BACLOFEN ANTAGONIZES INTRAVENOUS SELF-ADMINISTRATION OF NICOTINE IN MICE AND RATS

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Abstract — Aims: γ-Aminobutyric acid (GABA)-ergic transmission plays an important role in modulating reinforcing effects of different drugs of misuse. In particular, stimulation of GABA_B receptors negatively influences self-administration of cocaine, heroin, nicotine, alcohol and γ-hydroxybutyric acid. The effect and specificity of the GABA_B agonist baclofen on nicotine misuse were studied on two animal models of self-administration. Methods: The effects of RS baclofen and the two isomers R baclofen and S baclofen were studied on the acute nicotine self-administration in drug-naïve mice. The effect of RS baclofen was also studied in rats trained to chronically self-administer nicotine under a continuous reinforcement (FR1) schedule. Results: RS baclofen antagonizes nicotine intravenous self-administration at doses of 1.25–2.5 mg/kg intraperitoneally (i.p.). Furthermore, this effect is stereospecific. R baclofen completely prevented nicotine self-administration at the dose of 0.625 mg/kg i.p., whereas S baclofen was inactive up to the dose of 2.5 mg/kg i.p. In rats trained to self-administer nicotine, pretreatment with RS baclofen at the dose of 2.5 mg/kg i.p. significantly increased the rate of responding for nicotine. This effect was similar to the effect obtained when rats were pretreated with the nicotine central receptor antagonist mecamylamine (1 mg/kg i.p.). Conclusions: These data show that baclofen is able to antagonize nicotine-rewarding effects in mice and rats and suggest its potential clinical utility for the treatment of nicotine misuse.

INTRODUCTION

In recent years, a large body of experimental evidence has supported the hypothesis that γ-aminobutyric acid (GABA)-ergic transmission plays a crucial role in modulating reinforcement of several drugs of misuse by an inhibitory action on mesolimbic dopaminergic neurons.

GABAergic projections from nucleus accumbens and GABAergic interneurons are located within the A10 region of the ventral tegmental area (VTA), from which mesolimibic dopaminergic neurons originate. It has been suggested that GABA_A receptors are located on both mesolimbic dopamine cell bodies and GABAergic interneurons (Kalivas et al., 1990; Klitenick et al., 1992), whereas GABA_B receptors are confined to dopaminergic cell bodies (Kalivas et al., 1993). Although a GABA_A receptor participation in the modulation of reinforcement cannot be completely ruled out (Goeders et al., 1989; Hodge et al., 1996; Shoaib et al., 1998), the role of GABA_A receptors appears to be of particular interest. Different laboratories have provided evidence that stimulation of GABA_B receptors by specific GABA_B agonists negatively influences self-administration of cocaine (Roberts et al., 1996; Roberts and Andrews, 1997; Shoaib et al., 1998; Brebner et al., 2000), heroin (Xi and Stein, 1999, 2000), nicotine (Corrigall et al., 2000, 2001), gamma-hydroxybutyric acid (Fattore et al., 2001) and alcohol (Colombo et al., 2000).

Results of these studies suggest GABA_B agonists as potential therapeutic agents for clinical treatment of drug addiction. Among the GABA_B agonists, baclofen, a lipophilic analogue of GABA, seems thus far the most suitable for clinical use (Cousins et al., 2002). Moreover, preliminary clinical studies indicate baclofen as an active anti-craving medication for nicotine (Ling et al., 1998) and alcohol (Addolorato et al., 2000).

In order to obtain more information on the effects and specificity of baclofen on nicotine misuse, we tested it on two models of nicotine i.v. self-administration (IVSA): the model of acute IVSA in drug-naïve mice and that of chronic IVSA in trained rats.

MATERIALS AND METHODS

Animals

Male mice (28–30 g; Harlan Nossan, Udine, Italy) were housed six per cage with free access to food and water and were allowed to acclimatize to laboratory conditions (12 h light:12 h dark cycle, lights on at 08:00, 21 ± 1°C room temperature, 60% relative humidity) for ≥7 days after arrival. Food and water were available ad libitum until the time of testing. Male Long–Evans rats (Charles River, Como, Italy), weighing 250–280 g at the beginning of the study, were housed four per cage under the same environmental conditions and handled daily for ~10 min during the first week after arrival. Food and water were available ad libitum until the time of testing.

Drugs

Nicotine bitartrate (Sigma, Milan, Italy) was freshly dissolved in saline solution. Doses are referred to as the salt. RS/S/R-Baclofen (Tocris, Bristol, UK) and mecamylamine–HCl (Sigma) were freshly dissolved in saline solution and administered intraperitoneally (i.p.).

Intravenous self-administration in drug-naïve mice

Mice were tested in pairs in identical test cages: each presenting a frontal hole provided with an infrared detector that activated a cumulative recorder (Coulbourn Instruments, Allentown, PA, USA) and operated a syringe pump (Life
Science Instruments, Woodland Hill, CA, USA) to deliver drug solution (1 µl) contingent on a nose-poke response. Each mouse’s tail was extended outside the box and taped to a horizontal surface allowing access to the lateral tail veins through a 27G winged needle connected to the syringe with a Teflon tubing.

Mice were first placed in the test cage for 10 min of habituation with their tails taped, but no needle inserted. Based on the similarity in the baseline activity, mice were then paired, one defined as active and the other passive, and needles inserted in lateral tail veins. Each nose-poke of the active mouse resulted in a contingent drug injection, both to the active and the yoked passive mouse, so that both animals received the same amount of drug at the same time intervals. Nose-pokes of the yoked control mouse were counted but had no programmed consequences.

**Intravenous self-administration in trained rats**

Under anaesthesia with chloral hydrate (400 mg/kg i.p.), rats were surgically implanted with a chronic catheter. Briefly, under sterile conditions, a silastic catheter was inserted into the right jugular vein, tunneled under the skin and exited in the midscapular region, and was anchored to the back of the neck with two sutures. After surgery, each animal recovered in its home cage with food and water freely available, and, for the following 5 days, received a daily infusion of 0.2 ml of a sterile solution containing heparin (1%) and gentamycin (0.08 mg/ml).

The i.v. self-administration apparatus consisted of eight Plexiglas operant cages (30 × 30 × 30 cm; Med Associates, St Albans, VT, USA). Two holes, provided with photobeam detectors, were made 2 cm above the floor, 15 cm apart. At the time of the IVSA session, the catheter was connected to a swivel system through a metal spring, which was in turn connected to an infusion pump via a plastic tube; the swivel system allowed the animal to move freely in the operant cage. Nose-poking in one of the holes (defined as active) switched on the infusion pump, injecting the drug solution into the animal’s venous system. Nose-poking in the other hole (defined as inactive) had no effect on the pump. Assessment of experimental schedule and data collection was programmed through PC software. By using dual-hole operant chambers, specificity of responding on the drug-reinforced hole was assessed. Indeed, nose-pokes in the inactive hole were always recorded in order to verify whether self-administered nicotine produces a non-specific effect in animals.

Seven days following surgery, animals were first deprived of food for 24 h and then allowed 3 h daily access to nicotine under a continuous reinforcement (FR-1) schedule. Rats were kept in food-restriction conditions and fed with ~20 g of chow each day for the entire duration of the experiments.

Each nose-poke resulted in an i.v. infusion of 0.1 ml of drug solution delivered over a period of 5 s. Coincident with onset of the infusion, a stimulus light was turned on for 10 s, during which time nose-pokes were recorded but had no consequences (time-out period). Each rat was given a priming infusion in the experimental chamber before starting the daily session. IVSA sessions occurred once daily Monday to Saturday and took place at the same time each day during the dark phase of the cycle (between 09:00 and 00:30).

Experiments were carried out in strict accordance with both the *Guide for the Care and Use of Laboratory Animals* (NIH) and the European Union regulations for animal use in research (CEE no. 86/609).

**RESULTS**

As shown in Fig. 1, when nicotine (0.075 mg/kg/injection) injections were contingent upon nose-poking response, active mice significantly increased their rate of nose-poking with respect to the corresponding yoked passive mice. Pretreatment with the lowest dose of the racemic solution (RS) of baclofen tested (0.625 mg/kg) did not modify nose-poking response either in the active or passive mice with respect to the vehicle-pretreated mice.

When mice were pretreated with higher doses of RS baclofen (1.25 and 2.5 mg/kg), nose-poking response of active

![Fig. 1. Effect of different doses of RS baclofen (administered in a volume of 0.2 ml 20 min before starting the experimental session) on nicotine intravenous self-administration in drug-naïve mice.](image)

Each bar represents the mean ± SEM of the cumulative number of nose-poke responses of the active and passive mice in a 30 min session. Number of pairs of animals for each treatment group is indicated in parentheses. **P < 0.01 (ANOVA followed by Newmann–Keuls test).
mice was not significantly different from yoked passive controls. It is important to note that nose-poking activity of passive mice was not modified by any dose of RS baclofen tested with respect to vehicle-treated passive mice, indicating that NP response was not affected by non-specific effects.

Figure 2 shows that the effect of baclofen on nicotine self-administration is stereospecific. Thus, the R isomer antagonized nicotine-induced nose-poking response in active mice while the same dose of racemic compound did not modify nose-poking response with respect to vehicle-pretreated mice. By contrast, the S isomer was ineffective, even at a dose as high as 2.5 mg/kg.

Figure 3 shows the effect of two different doses of RS baclofen in rats chronically self-administering nicotine (0.06 mg/kg/injection). RS baclofen at the dose of 2.5 mg/kg significantly increased the rate of responding for nicotine, an effect very similar to that obtained with the nicotinic central receptor antagonist mecamylamine (1 mg/kg, i.p.). However, pretreatment with the higher dose of RS baclofen (5 mg/kg, i.p.) significantly decreased the rate of responding for nicotine.

DISCUSSION

In this study we show that the GABA<sub>B</sub> receptor agonist baclofen antagonizes nicotine IVSA both in drug-naïve mice and in rats trained to chronically self-administer nicotine. The model of IVSA in drug-naïve mice represents a situation where mice are exposed to the drug for the first time, therefore the response of mice to the drug is not conditioned by any previous drug experience or training. On the contrary, the ‘classical’ model of IVSA in rats represents a situation where the animals are trained to self-administer the drug and their response to the drug effects is conditioned by previous experiences. From this viewpoint, the model of IVSA in drug-naïve mice could be likened to a phase of 'acquisition'. This model has been validated by using several drugs of misuse, including nicotine (Martellotta et al., 1995), cocaine, morphine (Kuzmin et al., 1992), amphetamine (Cossu et al., 2001), cannabinoids (Martellotta et al., 1998a) and γ-hydroxybutyric acid (Martellotta et al., 1998b). Our results show that baclofen exerts an antagonistic effect on nicotine IVSA in drug-naïve mice. In fact, pretreatment with baclofen at doses of 1.25–2.5 mg/kg antagonized the nose-poking response in active mice. The specificity of this effect is supported by the finding that nose-poking response of passive mice is unaffected by baclofen pretreatment and by previous observation that baclofen itself is devoid of any reinforcing or aversive effect which could indirectly interfere with nicotine IVSA (Fattore et al., 2001). These data clearly indicate that baclofen is able to cancel nicotine-reinforcing effects in drug-naïve mice. Moreover, we show here that this effect is stereospecific. In fact, a comparison of the effect of R (+) and S (–) baclofen indicates that only the R (+) isomer is able to reduce nicotine-induced IVSA in active mice.

In addition, when we tested RS baclofen in rats trained to chronically self-administer nicotine, we found that the dose of 2.5 mg/kg significantly increased the rate of responding.
for nicotine. This effect is similar to that obtained when rats were treated with the nicotinic central receptor antagonist mecamylamine. Therefore this response to baclofen can be interpreted as an attempt by the rat to overcome the diminished effect of nicotine by increasing nicotine intake. When RS baclofen was tested at a higher dose (5 mg/kg) we found a decrease in the rate of responding. However, we cannot exclude that this response could be mainly due to an impairment of motor activity.

The results of this study further confirm and extend the hypothesis that the GABAergic system, in particular through GABA_B receptor type, plays a significant role in the modulation of the central effects of drug misuse. Although the mechanism of action is not completely understood, experimental evidence suggests the VTA as an important site where GABA_B receptors may modulate mesolimbic dopaminergic neuroactivity (Bonci and Malenka, 1999; Corrigall et al., 2000). Since the mesolimbic dopaminergic system is supposed to be the most important mediator of the reinforcing properties of several drugs of misuse, this could reasonably explain the antagonistic effect of baclofen on different drugs of misuse. Although this interpretation could be considered over-simplified, the potential clinical utility of baclofen, particularly in poly-drug misuse situations, which are considered as a clinical problem of wide dimension, remains of considerable interest.

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REFERENCES


