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ORIGINAL ARTICLE

GABAB Receptor Agonist Attenuates the Expression of Dextroamphetamine-Induced Conditioned Place Preference in Wistar Rat

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ABSTRACT
Background: Preclinical studies support the hypothesis that alterations in GABA neurotransmission can alter brain reward system function and, rewarding effects of psychostimulants. Objectives: This study aims to investigate if baclofen, a GABAB receptor agonist, has any effect on amphetamine craving behavior. Methods. 5-day conditioning schedule was performed with dextroamphetamine (0.1, 0.5, 1 mg/kg; IP) to determine the maximum effective dose of the drug. Another pilot test was performed with baclofen (1.25, 2.5, 5 mg/kg; IP) to determine its potential to induce either positive or aversive conditioning. Finally rats were conditioned with dextroamphetamine (1 mg/kg; IP) and then treated on 2 consecutive days with baclofen (1.25, 2.5, 5 mg/kg; IP) or saline and then they are assessed for maintenance of place preference behavior. Results. dextroamphetamine at 1 mg/kg was found to be most effective for conditioning behavior ($p<0.001$). Baclofen itself had no effect neither on place preference nor on aversive preference ($p=0.45$). When it was administered in two consecutive days, baclofen (1.25, 2.5 and 5.0 mg/kg, IP) attenuated the expression of conditioned response ($p<0.001$). This inhibitory effect was not the consequence of altering animals' motor activity. Conclusion. . These results indicated that baclofen suppressed the memory maintenance of cues associated with dextroamphetamine; baclofen also might be effective via decreasing the hyperactivity of dopamine activity in reward system and attenuates craving for drug of abuse. It may be potentially effective in the treatment of amphetamine craving.
Keywords; *Baclofen, Dextroamphetamine, Craving, Conditioned place preference, Cue-associated memory*

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INTRODUCTION

There is an increasing concern about the use of psychostimulats including amphetamines as a major public health issue worldwide. Use of amphetamine-like stimulants has significant psychiatric and medical consequences [1-3].

Animal studies have had a major role in understanding the biology and pathophysiology of drug dependency and abuse. In contrast to clinical studies, controlling various variables among the subject population is easier. These models often focus on the ability of the drugs to directly control an animal's specific behavior, which is consistent with specific addiction related behavior in humans. Various paradigms are designed to investigate any specific addiction related behavior. Conditioned place preference (CPP) paradigm is one of them defined to investigate drug craving behavior [4,5].

Reinforcing effect of amphetamine is principally mediated via stimulation of the corticomesolimbic dopamine (DA) system. Amphetamine administration increases concentration of monoamines in the

synapses by inhibition of MAO, blockade of their reuptake and promotion of their release [6]. DA is the crucial neurotransmitter in reward process which acts mainly in Nucleus Accumbence (NAc) [7, 8]. Treatment with DA antagonists as well as destroying the DA system both attenuates the self-administration of amphetamine in the self-administration paradigms in animal models of amphetamine dependence [9, 10]; although results in open-label human studies were hope-giving, later double blind clinical trials were not satisfactory and yet there are no approved pharmacological treatments for psychostimulant misuse [11, 12].

GABA is the major inhibitory neurotransmitter in the brain which modifies the function of many other systems, including those involved in reward system. Mesolimbic Dopaminergic neurons receive inhibitory GABAergic inputs from ventral pallidum and NAc. Preclinical studies support the hypothesis that alterations in GABA neurotransmission, can alter brain reward system function and, hence, the motivational, behavioural, and rewarding effects of drugs of abuse including psychostimulants [13, 14]. In animal models it's been shown that pretreatment with GABAB agonist, Baclofen, can inhibit rewarding effect of opiate in CPP paradigm [15]. Furthermore preclinical and clinical evidence show that GABAB agonists may be useful in treatment of cocaine addiction [16,17]. Although amphetamine and cocaine are both considered as psychostimulants, their pharmacological actions of amphetamine is quite different from other stimulants such as cocaine as the mechanism of its action is mainly via displacement of dopamine and norepinephrine from their storage sites and increase their release to the synaptic space [18].

GABAB receptor positive allosteric modulators can inhibit the expression of amphetamine-induced CPP [19]. Also GABAB positive allosteric modulators and Baclofen blocked the acquisition of amphetamine-induced CPPwhen used before the behavior is learned by the animals [20]. This drugs also facilitates the extinction of methamphetamine-induced CPP when added to extinction training [21].

Current study is performed to investigate if Baclofen as an available drug in market can reduce CPP of amphetamine after conditioned-reward has established which is the corresponding of craving behavior in amphetamine dependency in human.

MATERIALS AND METHODS

Animals

Male Wistar rats obtained from Pasteur Institute of Iran. All animals weighted 220-280 grams at the beginning of experiments. They were housed in room temperature of 22° C and in 12/12 hours light/ dark cycle; lights turn on at 7:00 AM. They had food and water *ad libitum*. Animals were allowed to habituate to their new environment for one week after arrival to laboratory.

Apparatus

1-A conditioned place preference (CPP) apparatus, consisted of two 30×30×60 cm chambers, connected by a guillotine gate to corridor space 10×30×60 cm. Two chambers were different in their color (black or white) and floor texture (textured or smooth). Chambers are observed by a camera system and time spend in each chamber were calculated manually by an observer.

2- Locomotion chamber device has been used to measure locomotor activity.

Conditioned place preference procedure

The 5-day condition place preference consisted of three phases:

1- Pre-conditioning test: in this phase natural preference to one of the chambers is tested. Animals were put in the corridor space while the guillotine gate is open so they can move freely in both chambers. They were observed for 30 minutes. The compartment occupied more was designated as the more preferred side, and if the animal spend nearly all the time in one chamber, was excluded from the study because of natural bias.

2- Conditioning phase: this phase performed in three consecutive days. On first day (day 2) morning, dextroamphetamine was injected intraperitoneally and the animal were placed in the less preferred side of the apparatus for 45 minutes while the guillotine gate is closed. The goal was to pair the dextroamphetamine effect with the color and texture of the chamber. 6 hours later on the same day saline was injected and the animal was placed on the opposite site. On second day (day3) the time order of dextroamphetamine and saline was reversed. The third day (day4) protocol is the same as the first day.

3- Post-conditioning test: on day 5 animals were placed in the corridor space while guillotine gate was open and observed for 30 minutes. The time spent in each chamber was recorded. Minimum 10% increase in time spent in drug-paired chamber before and after the conditioning procedure, is necessary but not sufficient to define successful conditioning.

Drugs

Dextroamphetamine HCl (DXAMPH) and Baclofen were obtained from Tocris bioscience (Bristol, UK) and dissolved in physiologic saline. Both drugs were injected subcutaneously in a volume of 1 ml/kg.

Experiment design

1- Dexroamphetamine dose-response pilot test: 4 group of rats (n=6) were given dextroamphetamine (0.1, 0.5, 1 mg/kg) or Saline and CPP protocol was performed. Conditioned behavior was assessed on day 5 and also on day 8.

2- Baclofen preferred or aversive conditioning test: 4 group of rats (n=6) were given Baclofen (1.25, 2.5, 5 mg/kg) or saline and CPP protocol was performed. In these series of experiments because primarily the aversion of the Baclofen-paired chamber had to been measured, animals were placed in the more preferred side after drug administration.

3- Effect of Baclofen treatment on established dextroamphetamine-induced conditioned place preference: four groups of animal (n=32) were conducted with dextroamphetamine (1 mg/kg, IP); on day 6 and 7, they were given saline or Baclofen (1.25, 0.5, 5.0 mg/kg, IP) in order to assess the effect of this GABAB receptor agonist on the established place preference (Summary of the study design is illustrated in figure 1).

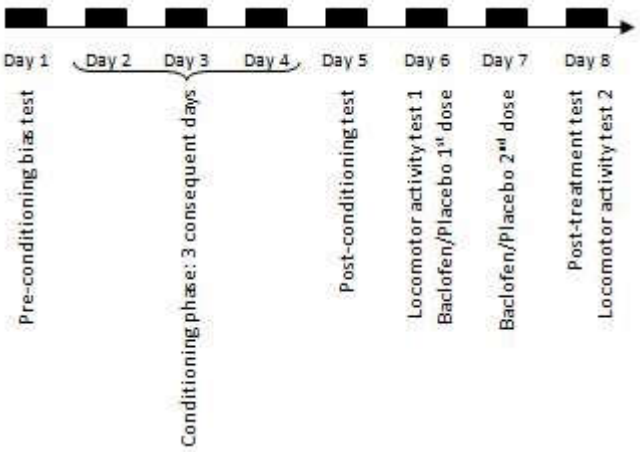

Figure 1- illustration of study protocol. Drug-free pre-test was done on day 1. Then rats received DXAMPH (1 mg/kg- IP) and conditioning process was performed on day 2 to 4. CPP test was done on day 5 and to verify if conditioning has occurred. Baclofen or placebo was administered on 2 consecutive days (day 6 and 7). The final CPP test was performed on day 8 to determine the capacity of Baclofen to attenuate the expression of DXAMPH-induced CPP. Locomotor activity was assessed before and after the administration of Baclofen (day 6 and 8) to determine if alteration in locomotor activity can mediate the effect of Baclofen on CPP.

DATA ANALYSIS

CPP was defined as spending significantly more time in the Meth-paired vs. saline-paired chamber. This was presented as the means ± S.E.M. and was evaluated statistically with a one-way analysis of variance (ANOVA) and post hoc Newman–Keuls. The acceptable level of statistical significance was $p < 0.05$. Data analysis performed by IBM SPSS statistics 19.

Ethical issues

This research has the approval of the Ethical committee of Tehran University of Medical Sciences and conducted in accordance with highest ethical and humane standards.

RESULTS

A pilot Dextroamphethamine (DAMP) dose–response was conducted with 0.1, 0.5, and 1 mg/kg, IP (n = 6 in each group). The most robust CPP was obtained with 1mg/kg. Comparing time spent (seconds) in the saline-paired vs. time spent in the DAMP-paired chamber for each dose resulted in the following: 0.1 mg/kg, 604±405 vs. 826±124; 0.5 mg/kg, 523±226 vs. 1154±232; and for 1mg/kg, 296±132 vs. 1347±149. DAMPH (0.5–1.0 mg/kg, IP) produced significant place preference [$F(3,20)= 13.363$,$p < 0.001$]. A post hoc Newman–Keuls test demonstrated significant CPP (i.e., a significantly greater amount of time spent in DAMP-paired chamber compared to the saline-paired chamber) for those rats conditioned with 1mg/kg DAMP. Thus, we choose the 1 mg/kg dose to induce DAMP-induced CPP for the rest of the study.

Another pilot study was performed to investigate if Baclofen alone can produce aversion or preferred conditioning. Baclofen did not produce not aversive nor preferred conditioning behavior [F(3,20)= 0.918, p= 0.45].

The 5-day conditioning protocol resulted in a significant preference for the DAMP-paired chamber. As a group, the 32 rats tested expressed CPP during Test 1 (day 5) (time spent in DAMP-paired chamber, 1241±296 s; time spent saline-paired chamber, 236±193 s; paired t-test: $p < 0.001$). 3 rats did not demonstrate a 10% (180 s) increase in time spent in drug-paird chamber; hence they were excluded from the rest of the study. This was done to assure us that only those rats that acquired the conditioned behavior were used to determine the potential for GABAB receptor agonist to subsequently reduce the acquired preference. Post-conditioning administration of saline did not impact the ability of drug free rats to express a preference for the DAMP-paired chamber. While administration of Baclofen in two consecutive days after test 1 and before test 2 attenuates the established place preference ($F(3, 25)= 7.241$, $p= 0.001$). Post-hoc Newman-Keuls test revealed that Baclofen in doses 1.25, 2.5 and 5 mg/kg IP, had this effect (figure 2). No difference in the magnitude of this effect was seen among three doses of baclofen ($p= 0.628$).

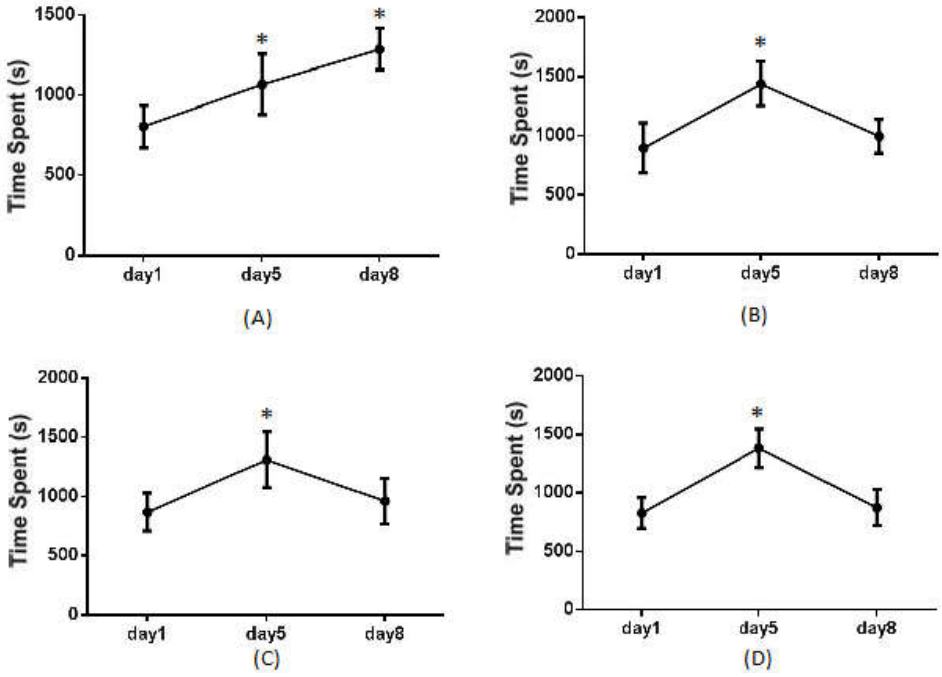

Figure 2- Dextroamphetamine-induced CPP is attenuated by post-conditioning administration of Baclofen. Rats assigned to Salin group expressed CPP on day 5 and day 8 (A). Rats administered Baclofen 1.25 mg/kg (B), 2.5 mg/kg (C) or 5 mg/kg (D) did not maintain conditioned place preference behavior for the dextroamphetamine-paired chamber on CPP Test 2 (Day 8) . *$p<0.05$

In addition, we have determined that the inhibitory effect of baclofen on maintenance of DAMP-induced CPP is not the consequence of altering motor activity. Locomotor behavior as measured by a photobeam activity system was not different between different groups (Saline, 56.8±10.56; Baclofen 1.25 mg/kg, 46±16.92; Baclofen 2.5 mg/kg, 57.88±15.41; Baclofen 5 mg/kg, 61.88±9.59; one-way ANOVA, $p = 0.152$).

DISCUSSION

Pairing of DAMP (0.5, 1 mg/kg, IP) with the primarily less preferred side of the apparatus produced a significant conditioning for the drug-paired side. The observed DAMP-induced preference was resistant to extinction; as repeated re-exposure for at least 3 days after conditioning to the chambers did not extinguish the preference in saline treated rats.

Current study revealed that systemic use of baclofen (1.25, 2.5, 5.0 mg/kg, IP) attenuates the expression of DAMP-induced place conditioning.

Locomotor activity was not significantly altered by Baclofen administration; so, the place preference results shall not be the consequence of an alteration in the capacity of rats to successfully perform the task.

Based on these findings it can be concluded that augmenting GABAB receptor signalling facilitates the extinction of DAMP induced CPP.

The present findings are in accordance with previous studies demonstrating effect of Baclofen on CPP induced by other substances including cocaine [16], and morphine [22, 15].

Li et al and Halbout et al also showed that systemic use of GABAB receptor agonist, baclofen, immediately after conditioning sessions with psychostimulants suppressed the expression of DAMP-reinforced place conditioning.

In this study baclofen was not presented on the day of CPP testing, as we concerned with the enduring effect of baclofen rather than the acute effect.

Because in this study baclofen was administered systemically, the site of its action remains unclear. But it is possible that this effect is attained by its modulatory effect on mesolimbic dopaminergic system. Dopaminergic neurons projecting from the ventral tegmental area to the NAc and Amygdala are the mainstay of brain reward system [23]. Specifically, the reinforcing properties of amphetamine derivatives are linked to their ability to displace dopamine and norepinephrine from their storage sites and increase their release to the synaptic space [24]. In support of this, there are ample of evidence indicating lesions of dopaminergic neurons or dopamine depletion reduces the reinforcing effects of amphetamine [25]. Despite the captious role of mesolimbic dopaminergic system in the rewarding effects, dopamine receptor blockers has limited utility as practical treatments for psychostimulant misuse [10].

Mesolimbic dopaminergic neurons receive descending inhibitory GABAergic inputs from the ventral pallidum and NAc [26, 14]. GABAergic neurons of NAc project to DAergic neurons within the VTA; therefore it seems rational to evaluate GABAmimetic drugs, as modulators the midbrain dopamine system and attenuating reinforcing and rewarding effect of DAMP in this study [14].

From another point of view baclofen can interfere with cue-associated, memory consolidation. When a memory is recalled but not successfully reconsolidated, extinction may occur [27]. Reconsolidation can be interfered with inhibiting glutamatergic neurotransmission [28], which itself can be done with GABAB receptor activation [29]. This process is shown to be effective in reducing cocaine craving [30].

In conclusion, we have found that systemic administration of the GABAB receptor agonist, baclofen, attenuates recently DAMP-induced conditioned place preference; together, these findings suggest that baclofen has potential as a therapeutic drug for amphetamine type stimulants abuse and dependence. Further human studies based on this finding are recommended. Also further studies using specific receptor agonists/antagonists and intra nuclear administration of drugs is suggested to better delineate the molecular mechanisms and brain pathways involved in reward process.

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