The Effect of Orphenadrine on Rewarding Property of Morphine-Induced Conditioned Place Preference

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Abstract

Objective: Orphenadrine (Orp), an anti-cholinergic drug, is subjected to drug abuse and dependence in recent years. This experiment was designed to study the effect of Orp on rewarding and reinforcing properties of morphine-induced conditioned place preference (CPP).

Methods: Rats were randomly divided into six groups as control (serum physiological), morphine (10 mg/kg), Orp (20 mg/kg and 30 mg/kg), and Orp + morphine (20 mg/kg, 30 + 10 mg/kg, respectively). After pretest, the rats were given multiple intraperitoneal injections of saline solution and Orp on alternative days.

Results: Morphine significantly enhanced the preference scores in the drug-paired side ($p<0.001$), whereas both groups with doses of Orp (20 mg/kg and 30 mg/kg) did not exhibit any preference compared with the control group. Orp (30 mg/kg) + morphine showed no preference, whereas Orp (20 mg/kg) + morphine significantly decreased the CPP when compared with morphine group ($p<0.01$). The combined used of Orp (20 mg/kg) + morphine reduced the preference, whereas Orp (30 mg/kg) + morphine did not exhibit acquisition of CPP in rats.

Conclusion: The acquisition of CPP is thought to be due to N-methyl D-aspartate receptor antagonistic effects of Orp at low dose. It is thought that the rewarding feature or abuse potential should be analyzed in further investigation.

Keywords: Morphine, orphenadrine, drug dependence, addictive behavioral

Morfinin Ödüllendirici Özelliği ile Oluşan Koşullanmış Yer Tercihine Orfenadrin'in Etkisi

Öz

Amaç: Orfenadrin (Orp), son yıllarda bağımlılık ve kötüye kullanımı olan antikolinerjik bir ilaçtır Mevcut deney, orfenadrinin morfin kaynaklı koşullu yer tercihinin (CPP) ödüllendirici ve pekiştirici özellikleri üzerindeki etkisini incelemek üzere tasarlanmıştır.

Yöntemler: Sıçanlar, Kontrol (Serum Fizyolojik), Morfin (10 mg/kg), Orp (20 ve 30 mg/kg) ve Orp + Morfin (sırasıyla 20, 30 +10 mg/kg) olarak altı gruba ayrıldı. Ön testi takiben, sıçanlara alternatif günlerde çoklu intraperitoneal enjeksiyonlar (i.p.) ile salin çözeltisi ve orfenadrin verildi.

Bulgular: Morfin, ilaç eşleştirilmiş taraftaki tercih süresini önemli ölçüde artırdı ($p<0,001$), ancak orfenadrinin (20 mg/kg ve 30 mg/kg) iki dozunun da uygulandığı gruplar kontrol grubundan daha fazla tercih göstermedi. Orp 30 mg/kg + morfin değişim göstermezken, Orp 20 mg/kg + morfin morfin grubuna göre CPP'yi önemli ölçüde azalttı ($p<0,01$). Orp 20 mg/kg + morfinin bir arada kullanılması tercihi azaltırken, Orp 30 mg/kg +morfin grubu için böyle bir etki gözlenmedi.

Sonuç: CPP alımının, düşük dozda orfenadrinin NMDA reseptör antagonistik etkilerinden kaynaklandığı düşünülmektedir. Ödüllendirme özelliğinin veya kötüye kullanım potansiyelinin ileri araştırmalar ile incelenmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Morfin, orfenadrin, ilaç bağımlılığı, bağımlılık davranışı

Anti-cholinergic drugs have been confined to the amelioration of extrapyramidal side effects induced by anti-psychotic and anti-Parkinson medication. Literature showed intermittent reports concerned with the abuse of centrally acting anti-cholinergic compounds. A recent study showed that schizophrenic patients with anti-psychotic treatment may have higher potential for abuse [1]. Agents such as biperiden and trihexyphenidyl have been found to be associated with abuse and dependence owing to their euphoric potential, which leads to an increase in speech and self-confidence [2-6]. Orphenadrine (Orp) offers wide range of muscarinic cholinoceptor blockade effects and also acts as N-methyl-D-aspartate (NMDA) and H1 receptor antagonist [7].

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Physical and psychological dependence of opioid analgesics is a serious and frequently encountered problem. Despite potent analgesic properties, their use is limited owing to the rapid development of tolerance and withdrawal symptoms. Overwhelming consequences of opioid analgesics lead to the development of effective treatments. Till now, there is no enduring and effective pharmacological treatment to achieve absolute abstinence from opiates. Despite known harmful properties of these substances, the continuous requisition owing to exhilarating effects increases in drug-dependent patients [8], students, and street children [9]. The abuse of said drugs is more prominent in patients already dependent on opioids. The use of opioid is as old as the beginning of medicine, and it is especially known for its analgesic, anti-tussive, smooth muscle relaxant, sedative, anti-diuretic, and euphoric properties [2, 3, 6]. Morphine is one of the most effective analgesics; however, chronic administration of the drug results in the development of tolerance and physical dependence, which ultimately limits the treatment with morphine [5, 10]. It is imperative to understand the underlying mechanism of dependence and rewarding effects of opioids. In recent years, experimental animals have been extensively used due to the ease of handling and reliability for evaluating the rewarding and reinforcing properties by using conditioned place preference (CPP). This test showed the rewarding effects of many drugs such as morphine, cocaine, heroin, and amphetamines and alcohol that have been extensively used for abuse [11, 12].

The literature is scanty regarding the rewarding potential of Orp for its potential of abuse. In the light of the aforementioned facts, this study was performed in rats to determine the effect of Orp in the acquisition of CPP pattern and to assess the susceptibility of Orp to drug abuse.

Material and Methods

Animals

Wister Albino rats weighing 260-320 g were obtained from the Animal Care Center, Istanbul University, Turkey. The animals were housed in 4 cages with environmental conditioning (21°C and a 12 h light/dark cycle) and had free access to pulverized standard rat pellet diet and tap-water. Istanbul University Local Animal Research Ethics Committee gave approval for this study on February 01, 2013; Apo No 173.

CPP

The relatively simple CPP model has been widely used to evaluate the rewarding and aversive effects of drugs or natural products [13].

Animals in the presence of various stimuli such as drug-paired stimuli can depict the drug-seeking behavior, and this method (CPP) is useful for evaluating the conditioning scores. Aversive effects can be observed at the drug-paired side when it produces unpleasant effects. The experimental techniques and protocols can vary widely while investigating the effects of addictive substances. Various design for floors and walls can be used in the 2 compartments of the apparatus [13, 14]. Similarly, animals can be subjected to different stimuli in the apparatus. According to the characteristics of the experimental setup, biased or neutral design can be used to perform the experimental studies.

In this study, we used different designs for the floor and wall of both the compartments of the apparatus. The design of the box was taken into account from the previous studies [15]. In the subsequent experiment of CPP, the biased method was found to be appropriate with the design of the box. Similarly, the selected experimental protocol was designed by taking into consideration different studies [16]. This study was conducted to examine the drive-reduction hypothesis of addiction in rats.

Apparatus

The test apparatus was a rectangular box made of plexiglass (30 cm long × 60 cm wide × 30 cm height). A partition with a small sliding guillotine door divided the box into 2 chambers of equal size: one compartment was white in color with a mesh floor and the other was a black rod floored with stainless steel.

Drug administration

Morphine hydrochloride (10 mg/kg) (Macfarlan Smith LTD., Edinburgh, UK) and Orp (20 mg/kg and 30 mg/kg) (Sigma Aldrich St. Louis, MO, USA). were dissolved in serum physiological solution and administered intraperitoneally to animals. The solutions were freshly prepared on the experiment day. It has been reported that Orp (35 mg/kg) produces seizure-like effect, therefore, higher doses were not tested in this study [17].

Experimental procedure and treatment

The experiment was conducted for a total period of 15 days (between 9:00 and 16:00) and was setup as 8 conditioning phases. The experiment consisted of habituation, pretest, conditioning phase, and posttest trials.

Habituation: Animals were habituated to the CPP apparatus before the start of the experiment (day 0) for a duration of 5 min for the novelty of the experiment.

Pretest: It was conducted without the administration of drug, and animals were placed in the apparatus for 15 min to determine the place preference score (day 1).

Conditioning phase: In this experiment, rats were subjected to drug and serum physiological solution on alternative days. On days 2, 4, 8, and 10, rats were given saline solution in black compartment, whereas on days 3, 5, 9, and 11, drug was administered to animals. During the conditioning phase, rats received 2 injections with an interval of 15 min, and soon after the second injection, rats were placed into the apparatus to assess the CPP. After 40 min of conditioning, rats were placed back in their cages. Schedule of drug administration is shown in Table 1. The place preference was determined by the time spent by each rat in the white side of the box.

Table 1. Experimental protocol

Groups	Injection (1)	Injection (2)
Control	Saline	Saline
Morphine (10 mg/kg)	Saline	Morphine
Orp (20 mg/kg)	Orp	Saline
Orp (30 mg/kg)	Orp	Saline
Orp (20 mg/kg) + Morphine	Orp	Morphine
Orp (30 mg/kg) + Morphine	Orp	Morphine

Orp: orphenadrine

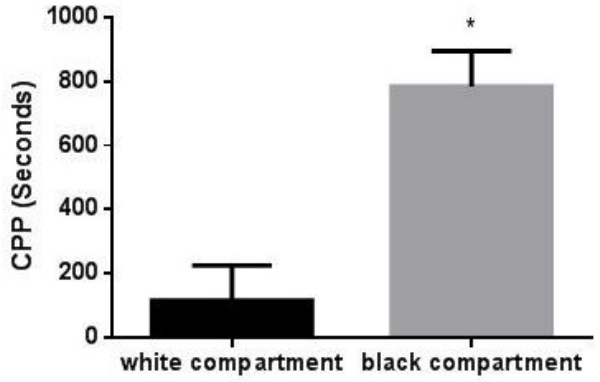

Figure 1. Pretest showing compartment preferences. Values are expressed as mean ± standard error
*p<0.001, Student's t test

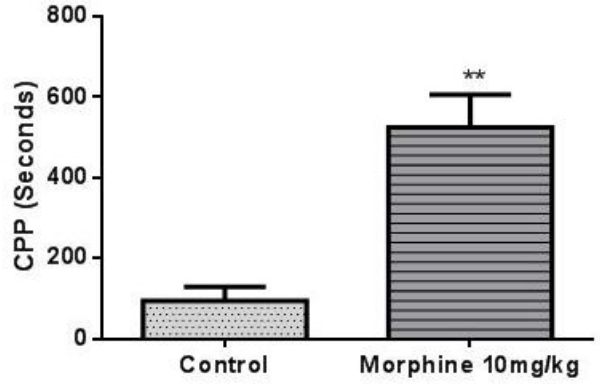

Figure 2. Shows difference between control and morphine group. Values are expressed as mean ± standard error
*p<0.001, Student's t test.

Posttest: The place preference was determined on 12th day without the administration of the drug, and the time spent by the animals in each box was monitored for 15 min.

Statistical analysis

Results were first compared with one-way analysis of variance and followed by post hoc Newman-Keuls test for multiple comparisons. Data were showed as mean ± standard error of mean, and $p<0.05$ was accepted as significant. Student *t*-test was used for comparison between the 2 groups (GraphpadPrism software, San Diego, California USA).

Results

In this paradigm, we used biased procedure to evaluate the rewarding and reinforcing effect of Orp alone or in combination with morphine. The time spent by rats in the white and black compartments during CPP pretest is shown in Figure 1.

Animals were placed in CPP apparatus without the administration of any drug, and the average time spent by each rat in the black compartment was found to be significantly higher ($p<0.001$) than the time spent in the white compartment (biased design).

The rats treated with morphine (10 mg/kg) spent more time in the white compartment than the control group ($p<0.001$), which showed the rewarding properties of morphine (Figure 2).

Both the doses of Orp (20 mg/kg and 30 mg/kg) did not show any significant difference in comparison with the control group in terms of time spent by rats in the white compartment (Figure 3). The time spent by rats treated with morphine (10 mg/kg) + Orp (30 mg/kg) was significantly higher than the control group rats

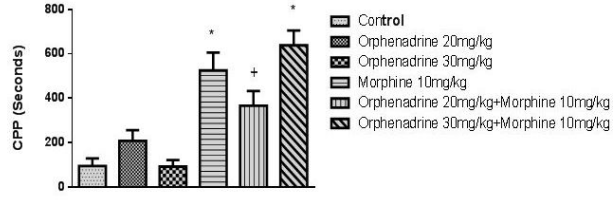

Figure 3. Time spent by rats in drug paired compartment during conditioning phase. values are expressed as mean ± standard error
*p<0.001 relative to control, + p<0.05 relative to morphine group Newman-Keuls test

($p<0.001$). whereas morphine (10 mg/kg) + Orp (20 mg/kg) group showed no significant difference when compared with the control ($p<0.05$).

Orp (20 mg/kg) + morphine group potently reduced the rewarding effects, whereas Orp (30 mg/kg) + morphine did not produce place preference compared with the morphine group alone ($p<0.05$; Figure 3). The locomotor activity did not show any significant difference; thus, the data are not presented here.

Discussion

Orp used in clinical practice as a skeletal muscle relaxant affects cholinergic, histaminergic, and glutaminergic systems [7]. Morphine shows the rewarding properties through similar systems [18]. Therefore, in this study, we evaluated the rewarding and reinforcing properties of Orp alone or in combination with morphine. The results of the pretest revealed that animals spent more time in the black compartment than in white, which led to the development of a biased experimental design. The data showed that morphine produced potent place preference, and the results were in accordance with the previous studies conducted on morphine rewarding effects in both biased [19] and unbiased [20] experimental designs. However, Orp did not produce CPP in rats after treatment with 20 mg/kg and 30 mg/kg doses. Several studies showed that anti-cholinergic agents possess rewarding properties [21], whereas the experiments conducted on biperiden and trihexyphenidyl [22] did not exhibit CPP, and these findings support our results.

We found that Orp (30 mg/kg) + morphine did not affect the morphine-induced CPP. Previous studies indicated that anti-histaminic and anti-cholinergic agents have rewarding potential; an increase in morphine efficacy was observed when used in combination with these agents [23]. Many studies indicated that histamine reduced the rewarding effect of drugs used in CPP. The rewarding properties can be explained as a decrease in histamine levels in ventral tegmental area (VTA) and nucleus accumbens (NAc) by morphine, and this results in an increase in dihydroxyphenylalanine to 3,4-dihydroxyphenylacetic acid ratio. Histaminergic system plays an inhibitory role on rewarding properties of morphine [24]. Histidine is shown to inhibit the morphine-induced CPP [25]. However, Zarrindast et al. [26] showed that histamine produced dose-dependent place preference, and pyrilamine H1 receptor antagonist decreased the histamine response. Despite Orp anti-histaminic properties, the high dose of Orp produced CPP, which can be subjected to other underlying mechanisms that require further experiments. As explained below, literature showed variation in terms of rewarding properties produced by anti-histaminic and anti-cholinergic agents [12, 26-28].

Orp (20 mg/kg) + morphine significantly reduced the morphine-induced CPP. The following decrease in acquisition of morphine-induced CPP can be explained by Orp behaving as NMDA ionotropic glutamate receptor antagonist. NMDA receptors play an important role in modulating the physical dependence caused by opioids [15, 29, 30]. It has been found that glutamate receptor antagonists prevent the development of place preference along with the rewarding effects of drugs and that NMDA receptors in VTA play a significant role in dopamine-mediated hippocampal synaptic potential [31]. Memantine NMDA receptor antagonist blocked the expression and development of acute opioid dependence when assessed by withdrawal-potentiated startle and hyperalgesia [32].

Involvement of NMDA receptors in modulation of neural plasticity in the learning process describes dependency mechanism. Previous studies showed a strong relationship between NMDA receptors and learning development, and CPP is a learning paradigm [15]. Similarly, strong ties have been found between dopamine and glutamate signaling in VTA and NAc on rewarding properties of opioids. Opioids activate the μ receptors in the VTA, and μ receptors indirectly stimulate the mesocorticolimbic dopamine system [33].

In conclusion, Orp alone at both the doses did not produce any CPP; moreover, 20 mg/kg of Orp significantly reduced the morphine-induced CPP. Decrease in acquisition of CPP can be due to the role of Orp as NMDA receptor antagonist, whereas (26) Orp (30 mg/kg) + morphine did not produce reinforcing properties or CPP. Orp possesses anti-cholinergic, anti-histaminic, and anti-glutamatergic properties. To understand the exact underlying mechanism of the agonists and antagonists of these systems, further studies are needed.

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