

Received 2017-02-13
Revised 2017-04-10
Accepted 2017-04-25

DOI: 10.22086/GMJ.V6I2.825

Baclofen Reversed the Nicotine-Induced Anxiety-Like Behavior: A Beneficial Therapeutic Strategy for Smoking Cessation Aid

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Abstract

Background: Nicotine dependence is the most widespread form of substance abuse. Anxiety is one of the prominent symptoms in withdrawal syndromes of nicotine. Nucleus Accumbens (NAc) is a principle area which participates in several behavioral responses (e.g., anxiety), and several studies reported that receptors are involved in the modulation of anxiety. This study was aimed to examine the interaction of baclofen and nicotine on anxiety behavior in NAc shell. **Materials and Methods:** Rats weighing between 220 and 250g were used. The guide cannulas were implanted bilaterally into the shell of the NAc. Nicotine (0.025, 0.05 and 0.5 µg/rat) and baclofen (0.25, 0.5 and 2 µg/rat) and the co-administration of nicotine (0.025, 0.05 and 0.5µg/rat) with baclofen (0.25 µg/rat) were microinjected through the infusion cannula. The elevated plus-maze (EPM) was used to evaluate the anxiety-like parameters, and all data were recorded and analyzed. **Results:** Intra-NAc administration of nicotine (0.5µg/rat) decreased anxiety ($P < 0.001$). Microinjections of baclofen (0.5 and 2 µg/rat) decreased anxiety ($P < 0.001$). However, there were no significant changes in anxiety-like parameters at the lowest dose of baclofen (0.25µg/rat). Co-administration of an ineffective dose of baclofen (0.25 µg/rat) with 3 doses of nicotine (0.025, 0.05 and 0.5 µg/rat) reversed the effects of nicotine in an effective dose (0.5 µg/rat). **Conclusion:** Our result indicated that GABAergic agonist baclofen in ineffective doses could reverse the effects of nicotine on anxiety symptoms. Furthermore, our result suggested that enhancement of GABA transmission through activation of GABA-B receptors on NAc shell could reverse nicotine anxiety-related effects. It may be a strategy of treatment for smoking cessation aid. [GMJ.2017;6(2):118-27] DOI: 10.22086/GMJ.V6I2.825

Keywords: Nicotine; Baclofen; Smoking Cessation Aid; GABA-B Receptor Agonists



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Introduction

Tobacco dependence in the form of cigarette smoking causes morbidity and mortality all over the world [1]. Nicotine is the main psychoactive substance in tobacco. Nicotine dependence is the most widespread form of substance abuse and is addictive like heroin, cocaine or alcohol [2,3]. Additionally, 80% of habitual smokers want to give up smoking, but definite cessation is hard, therefore; most of them are unsuccessful [4]. In fact, the reason for their recurrence is to eliminate the several unpleasant symptoms of smoking withdrawal such as anxiety [5].

A principle area of the basal forebrain which participates in several behavioral responses (e.g., anxiety) is Nucleus Accumbens (NAc) [6]. The NAc receives most of the dopaminergic neurons originated from ventral tegmental area (VTA) [7] and the afferentations of its shell participate in physiological responses to anxiety [8,9]. Several studies demonstrated that GABA-B receptors are also involved in the modulation of anxiety [10-13]. Also, recently it was indicated that the administration of baclofen at high doses (20 mg q.i.d.) significantly reduces the number of cigarettes smoked per day in a smoking reduction trial [14]. Baclofen is a GABA-B agonist which is approved by Food and Drug Administration (FDA) of USA and marketed as an antispasmodic and muscle relaxant [15]. It has few side effects, easily accessible in most pharmacies, a safe and tolerated the medication [15-19]. Thus many preclinical studies evaluated the effects of baclofen on drug-reinforced behavior [20-23].

According to the reports, GABA-mediated neurotransmissions play a principal role in anxiety, but data on the specific role of GABA-B receptors are few and variable [24]. Considering the following statements; 1. anxiety is one of the major symptoms in withdrawal syndromes of some substance abuses (including nicotine); 2. several studies reported GABA-B receptor had also been implicated in anxiety behaviors; 3. NAc is a principle area of the basal forebrain which participates in anxiety behavior. To our knowledge, there are no published studies examining the inter-

action between intra-NAc microinjection of baclofen and nicotine in NAc on anxiety level by elevated plus maze (EPM) [25-27].

Baclofen might be a beneficial therapeutic strategy for smoking cessation aid. Therefore, the aim of present study was to examine whether there is interference between baclofen and nicotine in NAc shell.

Materials and Methods

Drug Preparation

The drugs injected in the experiment were (-)-Nicotine hydrogen tartrate salt ([-]-1-methyl-2-[3-pyridyl] pyrrolidine) (Sigma, Poole, Dorset, UK) and (±)- baclofen (Ciba-Geigy, Switzerland; Sigma, Poole, Dorset, UK). Nicotine solutions were provided in saline, and the pH adjusted to 7.2 ± 0.1 with sodium hydroxide and baclofen was dissolved in isotonic saline solution (NaCl 0.9%). The drugs were used in a volume of $0.6 \mu\text{l}/\text{rat}$ into the NAc shell. Total doses of the drug are expressed as $\mu\text{g}/\text{rat}$. Control animals received saline instead of drug solution.

Animals

Male Wistar rats (obtained from Pasteur Institute, Iran), weighing 220-250g at the start of surgery, were housed in Plexiglas-walled cages (4 rats per cage) in a standards temperature room (22 ± 2 °C) on a cyclic 12/12-h dark/light with the light on at 7 a.m. All the rats had free access to food and water except during the brief test periods and were allowed to acclimate to the laboratory conditions for at least one week before surgery. A total of eight animals were used in each group and each animal was used only once. All procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the ethics committee of the Islamic Azad University, Tehran Medical Sciences Branch (code: IR.iau.tmu. Rec1395.24)

Surgery and Microinjection

We anesthetized the rats with intraperitoneal (i.p.) injections of ketamine hydrochloride (50 mg/kg) plus xylazine (4 mg/kg) and fixed them in a Kopf stereotaxic instrument.

The skull was exposed by incisor bar–3.3 mm, the stainless steel guide cannulas (22 gauge, 13 mm in length) were implanted bilaterally into the shell of the NAc. The assigned stereotaxic coordinates measured in -3.6 mm from Bregma. The cannulas were inserted into the skull with the dental cement, and then stainless steel stylets (20-gauges) were anchored into the guide cannulas to keep them patency before microinfusions. According to Paxinos and Watson 2007 [28], stereotaxic coordinates for the NAc shell were measured identically as antero-caudal: +1.68 mm from Bregma; lateral: +0.8 mm from the sagittal suture; vertical: 5.47 mm from Dura. After completing surgery, to get cleared from the anesthetic effects, rats had recovered for a minimum of 7 days before experiments were carried out.

Bilateral microinjection of 0.3 µl/side on both side of NAc shell were made over the course of 60-90s through 27-gauge infusion cannulas (Supa. Co, Iran), the tips extended 2 mm more than the length of the guide cannulas in order to sit the tip exactly on the shell of NAc. Then, 2 µl Hamilton microsyringe, attached to polyethylene tube, was used for injection and to the prevention of reflux, the injection lasted 1 min.

Behavior Assessment

One of the most common tests for the study and evaluation of anxiety in rodents is the EPM [29]. This test is based on rodents' avoidance of height and open spaces and their tendency to prefer closed places. This wooden appliance is made of four arms in the shape of a plus sign (+). Two of the arms without any side or end walls are called open arms (50×10). To prevent neonatal rats from falling from the open arm, we attached a 1cm high glass wall at the end of the open arm. Each of the other two arms has two side walls, an end wall, and an open top, which are called closed arms (50×10×40). These four arms are connected through a squared platform called the central area (10×10). The maze is elevated with metal bases to a height of 50 cm. One 100-watt lamp provides appropriate light at the height of 120 cm over the central area of the maze. Rats are placed in one of the open

arms, facing the central area of the maze. The rat has 5 minutes to move freely and investigate different parts of the maze. Special parameters, such as the percentage of open arm time (%OAT), the percentage of open arm entry (%OAE), rearing, head dipping, and so on, are observed and recorded. For each rat, %OAT and %OAE are calculated as follows:
 $\%OAE = \text{ratio of entries into open arms to total entries} \times 100$

$\%OAT = \text{ratio of time spent in the open arms of total time spent in any arms} \times 100$

A significant increase in these two parameters represents a decrease in the anxiety levels of the rodents. In comparing the importance of these two parameters in recording the anxiogenic and anxiolytic behaviors, the %OAE is less sensitive than the %OAT. Other behavioral parameters are evaluated and recorded, too. All of the EPM tests were conducted in an area with sound insulation.

Study Design

1. Experiment 1: Effects of Intra-Nac Microinjections of Nicotine On Anxiety-Like Behavior

Four groups of animals received saline (0.6µl/rat) and different doses of nicotine (0.025, 0.05 and 0.5 µg/rat). Then, %OAT, %OAE, and locomotor activity were evaluated 5 min after intra-NAc microinjections.

2. Experiment 2: Effects of Intra-Nac Microinjections of Baclofen On Anxiety-Like Behavior

Four groups of animals received saline (0.6µl/rat) and different doses of baclofen (0.25, 0.5 and 2 µg/rat). Then, %OAT, %OAE, and locomotor activity were evaluated 5 min after intra-NAc microinjections.

3. Experiment 3: Effects of Intra-Nac Administration of Baclofen On Nicotine-Induced Anxiolytic-Like Behavior

In this experiment, the animals received saline (0.6µl/rat) and different doses of nicotine (0.025, 0.05 and 0.5 µg/rat) 5 min after saline (four groups) or subthreshold doses of baclofen (0.25 µg/rat; four groups). Then, %OAT, %OAE, and locomotor activity were measured 5 min after second intra-NAc microinjections.

Histological Verification

Rats received bilateral microinjection of methylene blue (1%) 0.3 µl/side on both side of NAc shell for confirming the site of the drug injection. We killed the animals by anesthetic overdose; they were then decapitated, their brains separated and placed in formaldehyde (10%). After several days the brains were sliced with microtomes, injection sites justified according to Paxinos and Watson atlas (2007) [28]. Data from the animals with injection sites located outside the targeted sites (less than 5%) were discarded.

Data Analysis

Data were presented as mean±SEM. One-way analysis of variance (ANOVA) was applied to the evaluation of comparison between the effect of different doses of nicotine or baclofen with its control. Two-way ANOVA was used for evaluation of interactions between drugs. Following a significant F-value, post-hoc analysis (Tukey-test) was prepared for assessing specific inter-group comparisons. All statistical analyses were done using SPSS (version 19) and $P < 0.05$ was considered as statistically significant.

Results

Effects of Nicotine On Rats Behavior in the EPM

Table-1 show the effects of nicotine on anxiety-related parameters in the EPM. A one-way ANOVA revealed that nicotine (0.5µg/rat) in-

creased %OAT ($F(3,28) = 92.76, P < 0.001$) indicating an anxiolytic-like response to nicotine. No significant change in the %OAE ($F(3,28) = 0.99, P = 0.41$) and locomotor activity ($F(3,28) = 1.30, P = 0.32$) was observed following administration. The data showed that the anxiolytic-like effect was obtained by 0.5µg/rat of nicotine, while the administration of 0.05 and 0.025 µg/rat of nicotine had no significant effect in the EPM.

Effects of Baclofen On Rats Behavior in the EPM

Regarding Table-1, the animals that received baclofen (0.5 and 2 µg/rat), one-way ANOVA revealed significantly increased %OAT ($F(3, 28) = 30.75, P < 0.001$) indicating an anxiolytic response. No significant change in the %OAE ($F(3, 28) = 1.74, P = 0.18$) and locomotor activity ($F(3, 28) = 1.38, P = 0.26$) was observed following injection of (0.25 µg/rat) baclofen. The data may indicate that the baclofen induces an anxiolytic response.

Effects of Intra-Nac Administration of Baclofen On Nicotine-Induced Anxiolytic-Like Response

Figure-1 shows the effects of intra-NAc administration of nicotine alone or in combination with baclofen (0.25 µg/rat) on anxiety-related parameters in the EPM. In the group that received nicotine alone or with baclofen, two way ANOVA indicates a significant interaction between nicotine (0.025 ,0.05 and 0.5 µg/rat), baclofen (0.25 µg/rat) on %OAT (intra ef-

Table1. Rats Behavioral Measured in the EPM After Bilateral Microinjection of Nicotine and Baclofen Into NAc Shell.

Groups	%OAT	%OAE	Locomotor activity
	mean±SEM	mean±SEM	mean±SEM
Sham	28.1±1.32	44.2±16.40	8.0±1.2
Saline	27.3±4.92	43.3±17.67	7.0±1.9
Nicotine 0.025 (µg/rat)	28.12±6.46	51.25±16.37	8.8±2.1
Nicotine 0.05 (µg/rat)	33.75±1.98	54.44±13.55	7.6±2.4
Nicotine 0.5 (µg/rat)	63.37±5.50 ^a	55.9±15.88	10.2±3.1
Baclofen 0.025(µg/rat)	35.5±3.20	42.00±12.80	7.0±1.9
Baclofen 0.5(µg/rat)	56.87±17.60 ^a	45.37±12.86	8.8±2.1
Baclofen 2(µg/rat)	69.62±6.80 ^a	56.6±13.15	7.6±2.4

A: $P < 0.001$ compared to saline control Group

fect (group effect): $F(3, 56) = 9.81, P < 0.001$; inter effect (drug effect): $F(1, 56) = 54.21, P < 0.001$; intra-Inter effect (group – drug interaction effect): $F(3, 56) = 82.09, P < 0.001$, but not on %OAE (intra effect (group effect): $F(3,56) = 2.9, P=0.39$; inter effect (drug effect): $F(1, 56) = 0.57, P=0.45$; intra-inter effect (group – drug interaction effect): $F(3, 56) = 0.1, P=0.91$) and not on locomotor activity (intra effect (group effect): $F(3,56) = 1.69, P=0.17$; inter effect (drug effect): $F(1, 56) = 0.32, P=0.57$; intra-inter effect (group – drug interaction effect): $F(3, 56) = 2.05, P=0.11$).

Discussion

The present results show that intra-NAc microinjection of Nicotine in low dose ($0.5 \mu\text{g}/\text{rat}$) increases %OAT (the parameters of anxiety-related behavior) without %OAE and locomotor impairment in the EPM indicating an anxiolytic response, which is in agreement with the data in previous studies [30,31].

Several human and animal studies are demonstrating that nicotine alters anxiety-like behavior that could play a role in its addictive properties. In the rodents EPM test, contra-

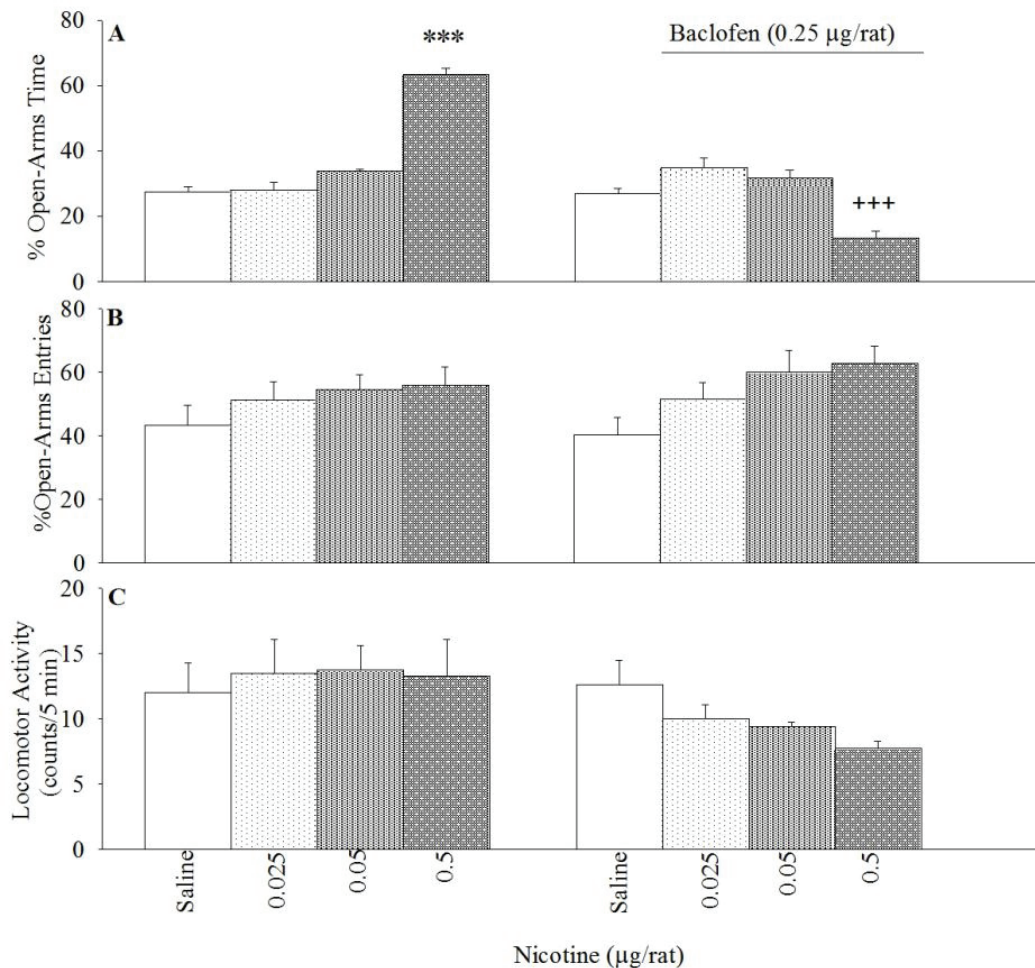


Figure 1. Effect of intra-NAc administration of nicotine along or with baclofen on rats behavior in the EPM. The animals received intra-NAc microinjection of saline and different doses of nicotine (0.025, 0.05 and $0.5 \mu\text{g}/\text{rat}$). After 10 min, they were injected with intra-NAc injections of baclofen, 5 min before testing. Each bar represents mean \pm SEM of %OAT (A), %OAE (B) and locomotor activity (C). *** $P < 0.001$, compared to saline group; +++ $P < 0.001$, compared to saline/nicotine group.

dicting results have been shown, variety in response to an acute administration of nicotine is reported. Relatively low doses of nicotine (0.1–0.4 mg/kg) have an anxiolytic [30] or anxiogenic effect [32] or causing no change on anxiety level [33,34]. In accordance with these studies and to be more accurate, nicotine in the low dose (0.05 mg/kg) has an anxiolytic-like response, while nicotine in high dose (0.8 mg/kg) induced an anxiogenic-like effect in mice by the EPM test [31,35,36]. These variances in rodents may depend on the dose, the time between injections and testing, the route of administration, the kind of behavioral test used, the genetic strain and the baseline anxiety level [35- 39].

Also, our results show that intra-NAc micro-injection of baclofen in NAc shell increased %OAT, the factors involved in anxiolytic-like behavior without %OAE and locomotor impairment in the EPM which demonstrated an anxiolytic response that is in line with previous studies [40-42]. In accordance with this, baclofen has anxiolytic-like responses in several preclinical tests. It has an anxiolytic-like behavior in a T-maze test [41].

It is clearly acknowledged that nicotine applies its behavioral effects by activation of nicotinic acetyl choline receptors (nAChRs). These nAChRs are situated predominantly presynaptically on dopaminergic neurons in VTA (i.e., Amygdala, Prefrontal Cortex and NAc) and affect on reinforcing locomotor stimulant by releasing dopamine [43-45]. Also, it seems that nAChRs receptors involved in the processes of learning and memory, reward, antinociception, and anxiety responses [46]. In line with this peripheral nicotine administration can increase extracellular dopamine levels for more than one hour in the rodents' NAc [47]. Activation of nAChRs within different areas of brain enhances release of several neurotransmitters including stimulatory (glutamate), modulatory (e.g., dopamine, norepinephrine, and serotonin) and inhibitory (e.g., GABA) [48,49]. The regulation of releasing these neurotransmitters which are induced by nicotine's effects depending on various postsynaptic receptors is the usual mechanism by which nicotine modulates behavior [50]. In the other hand, activation of GABA-B re-

ceptors in VTA lead to blocking dopamine releasing in NAc [51,52]. Also, previous studies provide clear evidence for the involvement of baclofen, as GABA-B receptors agonist, in decreases the withdrawal syndrome of ethanol and barbital [53, 54]. In accordance with this, we have observed in previous studies that baclofen prevented the somatic signs [55] and reestablished dopamine concentration in striatal and cortical [56] morphine withdrawal induced in mice. Nevertheless, other studies indicated that baclofen reduces the nicotine self-administration [20] and conditioned rewarding effects of nicotine [57]. Moreover, baclofen reversed the anxiogenic response induced by withdrawal from chronic diazepam or alcohol treatment [52,58,59].

Taken together in agreement with these reports, our result demonstrated microinjection of baclofen in NAc could reverse anxiety level which induced by nicotine in the male rats in EPM test. Clinically; baclofen reverses the anxiety associated with alcohol withdrawal [17]. Also, in rodent experiment baclofen was able to suppress alcohol withdrawal signs [60], post-traumatic stress [61], panic disorder [62] and traumatic spinal-cord lesions [63]. Moreover, a recent report showed baclofen dose-dependently blocked nicotine-induced reinstatement of self-administration in rats and dose-dependently extinguished nicotine-conditioned place preference in mice [64]. It is demonstrated that baclofen reduced nicotine-reinforced behavior at doses that do not affect responding for food, water, or locomotor activity. Also in other studies have been shown baclofen can reduce dopamine releasing and drug-reinforced behavior, suggesting that is not sedating and does not disrupt normal activities [20-23,65-67].

In accordance with all of them, the present experiment shows the same result. It is suggesting a sedative effect of baclofen is not causing its anxiolytic responses, because we microinjected baclofen via intra-NAc directly to CNS (i.e., NAc shell), its doses were very low and could not effect on rat's peripheral nervous system and movement. So it means baclofen did not effect on the locomotor activity of rats and did not effect on its anxiolytic responses in our EPM test.

Conclusion

Our results suggested that the dopaminergic neurons in NAc shell might have both nicotine and GABA_B receptor. These receptors could be under the control of baclofen, GABAergic systems, nicotine, and cholinergic systems; balances mean hyperactivation of cholinergic signaling which induced by nicotine, and it could also lead to anxiety symptoms. GABAergic agonist baclofen in ineffective doses could reverse the effects of nicotine on anxiety symptoms. Also, enhancement of GABA transmission through activation of GABA_B receptors on NAc shell could reverse nicotine anxiety-related effects. Moreover, in dopaminergic neurons of NAc shell might have both

nicotinic and GABA_B receptors and are under the balances between those two. Hence, our study suggests that baclofen could be a beneficial therapeutic strategy for smoking cessation aid.

Acknowledgments

This study was supported by a grant (No. 08/13/2925) from the Islamic Azad University, Tehran Medical Sciences Branch.

Conflict of Interest

The authors declare no conflict of interests.

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