European Journal of Pharmacology 627 (2010) 142–149

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

Effects of imipramine or $GABA_B$ receptor ligands on the immobility, swimming and climbing in the forced swim test in rats following discontinuation of cocaine self-administration

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ARTICLE INFO

Article history:
Received 3 July 2009
Received in revised form 25 September 2009
Accepted 14 October 2009
Available online 28 October 2009

Keywords:
Cocaine self-administration
Withdrawal
$GABA_B$ receptor ligand
Forced swim test
Chronic mild stress

ABSTRACT

We tested if discontinuation of cocaine self-administration can lead to the development of depressive-like symptoms in the forced swim test expressed as changes in immobility, swimming and climbing behaviors in rats. A "yoked" procedure in which rats were run simultaneously in groups of three, with two rats received the passive injection of cocaine or saline, was employed. Later, we examined whether acute treatment with the classical antidepressant imipramine or $GABA_B$ receptor ligands could alter the increases in immobility recorded after discontinuation of self-administered cocaine. We found a significant increase (44%) in the immobility time 3 days following discontinuation of cocaine (0.5 mg/kg/infusion/2 h daily) self-administration for 14 days; such enhancement resembled that observed in rats following the chronic mild stress. Acute administration with imipramine (15 or 30 mg/kg), the $GABA_B$ receptor agonists baclofen (0.125 mg/kg) and SKF 97541 (0.005 mg/kg), the positive allosteric modulator CGP 7930 (0.3 mg/kg) or the antagonist SCH 50911 (0.3 mg/kg) counteracted the cocaine discontinuation-induced enhancement in the immobility time. The enhanced immobility time in rats that self-administered cocaine (but not given cocaine passively) may reflect the motivated or cognitive processes of reinforced responding of cocaine and could be a potential driver of the addiction process *per se*. Moreover, either blockade or stimulation of $GABA_B$ receptors by their ligands in very low doses attenuated the enhanced immobility time in rats after discontinuation of cocaine self-administration and these findings extend preclinical studies demonstrating the potential involvement of $GABA_B$ receptor ligands to reduce cocaine craving.

1. Introduction

Cocaine is a potent brain stimulant and one of the most powerful addictive drugs of abuse. In humans, low doses of cocaine increase alertness, concentration and energy, whereas higher doses produce euphorigenic effects called as a "cocaine high" (Evans et al., 2002). The use of chronic cocaine in adults has also been linked to depressed mood and markedly diminished interest or pleasure in rewarding stimuli (i.e. "anhedonia") (Barr and Markou, 2005; Barr et al., 2002). The above symptoms appear during cocaine discontinuation and resemble core symptoms of major depressive disorder (Lynch and Leonard, 1978; Markou and Kenny, 2002; Markou et al., 1998).

Several recent studies have demonstrated that discontinuation of repeated treatment with drugs of abuse is also linked to the development with depressive-like symptoms in rodents. Thus, a dysphoric state — the major syndrome of depression — was found in rats withdrawn from repeated cocaine following conditioned place preference procedure (Ettenberg et al., 1999). In addition, repeated exposure to cocaine displayed reward deficits measured as elevations in brain reward thresholds in intracranial self-stimulation procedures which reflect the drug-induced anhedonia (Markou and Koob, 1991; Paterson et al., 2000). Discontinuation of daily cocaine injections resulted in a depressive-like behavior scored as significant increases in immobility in the forced swim test in rats (Filip et al., 2006). It should be underlined, that symptoms of cocaine withdrawal in human and rats can be attenuated by some antidepressant drugs (Gawin et al., 1989; Markou et al., 1992) further indicating that depressive-like processes may be involved (Pliakas et al., 2001).

Recent preclinical findings point an involvement of γ-aminobutyric acid (GABA) neurotransmission system (Brambilla et al. 2003; Krystal et al., 2002) and its metabotropic $GABA_B$ receptors as important brain targets in the pathophysiology of depression (Cryan and Kaupmann, 2005; Pilc and Nowak, 2005). In fact, several $GABA_B$ receptor antagonists have demonstrated antidepressant-like properties in a number of experimental models of depression including the forced swim test, learned helplessness, olfactory bulbectomy and chronic mild stress (Frankowska et al., 2007; Nakagawa et al., 1999; Nowak et al., 2006; Slattery et al., 2005). Antidepressant-like activity

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doi:10.1016/j.ejphar.2009.10.049

of the $GABA_B$ receptor antagonists is in accord with data obtained following genetic disruption of this receptor as the $GABA_B$ −/− animals exhibited reduced immobility in the forced swim test (Mombereau et al., 2005). Conversely, $GABA_B$ receptor agonists and positive allosteric modulators, at selected (high) doses, have been described to be ineffective in the forced swim test in mice and/or rats after subchronic (Borsini et al., 1986; Mombereau et al., 2004; Nakagawa et al., 1996a,b,c; Slattery et al., 2005) or chronic treatments (Nakagawa et al., 1996b), although controversy does exist. Thus, the $GABA_B$ receptor agonist baclofen given to mice or rats at relative low doses displayed anti-immobility effects in the forced swim test (Aley et al., 1990; Car and Wiśniewska, 2006; Frankowska et al., 2007). Altogether, these results indicate that either blockade or stimulation of $GABA_B$ receptors can induce antidepressant-like behaviors depending on a set of variable factors such as the drug dose, experimental procedures and probably the $GABA_B$ receptor neuroanatomical localization.

The present study aimed to verify the hypothesis that discontinuation of cocaine self-administration can develop depressive symptoms in the forced swim test in rats. To this end, we used a "yoked" procedure in which rats were run simultaneously in groups of three, with two rats (so-called "yoked" controls), received passive an injection of cocaine or saline, each time a response-contingent injection of cocaine was self-administered by the third paired rats. Such a procedure was used also to separate motivational from direct pharmacological effects of cocaine. Additionally, the responses of such animals in the forced swim test were compared to responses of animals underwent chronic mild stress. Furthermore, we examined whether acute treatment with a classical antidepressant imipramine or $GABA_B$ receptor ligands could alter the enhanced immobility time in rats withdrawn from self-administered cocaine.

2. Materials and methods

2.1. Animals

The experiment was performed on adult male Wistar rats delivered by a licensed breeder (Warsaw, Poland). The animals were housed individually in standard plastic rodent cages (45 cm × 25 cm × 15 cm), in a colony room maintained at 20 ± 1 °C and at 40–50% humidity under a 12-h light–dark cycle (lights on at 06:00). The rats had free access to food (Labofeed pellets, Kcynia, Poland) and water during the 7-day habituation period (self-administration procedures) or the 2-month habituation period (chronic mild stress procedures) before the start of experiments. Then, rats used in the cocaine self-administration procedures were maintained on limited water during initial training sessions (see below), while animals used in the chronic mild stress procedures were maintained on limited food and water intake for a period (14 h) preceding only each sucrose test (see below). All the experiments were carried out according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (publication no. 85-23, revised in 1985) and were approved by the Local Bioethics Commission. All efforts were made to minimize the number of animals used and their suffering. The animals were experimentally naïve.

2.2. Drugs

The following drugs were used (in parentheses: full chemical names and suppliers): (R)-baclofen (Tocris Cookson, Bristol, UK), CGP 7930 (3,5-*bis*(1,1-dimethylethyl)-4-hydroxy-β,β-dimethyl-benzenepropanol; Tocris Cookson, Bristol, UK), cocaine hydrochloride (National Institute on Drug Abuse, RTI International, USA), SCH 50911 ((+)-5,5-dimethyl-2-morpholineacetic acid hydrochloride; Tocris Cookson, Bristol, UK) and SKF 97541 (3-aminopropyl(methyl)phosphinic acid; Tocris Cookson, Bristol, UK). The drugs were dissolved in sterile 0.9% NaCl, except for CGP 7930 which was dissolved in 2 drops of ethanol and diluted as required in a 1% aqueous solution of Tween 80 (Sigma-Aldrich, USA). Baclofen, CGP 7930, SKF 97541 and SCH 50911 were injected *ip* in a volume of 1 ml/kg and cocaine was injected *iv* in volume of 0.1 ml/kg. Baclofen, CGP 7930 and SKF 97541 were given 30 min, SCH 50911 was given 45 min before the test. The doses of $GABA_B$ receptor ligands were chosen based upon the previous study (Frankowska et al., 2007).

2.3. Cocaine self-administration

Rats were trained to press the lever of standard operant conditioning chambers (Med-Associates, USA) under a fixed ratio 5 schedule of water reinforcement. Two days following "lever press" training and free access to water, the rats were chronically implanted with a silastic catheter in the external right jugular vein, as described previously (Frankowska et al., 2008; Przegaliński et al., 2008). Catheters were flushed everyday with 0.1 ml of saline solution containing heparin (70 U/ml, Biochemie, Austria) and 0.1 ml of solution of cephazolin (10 mg/ml; Biochemie GmbH, Austria). Catheter patency was tested periodically with the ultrashort-acting barbiturate anesthetic methohexital (10 mg/kg, *iv*; loss of consciousness within 5 s).

After a 10-day recovery period, all animals were water deprived for 18 h and trained to lever press to a fixed ratio 5 schedule of water reinforcement over a 2-h session. Then, subjects were given access to cocaine during 2-h daily sessions performed 6 days/week (maintenance) and from that time they were given *ad libitum* water. The house light was illuminated throughout each session. Each completion of five presses on the "active" lever complex (fixed ratio 5 schedule) resulted in a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml) and a 5-s presentation of a stimulus complex (activation of the white stimulus light directly above the "active" lever and the tone generator, 2000 Hz; 15 dB above ambient noise levels). Following each injection, there was a 20-s time-out period during which responding was recorded but had no programmed consequences. Response on the "inactive" lever never resulted in cocaine delivery. Acquisition of the conditioned operant response lasted a minimum of 10 days until subjects met the following criteria: minimum requirement of 20 reinforcements with an average of 6 days and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of < 10% of the average; this criterion was selected based on our prior experiments (Frankowska et al., 2008; Przegaliński et al., 2008). Once stable rates of responding were established, cocaine intake was discontinued on the following 1, 3, 10 or 30 days in separate groups of rats. During discontinuation of cocaine intake animals were kept in the home cages.

Rats were tested simultaneously in groups of three, with two rats serving as yoked controls that received an injection of either 0.5 mg/kg cocaine or saline which was not contingent on responding each time a response-contingent injection of 0.5 mg/kg cocaine was self-administered by the third paired rat.

2.4. Chronic mild stress

The chronic mild stress procedure was performed according to Papp et al. (1994). Briefly, rats were trained to consume a 1% sucrose solution. Following the final baseline test, rats were divided in two groups: one subjected to the chronic mild stress procedure and a second left unchallenged (control rats). The weekly stress regime consisted of two periods of food and water deprivation, 45° cage tilt, intermittent illumination, a soiled cage, paired housing, low intensity stroboscopic illumination, and two periods of no stress. Control rats and stressed animals, which were housed in separate rooms, had free access to food and water except for a period (14 h) preceding each sucrose test. Following 2 weeks of stress, both chronic mild stress and control sucrose tests were performed, 24 h after the last stress