 rats), 15/24 (62.5%) neuron inhibition, 5/24 (20.83%) neuron excitation, and 4/24 (16.66%) neurons without response (Fig. 3A₁₋₃) were found. In the group received ineffective doses (14 rats), 10/17 (58.82%) neuron inhibition, 4/17 (23.52%) neuron excitation, and 3/17 (17.64%) neuron without response (Fig. 3A₄) were considered. The proportion of neurons with excitatory response, to those with inhibitory or no response, was significant between the nicotine control group and the effective [$\chi^2(1) = 9.877$, $N = 36$, $P = 0.003$], and ineffective [$\chi^2(1) = 7.535$, $N = 29$, $P = 0.008$] groups.

The analysis of the percentage of change of the neurons firing rates indicated that the effective and ineffective groups could adjust the nicotine's effect [$F(3, 65) = 9.978$; $P < 0.001$] (Fig. 3B). Furthermore, when the duration of responses (just excitatory/inhibitory responses) was considered, the effective and ineffective groups modified the nicotine's effect [the one-way ANOVA ($F(3, 49) = 10.077$; $P < 0.001$) (Fig. 3C).



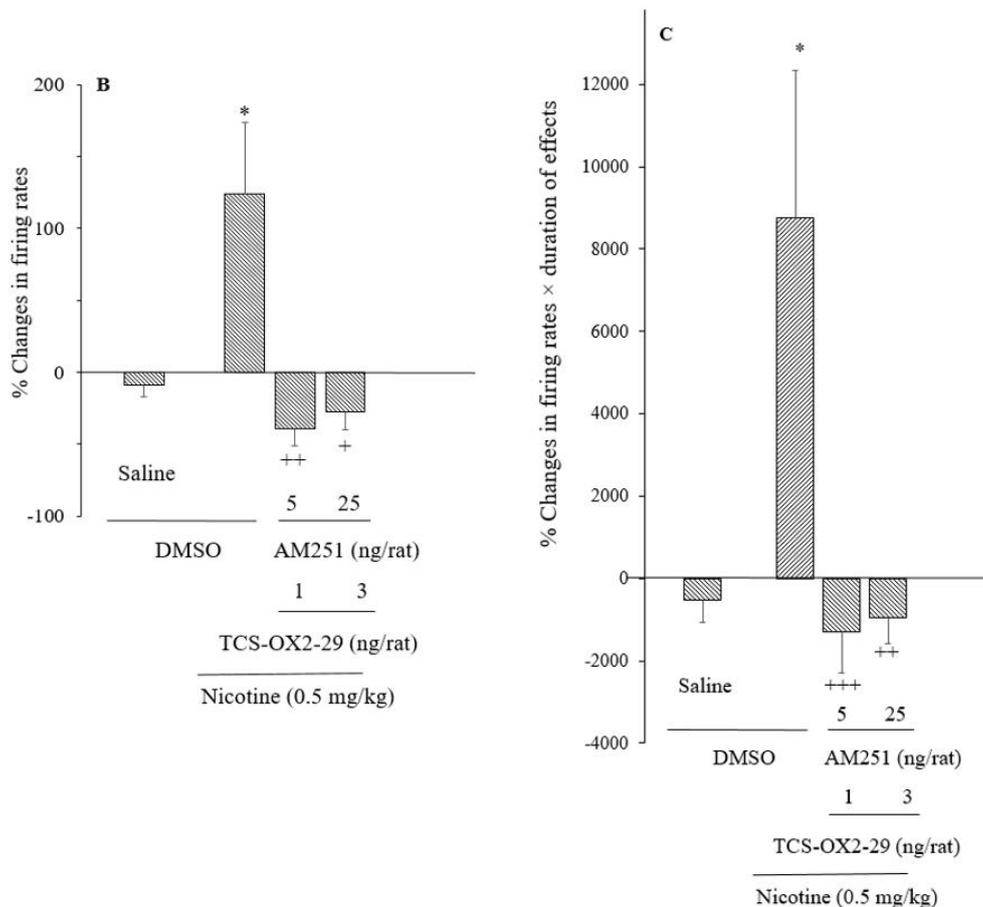


Fig. 3. (A) Histograms representing the spike frequency of the entire recording (90 min) of all neurons. The concurrent administration of (A₃) effective and (A₄) ineffective doses of AM251 and TCS-OX2-29 could prevent the nicotine-induced excitation. (B) The effect of concurrent blockade of the OX2R and CB1R of NAc on the percentage of increase/decrease activity of the NAc neurons, in response to nicotine. (C) The effect of concurrent blockade of the OX2R and CB1R of NAc on neuronal activity in response to nicotine, when the duration of the inhibition was taken into account. Data are expressed as mean ± SEM. **P* < 0.05 indicates significant differences compared to the saline control group; +*P* < 0.05 and ++*P* < 0.01, and +++*P* < 0.001 different from the nicotine control group. CB1R, cannabinoid receptor type 1; NAc, nucleus accumbens; OX2R, orexin-2 receptor.

DISCUSSION

A growing number of studies have shown that orexinergic (8,27-29) and cannabinoid (30-32) systems probably have a regulatory role in nicotine addiction. Both orexin receptors (OX1R, OX2R) are expressed in the NAc (17), but the OX1R levels are much lower, and OX2R is responsible for orexin's actions in this area (17,33), reviewed by Sharf *et al* (15). Also, the CB1Rs are found primarily in the brain (34) and expressed in the NAc (35,36), while the CB2R is found frequently in the peripheral nervous system (37). Based on the mentioned points, we tried to find the effect of CB1R on the firing rate of the NAc neurons in response to nicotine, and its probable crosstalk with the OX2R in this event, within this area.

The vast majority of the NAc neurons (90-95%) are GABA-containing medium spiny neurons (38), which display slow spike frequency (0.5 to 6.0 Hz) (19), confirmed by our results; and also the data from a previous work confirmed that the 0.5 mg/kg of nicotine increases the firing rate of the majority of NAc (19) and VTA neurons (32).

Studies showed that there are structural and functional interactions between nicotine and cannabinoid receptors, sharing several modulatory roles in some behavioral and physiological responses (10). A potential neurobiological substrate for this link is the colocalization of CB1R and nicotinic acetylcholine receptors in the brain (11). We detected that CB1R blockade in saline-treated animals, altered the ratio of neuronal responses,

but could not change the percentage of change. However, the CB1R blockade inhibited the nicotine's exciting effects on NAc neurons. These findings are consistent with the studies that have considered the CB1R blockade of VTA (8) or NAc (9) on the conditioned place preference induced by nicotine.

In addition, recent behavioral studies demonstrated that the OX2R has a modulatory action in nicotine addiction in the VTA (8) and NAc (9). Previous electrophysiological findings revealed that the OX2R blockade can inhibit the nicotine-induced increase of VTA (32) and NAc (19) neuronal responses. We showed that blockade of the NAc OX2R in the saline-treated animals can change the ratio of neuronal responses, without affecting the percentage of change. However, the OX2R blockade in the nicotine-treated rats prevented the nicotine's exciting effects on the NAc neuronal responses (19). Thus, it seems that the CB1R and OX2R blockade by themselves has no effect on the NAc neuronal activity, and they just modify the nicotine-evoked responses, and probably the NAc neurons response to nicotine is to some extent through orexin and endocannabinoid systems, as they can turn the nicotine preference to aversion and vice versa (9).

Several works support the idea that there is an interplay between cannabinoid and orexin systems, reviewed by Berrendero *et al.* (23) and Flores *et al.* (39). It has been shown that the OX2R and CB1R can interact with each other in the NAc but not in the VTA, in the development of the conditioned place preference induced by lateral hypothalamus stimulation (16); however, this interaction did not appear in a real model of addiction. Nonetheless, we recently showed that the CB1R can interact with the OX2R in the NAc (9), but not in the VTA (8,32), in response to nicotine.

Finally, the results showed that although there was no more impact of simultaneous administration of effective doses of OX2R and CB1R antagonists than the AM251 effective dose (25 ng/rat) alone on neuronal activity, the concurrent injection of the ineffective doses of both drugs reduced the nicotine-caused increase of NAc neuronal responses effectively, with a

significant difference, in comparison with the AM251 ineffective dose alone (5 ng/rat).

CONCLUSION

Taken together, it appears that orexin and cannabinoid by themselves presumably do not affect the neuronal firing rate of the NAc; nonetheless, their baseline action is essential and can moderate responses to nicotine more likely with an effect on nicotine receptors or other downstream organizations. Nonetheless, this possible link requires further examination to explain the essential mechanism of orexin and cannabinoid system action on the neurons of NAc. Also, it can be concluded that there is a potential interplay between the OX2R and CB1R in the NAc, at the receptor or the post-receptor levels, and these receptors seem to act through different pathways in the NAc, and it can be a result of the existence of diverse neurons' complex within the nucleus (16). In brief, the present results suggest that dependency and rewarding responses to nicotine can be extremely affected by the endocannabinoid and orexinergic systems in the NAc.

Acknowledgments

This research was financially supported by the Vice-Chancellery of Research of Isfahan University of Medical Sciences, Isfahan, I.R. Iran under Grant No. 395810.

Conflict of interest statement

All authors declared there is no conflict of interest in this study.

Authors' contribution

P. Reisi's contributed to the concept and design of the study, analyzed and interpreted the data, and revised the manuscript for important intellectual content. H. Alaei contributed to the concept and design of the study as well as the editing and revising of the manuscript for important intellectual content. R. Fartootzadeh contributed to literature search, experimental studies, data acquisition and analysis, and drafting the article. All authors participated in the final approval of the version of the article to be published.

REFERENCES

- Goniewicz ML, Delijewski M. Nicotine vaccines to treat tobacco dependence. *Hum Vaccin Immunother.* 2013;9(1):13-25. DOI: 10.4161/hv.22060.
- Ortells MO, Arias HR. Neuronal networks of nicotine addiction. *Int J Biochem Cell Biol.* 2010;42(12):1931-1935. DOI: 10.1016/j.biocel.2010.08.019.
- Mark GP, Shabani S, Dobbs LK, Hansen ST. Cholinergic modulation of mesolimbic dopamine function and reward. *Physiol Behav.* 2011; 104(1):76-81. DOI: 10.1016/j.physbeh.2011.04.052.
- Wu J, Gao M, Shen JX, Shi WX, Oster AM, Gutkin BS. Cortical control of VTA function and influence on nicotine reward. *Biochem Pharmacol.* 2013;86(8):1173-1180. DOI: 10.1016/j.bcp.2013.07.013.
- Beier KT, Steinberg EE, DeLoach KE, Xie S, Miyamichi K, Schwarz L, et al. Circuit architecture of VTA dopamine neurons revealed by systematic input-output mapping. *Cell.* 2015;162(3):622-634. DOI: 10.1016/j.cell.2015.07.015.
- Martini L, Waldhoer M, Pusch M, Kharazia V, Fong J, Lee JH, et al. Ligand-induced down-regulation of the cannabinoid 1 receptor is mediated by the G-protein-coupled receptor-associated sorting protein GASP1. *FASEB J.* 2007;21(3):802-811. DOI: 10.1096/fj.06-7132com.
- Jin L, Pan L, Guo Y, Zheng Y, Nie Z, Zhu R. Expression and localization of cannabinoid receptor 1 in rats' brain treated with acute and repeated morphine. *Acta Neurobiol Exp (Wars).* 2014;74(3):288-297.
- Azizi F, Fartootzadeh R, Alaei H, Reisi P. Effects of concurrent blockade of OX2 and CB1 receptors in the ventral tegmental area on nicotine-induced place preference in rats. *Neurosci Lett.* 2018;684:121-126. DOI: 10.1016/j.neulet.2018.07.017.
- Fartootzadeh R, Azizi F, Alaei H, Reisi P. Functional crosstalk of nucleus accumbens CB1 and OX2 receptors in response to nicotine-induced place preference. *Neurosci Lett.* 2019;698:160-164. DOI: 10.1016/j.neulet.2019.01.027.
- Lopez-Moreno JA, Gonzalez-Cuevas G, Moreno G, Navarro M. The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. *Addict Biol.* 2008;13(2):160-187. DOI: 10.1111/j.1369-1600.2008.00105.x.
- Viveros MP, Marco EM, File SE. Nicotine and cannabinoids: parallels, contrasts and interactions. *Neurosci Biobehav Rev.* 2006;30(8):1161-1181. DOI: 10.1016/j.neubiorev.2006.08.002.
- Alijanpour S, Rezayof A. Involvement of dorsal hippocampal and medial septal nicotinic receptors in cross state-dependent memory between WIN55, 212-2 and nicotine or ethanol in mice. *Neuroscience.* 2013;245:61-73. DOI: 10.1016/j.neuroscience.2013.04.030.
- Biala G, Kruk M, Budzynska B. Effects of the cannabinoid receptor ligands on anxiety-related effects of d-amphetamine and nicotine in the mouse elevated plus maze test. *J Physiol Pharmacol.* 2009;60(2):113-122.
- Dukes AJ, Fowler JP, Lallai V, Pushkin AN, Fowler CD. Adolescent cannabinoid and nicotine exposure differentially alters adult nicotine self-administration in males and females. *Nicotine Tob Res.* 2020;22(8):1364-1373. DOI: 10.1093/ntr/ntaa084.
- Sharf R, Sarhan M, Dileone RJ. Role of orexin/hypocretin in dependence and addiction. *Brain Res.* 2010;1314:130-138. DOI: 10.1016/j.brainres.2009.08.028.
- Yazdi F, Jahangirvand M, Pirasteh AH, Moradi M, Haghparast A. Functional interaction between OX2 and CB1 receptors in the ventral tegmental area and the nucleus accumbens in response to place preference induced by chemical stimulation of the lateral hypothalamus. *Pharmacol Biochem Behav.* 2015;139(Pt A):39-46. DOI: 10.1016/j.pbb.2015.10.012.
- Sahafzadeh M, Karimi-Haghighi S, Mousavi Z, Haghparast A. Role of the orexin receptors within the nucleus accumbens in the drug priming-induced reinstatement of morphine seeking in the food deprived rats. *Brain Res Bull.* 2018;137:217-224. DOI: 10.1016/j.brainresbull.2017.12.008.
- Kukkonen JP, Leonard CS. Orexin/hypocretin receptor signalling cascades. *Br J Pharmacol.* 2014;171(2):314-331. DOI: 10.1111/bph.12324.
- Fartootzadeh R, Azizi F, Alaei H, Reisi P. Orexin type-2 receptor blockade prevents the nicotine-induced excitation of nucleus accumbens core neurons in rats: an electrophysiological perspective. *Pharmacol Rep.* 2019;71(2):361-366. DOI: 10.1016/j.pharep.2018.12.013.
- Alger BE, Kim J. Supply and demand for endocannabinoids. *Trends Neurosci.* 2011;34(6):304-315. DOI: 10.1016/j.tins.2011.03.003.
- Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb Exp Pharmacol.* 2005;168:299-325. DOI: 10.1007/3-540-26573-2_10.
- Tsunematsu T, Yamanaka A. The role of orexin/hypocretin in the central nervous system and peripheral tissues. *Vitam Horm.* 2012;89:19-33. DOI: 10.1016/B978-0-12-394623-2.00002-0.
- Berrendero F, Flores A, Robledo P. When orexins meet cannabinoids: bidirectional functional interactions. *Biochem Pharmacol.* 2018;157:43-50. DOI: 10.1016/j.bcp.2018.08.040.
- Jääntti MH, Mandrika I, Kukkonen JP. Human orexin/hypocretin receptors form constitutive homo- and heteromeric complexes with each other and with

- human CB1 cannabinoid receptors. *Biochem Biophys Res Commun.* 2014;445(2):486-490.
DOI: 10.1016/j.bbrc.2014.02.026.
25. Taslimi Z, Haghparast A, Hassanpour-Ezatti M, Safari MS. Chemical stimulation of the lateral hypothalamus induces conditioned place preference in rats: Involvement of OX1 and CB1 receptors in the ventral tegmental area. *Behav Brain Res.* 2011;217(1):41-46.
DOI: 10.1016/j.bbr.2010.10.007.
 26. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates.* 6th ed. San Diego: Oxford: Academic Press; 2007. pp. 62.
 27. Dehkordi O, Rose JE, Dávila-García MI, Millis RM, Mirzaei SA, Manaye KF, *et al.* Neuroanatomical relationships between orexin/hypocretin-containing neurons/nerve fibers and nicotine-induced c-fos-activated cells of the reward-addiction neurocircuitry. *J Alcohol Drug Depend.* 2017;5(4):273-289.
DOI: 10.4172/2329-6488.1000273.
 28. LeSage MG, Perry JL, Kotz CM, Shelley D, Corrigan WA. Nicotine self-administration in the rat: effects of hypocretin antagonists and changes in hypocretin mRNA. *Psychopharmacology (Berl).* 2010;209(2):203-212.
DOI: 10.1007/s00213-010-1792-0.
 29. Hollander JA, Lu Q, Cameron MD, Kamenecka TM, Kenny PJ. Insular hypocretin transmission regulates nicotine reward. *Proc Natl Acad Sci U S A.* 2008;105(49):19480-19485.
DOI: 10.1073/pnas.0808023105.
 30. Gamaledin IH, Trigo JM, Gueye AB, Zvonok A, Makriyannis A, Goldberg SR, *et al.* Role of the endogenous cannabinoid system in nicotine addiction: novel insights. *Front Psychiatry.* 2015;6:41-52.
DOI: 10.3389/fpsy.2015.00041.
 31. Kudas E, Cohen C, Louis C, Griebel G. Cortico- limbic circuitry for conditioned nicotine-seeking behavior in rats involves endocannabinoid signaling. *Psychopharmacology (Berl).* 2007;194(2):161-171.
DOI: 10.1007/s00213-007-0813-0.
 32. Azizi F, Fartootzadeh R, Alaei H, Reisi P. Electrophysiological Study of the response of ventral tegmental area non-dopaminergic neurons to nicotine after concurrent blockade of orexin receptor-2 and cannabinoid receptors-1. *Brain Res.* 2019;1719:176-182.
DOI: 10.1016/j.brainres.2019.05.042.
 33. Marcus JN, Elmquist JK. Orexin projections and localization of orexin receptors. *The orexin/hypocretin system:* Springer; 2006. pp. 21-43.
DOI: 10.1385/1-59259-950-8:21.
 34. Pacher P, Bátkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev.* 2006;58(3):389-462.
DOI: 10.1124/pr.58.3.2.
 35. Morra JT, Glick SD, Cheer JF. Neural encoding of psychomotor activation in the nucleus accumbens core, but not the shell, requires cannabinoid receptor signaling. *J Neurosci.* 2010;30(14):5102-5107.
DOI: 10.1523/JNEUROSCI.5335-09.2010.
 36. Azizi P, Haghparast A, Hassanpour-Ezatti M. Effects of CB1 receptor antagonist within the nucleus accumbens on the acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized rats. *Behav Brain Res.* 2009;197(1):119-124.
DOI: 10.1016/j.bbr.2008.08.009.
 37. Pacher P, Mechoulam R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res.* 2011;50(2):193-211.
DOI: 10.1016/j.plipres.2011.01.001.
 38. Xia Y, Driscoll JR, Wilbrecht L, Margolis EB, Fields HL, Hjelmstad GO. Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. *J Neurosci.* 2011;31(21):7811-7816.
DOI: 10.1523/JNEUROSCI.1504-11.2011.
 39. Flores Á, Maldonado R, Berrendero F. Cannabinoid-hypocretin cross-talk in the central nervous system: what we know so far. *Front Neurosci.* 2013;7:256-272.
DOI: 10.3389/fnins.2013.00256.