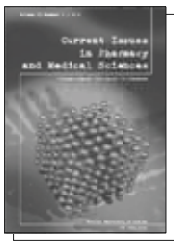

2012 Curr. Issues Pharm. Med. Sci. Vol. 25, No. 1

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy

Effects of bupropion and mecamylamine on motivational effects of drugs of abuse measured in CPP-reinstatement test in rats

BARBARA BUDZYŃSKA*, MARTA KRUK-SŁOMKA, PIOTR POLAK, GRAŻYNA BIAŁA

Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Poland

ABSTRACTS

In the present study, we investigated the mechanisms of cross-reinstatement of nicotine-induced place conditioning in rats. We used the conditioned place preference (CPP) paradigm and our data revealed that nicotine (0.175 mg/kg, base, i.p.) produced a place preference and once established, nicotine CPP was extinguished. Moreover the CB1 receptor agonist – WIN 55,212-2 (0.5 mg/kg, i.p.) or ethanol (0.5 g/kg, i.p.) reinstated a marked preference. Furthermore, we evaluated and compared the efficacy of the atypical antidepressant drug - bupropion (5, 10 and 20 mg/kg, i.p.) and a nonselective nicotinic receptor antagonist - mecamylamine (1 and 2 mg/kg, s.c.), in blocking the reinstatement of nicotine CPP provoked by WIN 55,212-2 and ethanol. Our results demonstrated that mecamylamine in all used doses and bupropion (except for dose of 10 mg/kg used in reinstatement induced by WIN 55,212-2) attenuated the reinstatement of nicotine-conditioned response induced by both drugs. Results obtained in the present studies may contribute to better understanding of the neurochemical mechanisms underlying nicotine addiction and the reciprocal relationships between nicotine, cannabis and ethanol.

Keywords: nicotine, WIN 55,212-2, ethanol, bupropion, mecamylamine, place conditioning

INTRODUCTION

Tobacco, alcohol and cannabis are frequently used together, and because of their numerous social and health-related consequences, this is a continuing source of national public policy debate. Previous studies in rodents have reported that the cholinergic system is involved in modulation of many functions within the central nervous system (CNS) e.g. anxiety, learning and memory, nociception, and that repeated administration of nicotine, an agonist of neuronal cholinergic nicotinic receptors (nAChRs), produces physical dependence [14,19]. Similarly, CB1 cannabinoid receptor ligands modulate locomotion, anxiety, memory, nociception and rewarding processes [9]. Moreover, behavioural experiments in animals showed that alcohol and nicotine produce similar effects in several behavioural models, i.e. sensitization, place preference or self-administration [2,17]. It has been well established that all major pharmacological effects of nicotine are mediated through several different types of nAChRs located within the brain [8]. In turn, ethanol alters the function of different ionotropic receptors [12] and cannabinoids produce their physiological effects by influencing cannabinoid (CB) receptors, especially the CB1 receptors found in the CNS [9].

Accordingly to the epidemiological data, the relapse to drug taking behaviour is a very important clinical problem. High rate of relapse to drug addiction is especially characteristic for people trying to quit tobacco. Moreover, smoking alcoholics are generally less successful at smoking cessation than are subjects without alcoholism [1].

Several laboratories have developed a reinstatement procedure based on the CPP, which is a simple non-invasive method, compatible with the classical Pavlovian conditioning [20]. In these studies, preference to one distinctive environment associated with a drug administration during conditioning can be extinguished by allowing animals to explore both compartments during a daily session in the absence of the drug. After extinction, a priming dose of drug or the exposure to drug-related environmental stimuli reinstate the extinguished CPP. Several animal studies have also demonstrated that drugs other than those previously received, can reinstate drug-seeking behaviour. This phenomenon, termed "cross-reinstatement", has been already described using drugs from different classes [16].

The aim of the present studies was to investigate the mechanisms committed to the co-abuse of nicotine, CB1 receptor agonists and ethanol. We explored the model of cross-reinstatement between nicotine, a synthetic CB1 receptors agonist (WIN 55,212-2) and ethanol in rats. For the purpose of better understanding the neurobiological mechanisms of relapse to nicotine taking and polydrug abuse, we investigated the influence of bupropion (an atypical antidepressant drug) and mecamylamine (a non-selective nicotinic receptor antagonist) on the reinstatement of nicotine CPP provoked by a priming dose of both WIN 55,212-2 and ethanol. The intention of this work is to aid in the development of

Corresponding author
* Department of Pharmacology and Pharmacodynamics,
Medical University of Lublin, 6 Chodzki Str., 20-093 Lublin, Poland
e-mail address: basia.budzynska@umlub.pl

more effective methods in the treatment of nicotinism and polydrug abuse.

MATERIALS AND METHODS

Animals:

The experiments were carried out on naive male Wistar rats weighing 250-300 g (Farm of Laboratory Animals, Warszawa, Poland) at the beginning of the experiments. The animals were group-housed, kept under standard laboratory conditions (12/12-h light/dark cycle) with free access to tap water, and adapted to the laboratory conditions for at least one week.

The rats were handled once a day for 5 days preceding the experiments. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Each experimental group consisted of 6-14 animals. The experiments were performed between 9.00 a.m. and 5.00 p.m. All experiments were carried out according to the *National Institute of Health Guidelines for the Care and Use of Laboratory Animals* and the *European Community Council Directive of 24 November 1986 for Care and Use of Laboratory Animals* (*86/609/EEC*), and approved by the local ethics committee.

Drugs:

The compounds tested were (-)-nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA), WIN 55,212-2 ([R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl] pyrolol [1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate], TOCRIS, USA), ethanol (95°, Polmos Poznań), mecamylamine hydrochloride (Sigma, St. Louis, MO, USA), bupropion hydrochloride (Sigma-Aldrich, St. Louis, MO, USA). The drugs were dissolved in saline (0.9% NaCl). WIN 55,212-2 was dissolved in one drop of TWEEN 80 and diluted in 0.9% NaCl. Ethanol was prepared for injections by diluting 95% ethanol to obtain a concentration of 10% (v/v). The pH of the nicotine solution was adjusted to 7.0. Fresh drug solutions were prepared on each day of experimentation. Agents were administered subcutaneously (*s.c.*) or intraperitoneally (*i.p.*) in a volume of 5 ml/kg. Except for nicotine, drug doses refer to the salt form. Control groups received saline injections at the same volume and by the same route.

Apparatus:

The testing apparatus for the CPP paradigm was already validated in our laboratory [2,3]. Each of six rectangular boxes (60 cm x 35 cm x 30 cm) was divided into three compartments: two large compartments (20 cm x 35 cm) were separated by removable guillotine doors from a small central area (10 cm x 10 cm). One of these had its walls and floor painted white, while the walls of the other were painted black. The central grey area constituted a "neutral" chamber, which serves as connection and a start compartment. The testing boxes were kept in a sound-proof room with neutral masking noise and dim 40-lx illumination.

Experimental procedure and treatment:

The CPP-reinstatement paradigm took place on 9 consecutive days and consisted of the following phases: pre-conditioning (pre-test), conditioning, post-conditioning (test), extinction and reinstatement.

Pre-conditioning

On the first day, each animal was placed separately in the neutral area with the guillotine doors removed to allow access to the entire apparatus for 15 min. The amount of time that the rats spent in each of the two large compartments was measured (a baseline preference), and observed on a monitor through a video camera system. All animals showed a moderate preference for the black compartment.

Conditioning

One day after pre-conditioning, the rats were randomized and subsequently conditioned with saline paired with the preferred (black) compartment (the morning sessions), and nicotine (0.175 mg/kg, base, i.p.) paired with the other (white) compartment (the afternoon sessions), for 30 min. Sessions were conducted twice each day, with an interval of 6–8 h, for 3 consecutive days (days 2–4). Injections were administered immediately before confinement in one of the two large compartments, as mentioned above. A dose of 0.175 mg/kg nicotine was chosen for conditioning because this is known to produce reliable conditioned place preference in rats, also under our experimental conditions [2,3,5]. The control group received saline every day. The neutral zone was never used during conditioning and was blocked by guillotine doors.

This method (biased design) was similar to that used in our previous experiments [3] accordingly to the data indicating that rewarding action of nicotine in the CPP paradigm can be observed after restricted doses and under specific biased conditions [5].

Post-conditioning (test)

On the day 5, conducted one day after the last conditioning trial, the test animals were placed in the neutral area with the guillotine doors removed and allowed free access to all compartments of the apparatus for 15 min. The time spent in the saline- and drug-paired compartments was recorded for each animal. No injections were given on the day of this preference test.

Extinction training

One day after the preference test, rats were given extinction testing daily for 3 days. On each trial, the rat was placed in the neutral area and allowed to explore both chambers for 15 min. No injections were given during this extinction period. The amount of time that rats spent in each chamber was measured on day 6 (Extinction 1), 24 h after initial preference test, and on day 8 (Extinction 3), 72 h after this preference test.

Reinstatement

One day after the last extinction trial (day 9), separate groups of rats received saline, bupropion (5, 10 or 20 mg/kg, i.p.) or mecamylamine (1 or 2 mg/kg, s.c.), 30 min before

a priming injection of WIN 55,212-2 (0.5 mg/kg, i.p.) or ethanol (0.5 g/kg, i.p.), and were immediately tested for reinstatement of CPP. During this reinstatement test, the rats were allowed free access to the entire apparatus for 15 min, and the time spent in each chamber was measured. Simultaneously, the number of passings through the central grey area was measured for 15 min.

Statistics

For the CPP paradigm, the data are expressed as means ± S.E.M of scores (i.e., the differences in seconds between post-conditioning and pre-conditioning time spent in the drug-associated compartment). Locomotor activity was expressed as the number of times passing through the central grey area (means ± S.E.M.). The statistical analyses were performed using repeated measure analysis of variance (ANOVA), with treatment as between subjects' variables, and session as within subjects' variable. Post-hoc comparison of means was carried out with the Tukey test for multiple comparisons, when appropriate. The confidence limit of $p<0.05$ was considered statistically significant.

RESULTS

The time spent in the initially less-preferred (white) and in the initially more preferred (black) sides did not significantly differ between groups on the pre-conditioning day. This side preference was not significantly changed when saline was paired with both compartments during the conditioning sessions.

Acquisition, extinction and reinstatement of nicotine-induced CPP

As shown in Fig. 1, in saline- and nicotine-conditioned rats, given saline or WIN 55,212-2 injection on the reinstatement test, two-way ANOVA analysis revealed that there was a significant effect of treatment and session [treatment: $F(1,90) = 253.23$, $p < 0.0001$; session: $F(4,90) = 36.40$, $p < 0.0001$; treatment x session: $F(4,90) = 37.14$, $p < 0.0001$]. On the test day, post-hoc analysis showed that there were significant differences in scores between saline-conditioned and nicotine-conditioned groups ($p < 0.001$, Tukey test). Fig. 1 also shows that the time spent in the nicotine-paired chamber gradually diminished over the days of repeated test training. The increase in time spent in the drug-paired compartment on day 6 (first test for extinction, Extinction 1, conducted 24 h after the preference test), was still greater for the nicotine-paired animals, than for the saline-paired animals ($p < 0.001$, Tukey test), whereas on day 8 (second test for extinction, Extinction 3, 72 h after the initial preference test), there was no difference in time spent in the drug-paired compartment between these two groups. This indicates that nicotine-CPP had been extinguished by repeated test trials. It can also be seen in Fig. 1 that the priming injection of WIN 55,212-2 (0.5 mg/kg, i.p.) reinstated the extinguished nicotine-CPP ($p < 0.001$ vs. the saline-conditioned group given saline injection during reinstatement test, Tukey test). What is more, the data shows differences in scores between nicotine-conditioned and WIN 55,212-2-primed rats and saline-conditioned and WIN 55,212-2-primed rats ($p < 0.001$, Tukey test), indicating that a prior CPP is necessary for a WIN 55,212-2 prime to produce an increase in time spent in the drug-paired compartment.

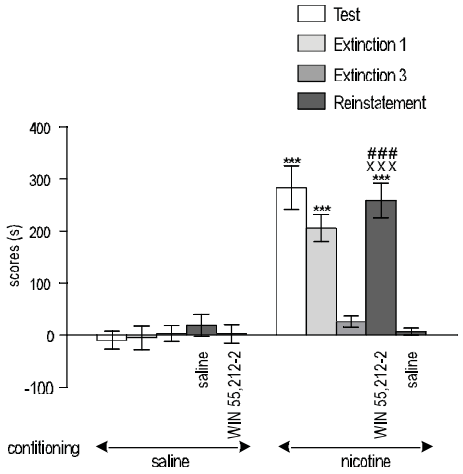

Fig. 1. Reinstatement of nicotine CPP in rats caused by a priming dose of WIN 55,212-2 (0.5 mg/kg, i.p.). Data represent means ±S.E.M., and are expressed as scores, i.e. differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=9-14. ***P < 0.001 vs. saline-conditioned group; ###P < 0.001 vs. saline-conditioned rats primed with WIN 55,212-2; xxxP < 0.001 vs. nicotine-conditioned rats primed with saline (Tukey test)

As shown in Fig. 2, in saline- and nicotine-conditioned rats, given saline or ethanol injection on the reinstatement test, two-way ANOVA analysis revealed that there was a significant effect of treatment and session [treatment: $F(1,90) = 449.16$ $p < 0.0001$; session: $F(4,90) = 56.38$, $p < 0.0001$; treatment x session: $F(4,90) = 61.90$, $p < 0.0001$]. On the test day, post-hoc analysis showed that there were significant differences in scores between saline-conditioned and nicotine-conditioned groups ($p < 0.001$, Tukey test). Fig. 2 also shows that the time spent in the nicotine-paired chamber gradually diminished over days of repeated test training. The increase in time spent in the drug-paired compartment on day 6 (first test for extinction, Extinction 1, conducted 24 h after the preference test), was still greater for the nicotine-paired animals, than for the saline-paired animals ($p < 0.001$, Tukey test), whereas on day 8 (second test for extinction, Extinction 3, 72 h after the initial preference test), there was no difference in time spent in the drug-paired compartment, between these two groups. In Fig. 2 it can also be seen that the priming injection of ethanol (0.5 g/kg, i.p.) reinstated the extinguished nicotine CPP ($p < 0.001$ vs. saline-conditioned group given saline injection during reinstatement test). What is more, the data shows differences in scores between nicotine-conditioned and ethanol-primed rats, and saline-conditioned and ethanol-primed rats ($p < 0.001$, Tukey test), indicating that a prior CPP is necessary for a ethanol prime to produce an increase in time spent on the drug-paired compartment.

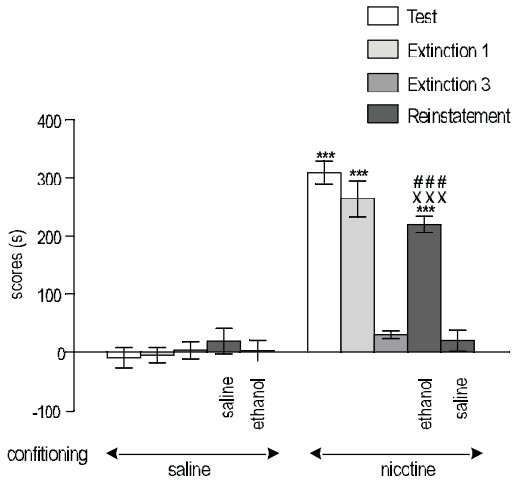

Fig. 2. Reinstatement of nicotine CPP in rats caused by a priming dose of ethanol (0.5 g/kg, i.p.). Data represent means±S.E.M., and are expressed as scores, i.e. differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=10-14. ***P < 0.001 vs. saline-conditioned group; ###P < 0.001 vs. saline-conditioned rats primed with ethanol; xxxP < 0.001 vs. nicotine-conditioned rats primed with saline (Tukey test)

The effect of bupropion on WIN 55,212-2 and ethanol-induced reinstatement

Pretreatment with bupropion (10 and 20 mg/kg, i.p.) influenced the priming effect of WIN 55,212-2 in nicotine-conditioned rats [treatment effect on the reinstatement test: $F(3,39) = 148.7$, $p < 0.0001$] (Fig. 3). Indeed, post-hoc individual comparisons indicated the significant effect of bupropion at a dose of 20 mg/kg ($p < 0.001$ vs. the WIN 55,212-2-reinstated group, Tukey test) which completely abolished the reinstatement of nicotine CPP previously established. Moreover, the dose of 10 mg/kg of bupropion intensified the priming effect of WIN 55,212-2 in this experimental procedure ($p < 0.001$ vs. the WIN 55,212-2-reinstated group, Tukey test).

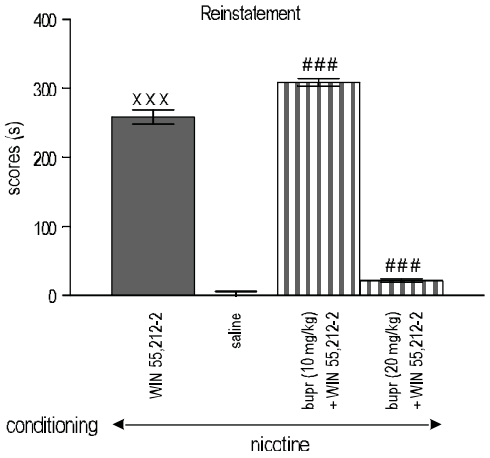

Fig. 3. Effects of bupropion (bupr) (10 and 20 mg/kg, i.p.) on the reinstatement of nicotine CPP caused by a priming dose of WIN 55,212-2 (0.5 mg/kg, i.p.). Data represent means±S.E.M., and are expressed as scores, i.e. differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=9-12. xxx P < 0.001 vs. nicotine-conditioned rats primed with saline; ###P < 0.001 vs. nicotine-conditioned rats primed with WIN 55,212-2 (Tukey test)

Bupropion also attenuated the priming effect of ethanol on nicotine-induced CPP [treatment effect on the reinstatement test in nicotine-conditioned rats: $F(3,45) = 216.8$, $p < 0.0001$]. A statistically significant effect was seen for both used doses of bupropion, i.e. 5 and 10 mg/kg ($p < 0.001$ vs. ethanol-reinstated group, Tukey test) (Fig. 4).

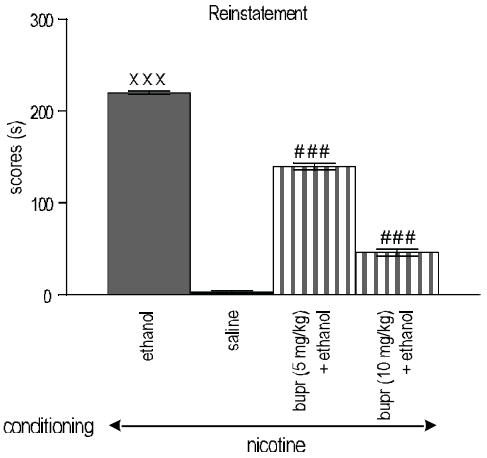

Fig. 4. Effects of bupropion (bupr) (5 and 10 mg/kg, i.p.) on the reinstatement of nicotine CPP caused by a priming dose of ethanol (0.5 g/kg, i.p.). Data represent means±S.E.M., and are expressed as scores, i.e. differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=8-14. xxxP < 0.001 vs. nicotine-conditioned rats primed with saline; ###P < 0.001 vs. nicotine-conditioned rats primed with ethanol (Tukey test)

The effect of mecamylamine on WIN 55,212-2 and ethanol-induced reinstatement

Pretreatment with mecamylamine (1 and 2 mg/kg, s.c.) decreased the priming effect of WIN 55,212-2 in nicotine-conditioned rats [treatment effect on the reinstatement test: $F(3,39) = 68.97$, $p < 0.0001$] (Fig. 5). Indeed, post-hoc individual comparisons indicated the significant effect of mecamy-

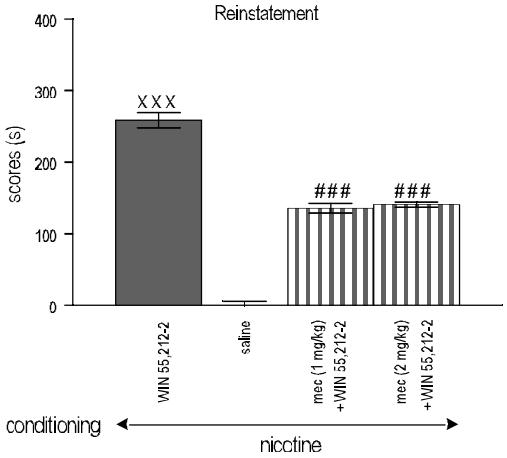

Fig. 5. Effects of mecamylamine (mec) (1 and 2 mg/kg, s.c.) on the reinstatement of nicotine CPP caused by a priming dose of WIN 55,212-2 (0.5 mg/kg, i.p.). Data represent means±S.E.M., and are expressed as scores, i.e. differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=8-12. xxxP < 0.001 vs. nicotine-conditioned rats primed with saline; ###P < 0.001 vs. nicotine-conditioned rats primed with WIN 55,212-2 (Tukey test)

lamine at a dose of 1 and 2 mg/kg ($p < 0.001$ vs. the WIN 55,212-2-reinstated group, Tukey test) which abolished the reinstatement of nicotine CPP previously established.

Mecamylamine also attenuated the priming effect of ethanol on nicotine-induced CPP [treatment effect on the reinstatement test in nicotine-conditioned rats: $F(3,45) = 227.7$, $p < 0.0001$]. A statistically significant effect was seen for both used doses of mecamylamine, i.e. 1 and 2 mg/kg ($p < 0.001$ vs. the ethanol-reinstated group, Tukey test) (Fig. 6).

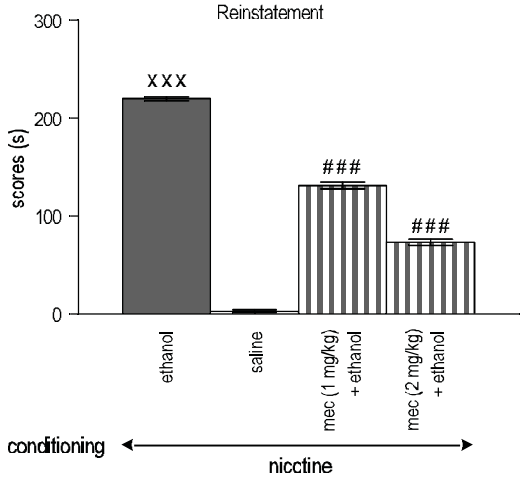

Fig. 6. Effects of mecamylamine (mec) (1 and 2 mg/kg, s.c.) on the reinstatement of nicotine CPP caused by a priming dose of ethanol (0.5 g/kg, i.p.). Data represent means±S.E.M., and are expressed as scores, i.e. differences (in s) between post-conditioning and pre-conditioning time spent in the drug-associated compartment; n=8-14. xxxP < 0.001 vs. nicotine-conditioned rats primed with saline; ###P < 0.001 vs. nicotine-conditioned rats primed with ethanol (Tukey test)

The effect of bupropion on locomotor activity

Pretreatment with bupropion in nicotine-conditioned rats primed with WIN 55,212-2 (0.5 mg/kg, i.p.) caused changes in locomotor activity [$F(2,21)=5.367$, $p < 0.05$]. A statistically significant decrease in locomotor activity was seen for the lower dose of bupropion (10 mg/kg) ($p < 0.05$ vs. the WIN 55,212-2-reinstated group, Tukey test) (Tab.1). Moreover, mecamylamine also attenuated the effect of a priming dose of WIN 55,212-2 in locomotor activity [$F=(2,20)=37.23$, $p < 0.001$]. A statistically significant effect was seen for the dose 2 mg/kg of mecamylamine, ($p < 0.001$ vs. the WIN 55,212-2-reinstated group, Tukey test) (Table 1).

Table 1. Effects of bupropion (bupr) (10 and 20 mg/kg, i.p.) and mecamylamine (mec) (1 and 2 mg/kg, s.c.) on locomotor activity of nicotine conditioned rats primed with WIN 55,212-2 (0.5 mg/kg) during reinstatement day

	Saline	WIN 55,212-2	Bupr (10)+ WIN 55,212-2	Bupr (20)+ WIN 55,212-2	Mec (1) + WIN 55,212-2	Mec (2) WIN 55,212-2
mean ± SEM	29.63±2.32	29.43±3.78	18.57±2.41 #	25.5±9.49	25.67±2.99	16.63±1.97 ###
N	14	7	7	8	6	8

Data represent means±S.E.M., and are expressed as the number of passings through the central grey area measured for 15 min. n=6-14. #P < 0.05 ###P < 0.001, vs. nicotine-conditioned rats primed with WIN 55,212-2 (Tukey test)

What is more, a pretreatment with bupropion in nicotine-conditioned rats primed with ethanol (0.5 g/kg, i.p.) caused changes in locomotor activity [$F(2,19)=21.95$, $p=0.0001$]. A statistically significant decrease in locomotor activity was seen for the both doses of bupropion (5 and 10 mg/kg) ($p < 0.001$ vs. the ethanol-reinstated group, Tukey test), (Tab. 2). Moreover, mecamylamine also attenuated the effect of a priming dose of ethanol in locomotor activity [$F=(2,21)= 4.104$, $p < 0.033$]. A statistically significant effect was seen only for the dose 1 mg/kg of mecamylamine, ($p < 0.05$ vs. the ethanol-reinstated group, Tukey test) (Tab. 2).

Table 2. Effects of bupropion (bupr) (5 and 10 mg/kg, i.p.) and mecamylamine (mec) (1 and 2 mg/kg, s.c.) on locomotor activity of nicotine conditioned rats primed with ethanol (0.5 g/kg) during reinstatement day

	Saline	Ethanol	Bupr (5) + ethanol	Bupr (10) + ethanol	Mec (1)+ ethanol	Mec (2)+ ethanol
mean ±SEM	30.05±2.32	36.67±3.06 xxx	23.83±3.09 ###	27.25±4.03 ###	30.67±3.04 #	33.86±5.57
N	14	6	6	8	9	7

Data represent means±S.E.M., and are expressed as the number of passings through the central grey area measured for 15 min. n=6-14. xxxP < 0.001 vs. nicotine-conditioned rats primed with saline; #P < 0.05 ###P < 0.001, vs. nicotine-conditioned rats primed with ethanol (Tukey test)

DISCUSSION

The results of the present studies confirm the complex interaction between nicotine, cannabinoids and ethanol. Our experiment shows that nicotine is capable of inducing CPP in definite conditions and that extinguished preference is reinstated by a priming dose of WIN 55,212-2 and ethanol. The dose of nicotine was chosen according to the narrow range of doses reported to produce CPP in rats [3,5]. The major findings of our studies were that a non-selective nAChRs' antagonist, mecamylamine, prevented the reinstatement of previously extinguished nicotine place preference caused by a priming dose of both WIN 55,212-2 and ethanol. Furthermore, we discovered that the atypical antidepressant drug, bupropion (5, 10 mg/kg, i.p.), blocked reinstatement of nicotine CPP provoked by ethanol. Also, the dose of 20 mg/kg of bupropion attenuated nicotine place preference caused by a priming dose of WIN 55,212-2. Surprisingly, however, the dose of 10 mg/kg of bupropion intensified the observed effect.

It has been already suggested that priming injections of drugs reinstate drug-seeking behaviour after extinction, because they activate the brain systems involved in their discriminative stimulus properties and/or their rewarding properties [10]. Tobacco and cannabis share some similar biological actions and are used frequently in combination, and therefore the study of their functional interactions is of special interest in the context of polydrug abuse, a quite frequent phenomenon. There is evidence that nicotine does not produce rewarding effects in CB1 knock-out mice, although this effect was observed in wild type animals measured in CPP paradigm [6]. However, in the context of our studies, biochemical data indicate that pre-treatment with Rimonabant (SR 141716) blocks nicotine-enhanced extracellular

dopamine levels in the shell of the nucleus accumbens (NAC) [7]. In keeping with the hypothesis that the mesocortical dopaminergic system plays a pivotal role in the rewarding effects of many psychoactive drugs, it seems to be essential to mention that CB1 receptors are widely distributed throughout the brain and participate in the regulation of dopamine synthesis, release and turnover. Interestingly, the co-localization of CB1 and nAChRs has been reported in several brain areas, such as the hippocampus and the amygdala. This supports the possibility of functional interactions between these two systems [9].

In our experiments, we also confirmed the phenomenon of cross-reinstatement between nicotine and ethanol. As already mentioned, several experiments showed that alcohol and nicotine produced similar effects in animal models, i.e. sensitization, place preference or self-administration, learning/memory and anxiety [2,17,23].

It has been suggested that ethanol's, as well as nicotine's, rewarding effects result from enhancing dopamine release in the NAC [12]. The ability of ethanol to increase the dopamine level results from it directly interfering with various ligand-gated ion channels, including the GABA-A/benzodiazepine receptor complex and the glutamatergic, serotoninergic [5-HT3] receptors, but this drug can also modulate (enhance or inhibit) the function of nAChRs [17]. In the context of our study, several findings show that voluntary ethanol intake enhances the extracellular level of acetylcholine in the ventral tegmental area (VTA). This in turn may interact with nAChRs localized in this area, and this subsequently, may stimulate dopamine overflow in the NAC [17].

The main objective of the present studies was to examine whether the atypical antidepressant drug – bupropion, or nAChRs' antagonist – mecamylamine, attenuate the motivational effects of a priming dose of WIN 55,212-2 and ethanol.

In the context of our studies, several findings indicate the role of bupropion on the behavioural effects of nicotine and other psychoactive drugs. It was shown that bupropion dose-dependently diminishes nicotine conditioned taste aversion [22]. Moreover, it is worth mentioning that our experiment shows that acute bupropion administration decreases nicotine self-administration in rats [21]. However, we have no knowledge of previous animal studies on the impact of bupropion on the motivational effects of CB1 receptor ligands or on ethanol.

Additionally, it is worth mentioning that mecamylamine blocks receptors that are pivotal for nicotine's motivational effects, the nAChRs located on the dopaminergic and the GABAergic neurons in the VTA [18]. Interestingly, a cross effect between CB1 receptor ligands and mecamylamine was also observed [15]. Moreover, a massive body of evidence has revealed the interaction between ethanol and mecamylamine (mecamylamine can attenuate ethanol intake and preference), as well as the ethanol-induced enhancement of dopamine release in the NAC [4].

Considering the discrepancy between the effects of bupropion and mecamylamine on the reinstatement of nicotine-induced CPP by priming injection of WIN 55,212-2, we should reflect on this drug's varied mechanisms. The neurochemical mechanism of the bupropion effective in the treatment of nicotine dependence is still unclear. Some studies suggest that these effects are related to its facilitation of dopamine release, as the dopaminergic system has been shown to be involved in the reward system [10]. On the other hand, it was shown that the effect of inhibiting dopamine re-uptake is rather weak in therapeutic doses of bupropion [13]. Therefore, this may suggest that an alternative mechanism is involved in the behavioural effects of bupropion in combination with nicotine. This drug has been reported to be a nAChRs' antagonist [11]. However, it was shown that bupropion, contrary to mecamylamine, does not block nAChRs entirely [13]. Additionally, bupropion has been reported to block the $\alpha_3\beta_2$, $\alpha_4\beta_2$, and α_7 subtypes of nAChRs with different selectivity, whereas mecamylamine is a non-selective antagonist of nAChRs. Therefore, we may suggest that its influence on the different types of cholinergic nicotinic receptors may be important in a cross-talk effect between nicotine and cannabinoids.

As mentioned earlier, both bupropion and mecamylamine alleviate the reinstatement of nicotine-induced CPP by a priming dose of ethanol. These findings may further confirm the interaction between nicotine and ethanol on the level of a reward system, as well as the participation of different subtypes of nAChRs in crossover effects between two agents. Moreover, obtained data possibly suggests that inhibition of dopamine and noradrenalin re-uptake by bupropion plays a more important role in the reinstatement of nicotine-induced CPP by a priming dose of ethanol than does WIN 55,212-2.

However, our studies demonstrated that changes of locomotor activity occurred after concomitant administration of bupropion (10 mg/kg) and WIN 55,212-2 or bupropion (5 and 10 mg/kg) and ethanol during the reinstatement day. Moreover, our studies showed that mecamylamine hampered locomotor activity when administered before WIN 55,212-2 (at the dose of 2 mg/kg) and before ethanol (at the dose of 1 mg/kg). Therefore, we can not exclude that the observed effects result from the influence of both bupropion and mecamylamine on locomotor activity in rats.

Taking all these results together, our data confirm the existence of interactions between nicotine, cannabinoid system and ethanol as measured in the CPP-reinstatement paradigm. Our studies show that mecamylamine (1 and 2 mg/kg) prevents the reinstatement of previously extinguished nicotine place preference caused by a priming dose of both WIN 55,212-2 and ethanol. Moreover, bupropion (5, 10 mg/kg, i.p.) blocks the reinstatement of nicotine CPP provoked by ethanol, while in a higher dosage of 20 mg/kg, it prevents reinstatement by a priming dose of WIN 55,212-2.

Our research may provide new insight into the mechanisms underlying the interaction between nicotine, cannabis

and ethanol. Results obtained in the present studies may contribute to better understanding of the mechanisms underlying nicotine addiction and the reciprocal relationships between nicotine, cannabis and ethanol, since co-abuse of these psychoactive compounds is a quite frequent phenomenon. Our findings may further indicate that the cholinergic system plays a pivotal role in the neurobiological processes underlying the relapse to drug addiction. Moreover, as reinstatement of drug-seeking is a factor for the development of dependence, bupropion and mecamylamine may be useful in the relapse-prevention phase of addiction treatment.

REFERENCES

1. Abrams D.B., et al.: Interventions for alcoholics who smoke. *Alcohol Health Res. World., 1994,* 20, 111-117.
2. Biala G.: Calcium channel antagonists suppress nicotine-induced place preference and locomotor sensitization in rodents. *Pol. J. Pharmacol*., 2003, 55, 327-335.
3. Biala G., et al.: Effects of varenicline and mecamylamine on the acquisition, expression, and reinstatement of nicotine-conditioned place preference by drug priming in rats. *Naunyn Schmiedebergs Arch. Pharmacol*., 2010, 381, 361-370.
4. Blomqvist O., et al.: Ethanol-induced locomotor activity, involvement of central nicotinic acetylcholine receptors? *Brain Res. Bull*., 1992, 29, 173-178.
5. Calcagnetti D.J. and Schechter M.D.: Nicotine place preference using the biased method of conditioning. *Prog. Neuropsychopharmacol. Biol, Psychiatry, 1994,* 18, 925-933.
6. Castane A., et al.: Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. *Neuropharmacology,* 2002, 43, 857-867.
7. Cohen C., et al.: SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav. Pharmacol*., 2002, 13, 451-463.
8. Corrigall W.A., et al.: The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology*, 1992, 107, 285-289.
9. Dewey W.L.: Cannabinoid pharmacology. *Pharm. Rev.,* 1986, 38, 151–178.
10. Di Chiara G.: The role of dopamine in behavioural actions of nicotine related to addiction. *Eur. J. Pharmacol*., 2000, 393, 295-314.
11. Fryer J.D. and Lukas R.J.: Noncompetitive functional inhibition at diverse, human nicotinic acetylcholine receptor subtypes by bupropion, phencyclidine, and ibogaine. *J. Pharmacol. Exp. Ther*., 1999, 288, 88-92.
12. Grant K.A.: Emerging neurochemical concepts in the actions of ethanol at ligand-gated ion channels. *Behav. Pharmacol*., 1994, 5, 383–405.
13. Helge T.D. and Denelsky G.Y.: Pharmacologic aids to smoking cessation. *Cleve. Clin. J. Med.,* 2000, 67, 21–24.
14. Hildebrand B.E., et al.: Behavioural and biochemical manifestations of mecamylamine-precipitated nicotine withdrawal in the rat, role of nicotinic receptors in the ventral tegmental area. *Neuropsychopharmacology,* 1999, 21, 560–574.
15. Hiranita T., et al.: Methamphetamine-seeking behavior is due to inhibition of nicotinic cholinergic transmission by activation of cannabinoid CB1 receptors. *Neuropharmacology*, 2008, 55, 1300-1306.
16. Itzhak Y. and Martin J.L.: Cocaine-induced conditioned place preference in mice: induction, extinction and reinstatement by related psychostimulants. *Neuropsychopharmacology*, 2002, 26, 130-134.
17. Larsson A. and Engel J.A.: Neurochemical and behavioral studies on ethanol and nicotine interactions. *Neurosci. Biobehav. Rev*., 2004, 27, 713-720.
18. Mansvelder HD, et al.: Synaptic mechanisms underlie nicotine- induced excitability of brain reward areas. *Neuron*, 2002, 33, 905–919.
19. Marubio M.L., et al.: Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. *Nature,* 1999, 398, 805–810.
20. Mueller D., et al.: Persistence and drug-induced reinstatement of a morphine-induced conditioned place preference. *Behav. Brain. Res*., 2002, 136, 389-397.
21. Rauhut A.S., et al.: Effect of bupropion on nicotine self-administration in rats. *Psychopharmacology*, 2003, 169, 1-9.
22. Rauhut A.S., et al.: Bupropion differentially alters the aversive, locomotor and rewarding properties of nicotine in CD-1 mice. *Pharmacol. Biochem. Behav*., 2008, 90, 598-607.
23. Rezvani A. and Levin R.D.: Nicotine – alcohol interactions and cognitive function in rats. *Pharmacol. Biochem. Behav*., 2006, 72, 865–872.