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SHORT COMMUNICATION

ROLE OF DOPAMINE D_3 RECEPTORS IN CONTROLLING THE EXPRESSION OF COCAINE SENSITIZATION IN RATS

Małgorzata Filip#, Iwona Papla, Klaudia Czepiel

Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences,
Smętna 12, PL 31-343 Kraków, Poland

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It is established that dopamine (DA) is an important brain mediator of the behavioral (i.e. sensitizing) effects of cocaine in rodents. Among DA receptors, recent findings point to engagement of DA D_3 receptors in cocaine addictive actions. In the present study, we attempted to determine the role of DA D_3 receptors in the expression phase of sensitization to cocaine in rats, using the selective ligands 7-OH-PIPAT (an agonist) and nafadotride (an antagonist) of these receptors. Repeated administration (1–5 days) of cocaine (10 mg/kg, *ip*) to male Wistar rats significantly enhanced the locomotor activation induced by its challenge dose given after 5-day withdrawal (on day 10). 7-OH-PIPAT (1 mg/kg, but not 0.01–0.1 mg/kg, *sc*) administered together with a challenge dose of cocaine significantly decreased the response to cocaine in rats treated repeatedly with cocaine. On the other hand, the expression of cocaine sensitization was increased when that drug was combined with nafadotride (0.4 mg/kg, *ip*) on day 10. The results indicate a role of DA D_3 receptors in controlling the expression of cocaine sensitization in rats, and may suggest an importance of DA D_3 receptor agonists in the therapy of cocaine abuse.

Key words: *7-OH-PIPAT, cocaine, nafadotride, behavioral sensitization, rats*

correspondence; e-mail: filip@if-pan.krakow.pl

INTRODUCTION

Behavioral sensitization to cocaine is a widely used animal model that is believed to reflect the drug-induced paranoia, craving and relapse in humans [20]. The above phenomenon is characterized by an augmentation of locomotor activity, stereotypy and positive reinforcing effects, after discontinuing a regimen of repeated, intermittent drug injections [20].

A lot of studies have demonstrated that brain dopamine (DA) neurotransmission is critical for the generation of cocaine effects, including behavioral sensitization, in rats [20]. In fact, the drug inhibits DA reuptake transport [12] and increases extracellular levels of the neurotransmitter in terminal areas, mainly of the mesolimbic DA pathway [13]. Since DA affects its target cells *via* an interaction with different receptor subtypes, it is likely that at least one of them is critically engaged in the DA-mediated behavioral effects of cocaine. Of at least five DA receptor subtypes [8], recent findings point to importance of DA D_3 receptors in the drug-evoked effects: 1) the transcript and protein for these receptors are densely located in the DA mesolimbic system [9], 2) activation of DA D_3 receptors decreases basal extracellular DA concentrations in the terminals of this system, i.e. the nucleus accumbens [19, 27], 3) agonists or partial agonists of DA D_3 receptors inhibit cocaine-seeking behavior and the effects triggered by presentation of drug-associated cues [21], conditioned place preference induced by the psychostimulant [11] as well as produce stimulus generalization to cocaine in drug discrimination model [6, 7, 26]. Using appropriate antagonists and knockout mice, it was found that DA D_3 receptors play an inhibitory role in the behavioral responses to cocaine and in the regulation of its striatal gene [3], however, other studies with DA D_3 receptor antagonists did not fully confirm this suggestion [2, 5]. Pharmacological and genetic findings of human studies implicate DA D_3 receptors in cocaine addiction [15]. It has also been reported that exposure to cocaine significantly increased the expression of DA D_3 receptor mRNA and protein in the human brains of cocaine-overdose victims [17].

In the present study, we investigated the ability of some DA D_3 receptor ligands to affect the expression of cocaine sensitization in rats. To that end we used 7-OH-PIPAT (an agonist; [14]) and nafadotride (an antagonist; [25]), which display 41- and 10-fold higher affinity for DA D_3 receptor sites compared to DA D_2 ones, respectively, and more than 50-fold D_3/other neurotransmitter binding sites selectivity [1, 14].

MATERIALS and METHODS

Animals

The experiment was performed on male Wistar rats (280–300 g). The animals had free access to food (Labofeed pellets) and water, and were kept at a room temperature of 20 ± 1°C under a 12-h light/dark cycle (the light on between 6.00–18.00 h). All the experiments were approved by the Committee for Laboratory Animal Welfare and Ethics and met the International Guidelines for Care and Use of Laboratory Animals.

Drugs

The following drugs were used (in parentheses: pre-session injection times, route of injections and suppliers): cocaine hydrochloride (–5 min, *ip*; Merck, Germany), (R,S)trans-7-hydroxy-2-(*N*-n-propyl-*N*-3'-iodo-2'-propenyl)aminotetralin (7-OH-PIPAT; –20 min, *sc*; Tocris, UK) and nafadotride (–35 min, *ip*; de l'INSERM, Centre Paul Broca, France). Cocaine and nafadotride were dissolved in saline (0.9% NaCl), while 7-OH-PIPAT was dissolved in 20% β-cyclodextrin (Sigma-RBI, USA). The drugs were injected in a volume of 1 ml/kg.

Locomotor activity measurement

The locomotor activity of rats was recorded individually for each animal as described previously [22]. Briefly, the rats' behavior was measured in Opto-Varimex cages (Columbus Instruments, USA). Locomotor activity associated with horizontal locomotion was defined as a distance traveled (cm).

Before recording the locomotor activity, the animals were allowed a 60-min habituation period after which they were taken out, injected with the drugs, and placed back in the boxes. Locomotor activity was recorded for 60 min. Eight rats per group were used.

Behavioral sensitization to cocaine

During the first 5 days of the experiment, the animals received the injections of saline (*ip*) or cocaine (10 mg/kg, *ip*). On day 8, the rats were given

saline (*ip*), whereas on day 10 they were challenged with saline (*ip* or *sc*) + cocaine (10 mg/kg, *ip*), 7-OH-PIPAT (0.01–1 mg/kg, *sc*) + cocaine (10 mg/kg, *ip*) or nafadotride (0.2–0.4 mg/kg, *ip*) + cocaine (10 mg/kg, *ip*). Locomotor activity was recorded on days 1, 5, 8 and 10.

Statistical analyses

The data are expressed as mean total activity counts (± SEM) for the 60-min observation period. To evaluate behavioral sensitization, the response to cocaine on day 10 was compared between the groups given repeated cocaine or repeated saline. The one-way ANOVA, followed by *post hoc* Dunnett's test, were applied to evaluate the effects of the treatments on days 8 and 10.

RESULTS and DISCUSSION

On day 10, cocaine challenge of rats previously repeatedly treated with the psychostimulant (days 1–5) produced an increase in locomotor hyperactivity (about 1.4-2-fold) compared to the effect of acute cocaine in saline-treated (days 1–5) animals (Figs. 1–2).

An ANOVA showed a significant effect of treatment with 7-OH-PIPAT + cocaine [$F(4,35) = 7.55$, $p < 0.01$] and with nafadotide + cocaine [$F(3,27) = 8.13$, $p < 0.01$]. Subsequent experiments were conducted on rats treated repeatedly (days 1–5) with cocaine. When those animals were given a challenge dose of the psychostimulant in a combination with 7-OH-PIPAT on day 10, a significant decrease in the locomotor activity of animals was found after 1 mg/kg (but not 0.01–0.1 mg/g) of 7-OH-PIPAT (Fig. 1). Following the injection of nafadotride (0.4, but not 0.2 mg/kg) in combination with systemic cocaine, a 2-fold enhancement of the locomotor response was observed (Fig. 2). When given alone, neither 7-OH-PIPAT (0.01–1 mg/kg) nor nafadotride (0.2–0.4 mg/kg) modified the rats' locomotor activity (data not shown).

The present results show sensitization to locomotor hyperactivity effect of cocaine in the rats that were treated for 5 days with the drug, and challenged with cocaine on day 5 after its withdrawal. Important observation from this study indicates that, when given concurrently with cocaine on day 10, the DA D_3 receptor agonist 7-OH-PIPAT (at a dose of 1 mg/kg) significantly reduced the expression of cocaine sensitization. This reduction seems to be specific, since 7-OH-PIPAT, when given alone, did not decrease locomotor acivity of the animals.

It was also found that pretreatment with nafadotride enhanced the locomotor hyperactivation evoked by cocaine on day 10, which indicated that this DA D_3 receptor antagonist enhanced expression of cocaine sensitization. Despite its only a 10-fold D_3/D_2 receptor selectivity *in vitro* [1], nafadotride did not increase *per se* the basal locomotor activity of animals (present study; [4]) and reportedly

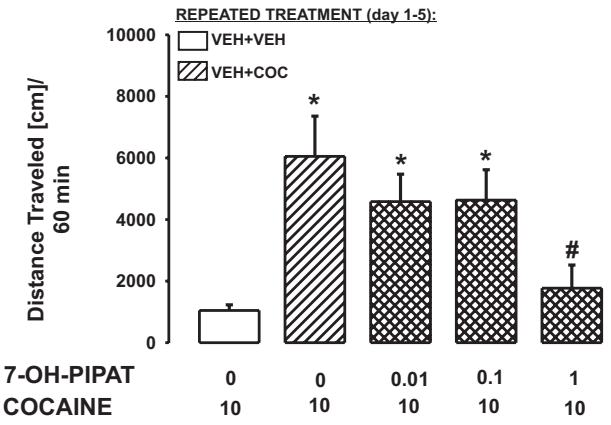

Fig. 1. Effects of 7-OH-PIPAT on the expression of cocaine sensitization. Rats were treated repeatedly with vehicle (VEH) or cocaine (COC; 10 mg/kg) daily for 5 days. On day 10, they were challenged with vehicle + cocaine (10 mg/kg) or 7-OH-PIPAT (0.01–1 mg/kg) + cocaine (10 mg/kg). * $p < 0.01$ vs vehicle-treated and cocaine-challenged group; # $p < 0.05$ vs cocaine-treated and cocaine-challenged group

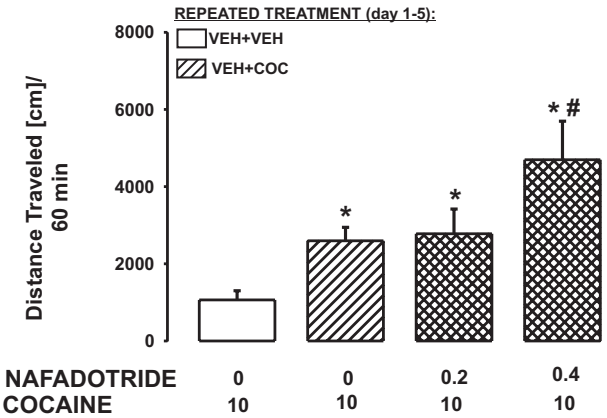

Fig. 2. Effects of nafadotride on the expression of cocaine sensitization. Rats were treated repeatedly with vehicle (VEH) or cocaine (COC; 10 mg/kg) daily for 5 days. On day 10 they were challenged with vehicle + cocaine (10 mg/kg) or nafadotride (0.2–0.4 mg/kg) + cocaine (10 mg/kg). * $p < 0.05$ vs vehicle-treated and cocaine-challenged group; # $p < 0.05$ vs cocaine-treated and cocaine-challenged group

it did not occupy *in vivo* DA D_2 receptor sites up to 3 mg/kg (*ip*) [16], therefore it seems that the blockade of DA D_3 receptors really contributes to the pharmacological effect of nafadotride, in particular to its enhancing effect on cocaine sensitization.

Apart from our findings, a lot of reports indicate a strong involvement of DA D_3 receptors in the behavioral actions of cocaine and other abused psychostimulants (see Introduction). Recent findings show that nafadotride inhibited development of locomotor sensitization to amphetamine [24], while this and other antagonists at DA D_3 receptors decreased rewarding effects of cocaine [23] and increased its locomotor hyperactivating effect [Filip and Czepiel, unpublished observation]. On the other hand, it has been reported that several DA D_3 receptor agonists (e.g. 7-OH-DPAT) enhanced the development of cocaine sensitization in rats [18], substituted for cocaine in a drug discrimination model [6, 7, 23], enhanced the reinforcing effects of self-administered cocaine [26] as well as attenuated the incentive motivational properties of amphetamine and cocaine as measured by conditioned place preference tests [10, 11].

In conclusion, our results indicate a role of DA D_3 receptors in controlling the expression of cocaine sensitization in rats. They can also suggest that the DA D_3 receptor agonists may be useful in the treatments of some consequences of repeated cocaine intake (i.e. paranoia, craving and relapse) in humans addicts.

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