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Involvement of cannabinoid CB_1 receptors in drug addiction: effects of rimonabant on behavioral responses induced by cocaine

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Abstract:
A lot of evidence indicate that endocannabinoids and cannabinoid CB_1 receptors are implicated in drug addiction. In the present study, we investigated the effect of the cannabinoid CB_1 receptor antagonist/partial agonist rimonabant on the cocaine-maintained reinforcement and relapse to cocaine seeking as well as on the cocaine challenge-induced hyperactivity in sensitized rats and on discriminative stimulus effects of cocaine in rats. We found that endocannabinoids were not involved in maintenance of cocaine reinforcement and its subjective effects since pharmacological blockade of cannabinoid CB_1 receptors altered neither self-administration nor discriminative stimulus effects of cocaine. On the other hand, withdrawal from repeated access or exposure to cocaine and then a reinstatement of cocaine-seeking behavior or a sensitized locomotor response to a single cocaine challenge, respectively, was potently reduced by pretreatment with rimonabant. The latter observations may show that repeated cocaine treatment and the drug withdrawal produce – apart from behavioral effects – also different neural consequences in the endocannabinoid systems in rats.

Key words:
cannabinoid CB_1 receptors, cocaine, drug addiction, rimonabant

Introduction

Cocaine is an alkaloid with psychostimulant action, and cocaine dependence still remains a serious medical and social problem [43]. This drug exhibits high affinity for dopamine, serotonin and norepinephrine transporters and inhibits the reuptake of those neurotransmitters into presynaptic nerve terminals [44]. It was established that enhanced dopamine neurotransmission and indirect activation of dopamine receptors within the dopaminergic mesolimbic pathway plays a significant role in the expression of the locomotor [16, 27], discriminative stimulus [8], and reinforcing effects [54] of cocaine.

Recently, it was shown that the cannabinoid system, composed of the endogenous substances (e.g. anandamide, 2-arachidonylglycerol) that interact with at least two cannabinoid receptor subtypes, CB_1 and CB_2 (for review see [49]), modulates synaptic neurotransmissions and is involved in the brain pathways implicated in addiction (for review see [78]). Several

studies point to a key role of cannabinoid CB_1 receptors in the behavioral and biochemical processes underlying drug addiction [72]. In fact, the cannabinoid CB_1 receptors are densely expressed within mesolimbic dopamine pathway [79] and are linked with rewarding aspects of not only Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient in marijuana [11, 46, 75], but also other abused substances, including cocaine [e.g. 18, 46, 72]. Thus, activation of cannabinoid CB_1 receptors is a permissive element for the expression of cocaine-induced rewarding effects in rats [23] and for the relapse to cocaine seeking behavior [18]. Contrary, it has been demonstrated that cannabinoid CB_1 receptors are engaged in acquisition, but not expression, of conditioned place preference induced by cocaine [10] and for the relapse induced by re-exposure to cocaine-associated conditioned stimuli and cocaine priming injections in rats withdrawn from cocaine self-administration [18]. Interestingly, pretreatment with the cannabinoid CB_1 receptor antagonist/inverse agonist rimonabant was unable to modify cocaine reinforcement/reward in self-administration paradigm in rodents [23, 48, but see also 72] and in monkeys [75] or brain-stimulated reward in rats [81], and genetic invalidation of cannabinoid CB_1 receptor did not alter cocaine self-administration [15, but see also 72] or cocaine-evoked conditioned place preference [52] in mice.

On the other hand, there are no available data on the role of cannabinoid CB_1 receptors in the cocaine-evoked behavioral sensitization or discrimination. To this end, we investigated the effect of the cannabinoid CB_1 receptor antagonist/partial agonist rimonabant [60] on the cocaine challenge-induced hyperactivity in sensitized rats and on discriminative stimulus effects of cocaine in rats. Sensitization is a phenomenon that is characterized by the increased response (e.g. locomotor hyperactivity) to the subsequent drug challenge after the repeated administration regimen is discontinued [42, 61] and is believed to mediate incentive salience or "drug wanting" [62]. The drug discrimination is an animal model widely used to reflect the subjective effects of drugs in humans [68]. Since there is only a single report in rats on the effects of rimonabant in cocaine self-administration paradigm [18], we conducted studies to further investigate the role of cannabinoid CB_1 receptors in cocaine-maintained reinforcement and relapse to cocaine seeking using different experimental protocols than De Vries et al. [18].

Materials and Methods

Animals

Male Wistar rats (280–300 g) delivered by a licensed breeder (T. Górzkowska, Warszawa, Poland) were housed either 8 per cage (locomotor activity studies), 2 per cage (drug-discrimination procedures) or individually (self-administration procedures) in standard plastic rodent cages 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 animals had free access to food (Labofeed pellets) and water, except for those used in self-administration and drug discrimination procedures which were maintained on limited amount of water during initial training sessions and had restricted access to water, respectively (see below). All experiments were conducted during the light phase of the light-dark cycle (between 08:00–15:00) and were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and with approval of the Bioethics Commission as compliant with the Polish Law (21 August 1997).

Drugs

Cocaine hydrochloride and rimonabant (SR 141716A) were used. Cocaine was dissolved in sterile 0.9% NaCl, while rimonabant was dissolved in a mixture of ethanol-emulphor-19% β-cyclodextrin (1:1:10). Cocaine was given either *iv* (0.5 mg/kg/injection) or *ip* (1 ml/kg). Rimonabant was injected *ip* in a volume of 1 ml/kg 45 min before cocaine.

Self-administration

Rats which drank limited amount of water during initial training sessions, were trained to press the lever in standard operant chambers (Med-Associates, USA) under a fixed ratio (FR) 5 schedule of water reinforcement. Two days following lever-press training and free access to water, the rats were anesthetized with ketamine HCl (75 mg/kg, *ip*; Biowet, Poland) and xylazine (5 mg/kg, *ip*; Biowet, Poland) and chronically implanted with a silastic catheter in the external right jugular vein, as described previously by Filip [26]. Catheters were flushed every day with 0.1 ml of saline solution containing heparin (70 U/ml, Biochemie

GmbH, 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*) for loss of consciousness within 5 s. During studies no catheter problems occurred.

Maintenance

Rats were allowed 10 days to recover before the start of the experiments. Initially, all animals deprived of water for 18 h were trained in one 2-h session to press the lever on an FR 5 schedule for water reinforcement. Then, subjects (n = 8 rats) began lever pressing for cocaine reinforcement and from that time they were given water *ad libitum* throughout the remaining period of the experiment. Rats were given access to cocaine during 2-h daily sessions performed 6 days/week (maintenance). The house light was on throughout each session. Each completion of an FR 5 schedule (i.e. 5 lever presses) on the "active" lever resulted in a 5-s injection of cocaine and a 5-s presentation of a stimulus complex, consisting of activation of the white stimulus light directly above the "active" lever and the tone generator (2000 Hz; 15 dB above ambient). Cocaine was delivered at a dose of 0.5 mg/kg per 0.05 ml; this training dose was selected based on prior experiments indicating that rats readily acquire self-administration at this dose and do not display differences in cocaine intake [26]. 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. Each training trial lasted for 2 h or until the subject had self-administered 24 infusions of cocaine. Acquisition of the conditioned operant response lasted a minimum 10 days and until subjects met the following criteria: minimum requirement of 24 reinforcements and active lever presses with an average of 3 consecutive days and a standard deviation across those 3 days to vary by 10%. Once stable rates of responding were established, subjects were pretreated with either vehicle or rimonabant (5–10 mg/kg) before the test sessions. The order of injections was counterbalanced according to a Latin square design, and test sessions were separated by at least two-three baseline days of cocaine self-administration.

Reinstatement

A separate group of rats (n = 7–8 rats/group) served to examine reinstatement of cocaine self-administration. After an average of 18 days of the training and maintenance sessions of self-administration, subjects met an acquisition criterion that required the number of reinforcements and active lever presses over 3 consecutive maintenance sessions to vary by 10%. Then the extinction procedure was instituted on the following day. During extinction trials subjects had 2-h daily training sessions; however, active lever presses now resulted in neither the delivery of cocaine (saline was substituted for cocaine) nor the presentation of the conditioned stimulus. Once they reached the extinction criterion (a minimum of 10 extinction days with the responding on the active lever below 10% of the level observed during maintenance during at least 3 consecutive days), the rats were divided into two groups and were tested for response reinstatement induced by cocaine (10 mg/kg, *ip*; n = 7) or by the drug-associated cue (tone + illumination; n = 8). During reinstatement test (2-h session), active lever presses on the FR 5 schedule resulted only in an intravenous injection of saline. Rats were pretreated with either vehicle or rimonabant (5–10 mg/kg) before tests. Drug combinations were given in a randomized order and each rat was examined in three reinstatement tests that were separated by at least two-three extinction sessions.

Drug discrimination

Rats (n = 8) with restricted access to water during the daily training sessions (5–6 ml/rat/session), after test sessions (15 min), and over the weekends were trained to discriminate cocaine (10 mg/kg, *ip*) from 0.9% NaCl (*ip*), according to the procedure described previously [28]. Briefly, cocaine or saline was administered 15 min before daily (Monday-Friday) sessions (15 min) in two-lever standard operant chambers (Med-Associates; USA) under a FR 20 schedule of continuous water reinforcement and depending on the treatment left or right lever became active. That phase of training continued until the animals met the criterion (an individual mean accuracy of at least 80% of correct responses, before the first reinforcer during 10 consecutive sessions). During this phase, one rat was excluded from the study for failure to maintain performance at the criterion level (see above). Later, test

sessions were conducted once or twice a week, while cocaine and saline sessions intervened between the test sessions to maintain discrimination accuracy. Only the rats that met an 80% performance criterion during the preceding cocaine and saline sessions were used in the tests. After completion of 20 responses to either lever, or after the session time elapsed, a single reinforcer was delivered and the animals were removed from the chamber. Once in their home cages, all the rats were allowed 15 min of free access to water. In substitution tests, rats were tested with different doses of cocaine (0–10 mg/kg; –15 min) or rimonabant (5–10 mg/kg; –60 min). In combination tests, rimonabant (5 mg/kg) was given before different doses of cocaine (1.25–10 mg/kg).

Sensitization

During the first 5 days of the experiment, the animals received either saline or cocaine. On day 10, to assess effects of rimonabant on expression of cocaine sensitization, they were challenged with vehicle + cocaine (10 mg/kg, *ip*), or rimonabant (1.25–10 mg/kg) + cocaine (10 mg/kg, *ip*). Locomotor activity was recorded for 60 min and the test started immediately after cocaine injection. Additionally, separate groups of animals were used to study the effects of rimonabant on basal locomotor activity. The locomotor activity of rats was recorded for each animal as described previously [58]. Briefly, the locomotor activity was measured in Opto-Varimex cages (Columbus Instruments, USA). Horizontal locomotor activity, defined as distance travelled, was expressed in cm. Before locomotor activity was recorded, rats were habituated in the test cages for 2 h/day on each of the two days before the start of the experiment, and on the test day for 1 h before the start of the test session. Locomotor activity was recorded for 60 min and the test started immediately following the second (cocaine or saline) injection. Each group of rats consisted of 6–7 animals.

Statistical analyses

During maintenance of cocaine self-administration, the number of responses on the active and inactive lever (including time-out responding) and the number of infusions for each group pretreated with rimonabant were analyzed by a one-way analysis of variance (ANOVA) for repeated measures. During reinstatement of cocaine seeking behavior induced by cocaine priming or by the drug-associated cue, the number of responses on the active and inactive lever (including time-out responding) for each group pretreated with rimonabant was analyzed by a one-way ANOVA for repeated measures. *Post-hoc* Dunnett's test was used to analyze differences between group means.

In drug discrimination studies, accuracy was defined as a ratio of correct responses to the total number of responses before delivery of the first reinforcer. During test sessions, performance was expressed as the percentage of drug-lever responses to total responses upon completion of an FR 20 on either lever. Response rates (responses per s), regarded as a measure of behavioral disruption, were calculated as the total number of responses to either lever before completion of the first FR 20, divided by the number of seconds required to complete the FR. The data from animals that completed the FR 20 during the test sessions were used for analysis of percentage of drug-appropriate lever responses, while the data from all the rats used in tests were included for analysis of responding rate. A one-way ANOVA for repeated measures was used to compare the percentage of drug-appropriate lever responding and response rates during the test sessions with the corresponding values of the preceding drug session (substitution tests). *Post-hoc* Dunnett's test was used to analyze differences between group means. In combination experiments, a two-way ANOVA for repeated measures was used to analyze the effects of rimonabant (factor 1) and cocaine dose (factor 2).

In locomotor activity studies, the data are expressed as distance travelled during a 60-min observation period. The one-way analysis of variance (ANOVA), followed by *post-hoc* Dunnett's test, was used to evaluate the treatment group effect on acute locomotor activity as well as expression of cocaine sensitization.

Results

Maintenance of cocaine self-administration

Rats showed stable responding on levers during the last 3 self-administration maintenance sessions with an acquisition criterion requiring that the number of active lever presses and cocaine injections varied by less than 10%. The animals had self-administered

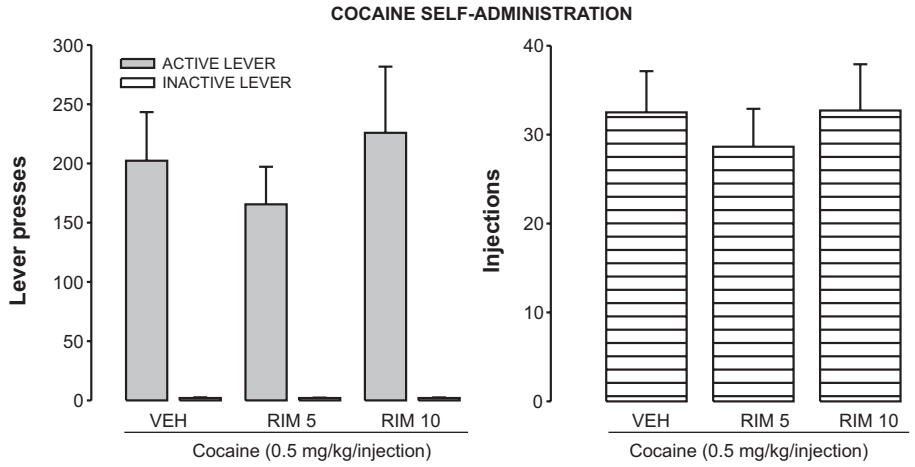

Fig. 1. Effects of rimonabant on maintenance of cocaine self-administration in rats responding under a FR 5 schedule of reinforcement. Number of the active (grey bars), inactive (white bars) lever presses and cocaine injections (stripped bars) following rimonabant (RIM; 5–10 mg/kg), and vehicle (VEH) during cocaine self-administration. During self-administration active lever responses resulted in a delivery of a cocaine injection (0.5 mg/kg per injection) and simultaneous presentation of a light + tone stimulus complex

24–36 injections of cocaine with the daily mean cocaine intake between 12–18 mg/kg. Rats practically did not respond on the inactive lever, independently of self-administration test day.

Rimonabant (5–10 mg/kg) neither changed the number of active [$F(2,21) = 0.5$] or inactive [$F(2,21) = 1.11$] lever presses, nor the number of cocaine injections [$F(2,21) = 0.25$] (Fig. 1).

Reinstatement of cocaine self-administration

After extinction trials during which active lever presses resulted in the *iv* delivery of saline without the presentation of the conditioned stimulus (cue), the rats were tested for response reinstatement induced by a priming dose of cocaine (10 mg/kg, *ip*) and by the presentation of the drug-associated cue. During cocaine priming and cue-induced reinstatement tests, rats responded more often on the active lever in relation to the inactive lever ($p < 0.05$) and to the extinction period ($p < 0.05$) (Fig. 2).

Rimonabant (5–10 mg/kg) dose-dependently reduced the response reinstatement induced by cocaine priming on active lever [$F(3,26) = 4.74$, $p < 0.01$]; the significant attenuation was observed after pretreatment with rimonabant (10 mg/kg) ($p < 0.05$). The

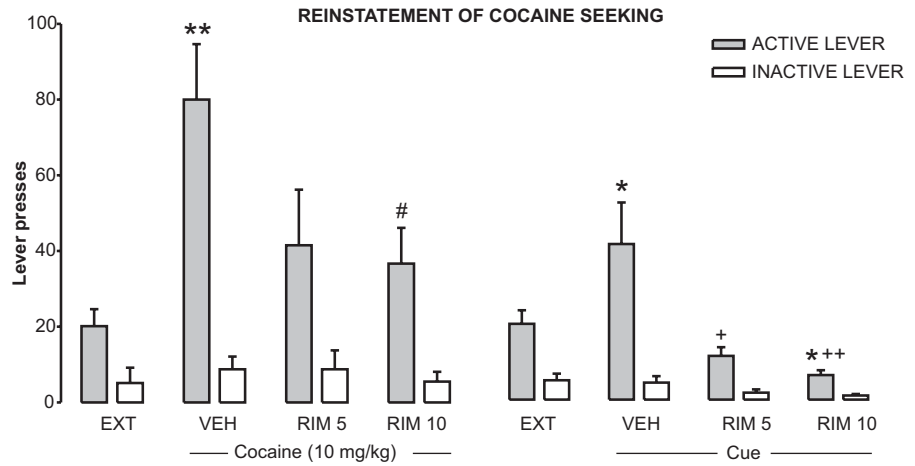

Fig. 2. Effects of rimonabant on the reinstatement of cocaine seeking behavior induced by cocaine-primed injections or the drug-associated cue. Number of the active (grey bars) and inactive (white bars) lever presses following cocaine priming injections (10 mg/kg, *ip*; left) or the cue (tone + illumination; right) are shown for pretreatment with rimonabant (RIM; 5–10 mg/kg), and vehicle (VEH) testing and baseline extinction (EXT) responding. * $p < 0.05$, ** $p < 0.001$ *vs.* extinction; # $p < 0.05$ *vs.* vehicle + cocaine; $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ *vs.* vehicle + cue

number of inactive lever presses was unaltered [F(3,26) = 0.25] (Fig. 2, left).

A significant reduction of the cue-induced reinstatement of cocaine seeking behavior on active lever was observed after pretreatment with 5 and 10 mg/kg of rimonabant [F(3,28) = 6.66, p < 0.01]. There was no significant effect of rimonabant pretreatment on inactive lever responding [F(3,28) = 2.23] (Fig. 2, right).

Cocaine discrimination

The acquisition of cocaine (10 mg/kg) *vs.* saline discrimination was reached in an average of 25 sessions (ranging between: 19–29). Administration of cocaine (1.25–10 mg/kg) produced a dose-dependent increase in the cocaine-appropriate lever responding (Fig. 3); drug-appropriate lever responding after cocaine, 1.25–10 mg/kg, ranged from 27 to 100%. Administration of saline evoked less than 10% of drug- appropriate lever responding. The response rates after all the test doses of cocaine did not differ from those recorded during the preceding cocaine session.

Following rimonabant (5 mg/kg) administration, neither substitution (less than 25% of cocaine-lever responding) nor alteration in the animals' response rates were found (Fig. 3), while 10 mg/kg of rimonabant induced behavioral disruption.

There was the main effects of rimonabant pretreatment [F(1,12) = 6.2, p < 0.01] and cocaine dose [F(3,36) = 20.9, p < 0.001], but no main effect of a rimonabant x cocaine dose interaction [F(3,36) = 1.3] as shown by a two-way ANOVA (Fig. 3, upper). There was no main effect of pretreatment with rimonabant [F(1,12) = 0.34], cocaine dose [F(3,36) = 0.7] or a rimonabant × cocaine dose interaction [F(3,36) = 0.6] on response rates (Fig. 3, lower).

Cocaine sensitization

A one-way ANOVA did not show significant differences in the rats' basal locomotor activation expressed as distance traveled after administration of rimonabant (5–10 mg/kg) [F(2,17) = 0.93] (vehicle = 457.7 ± 57.6; rimonabant, 5 mg/kg = 339.192.6; rimonabant, 10 mg/kg = 374.1 ± 83.4).

On day 10, the challenge with cocaine in rats treated repeatedly (days 1–5) with the psychostimulant produced a 2.2-fold increase in locomotor hyperactivity compared to the effect of acute cocaine injection to saline-treated (days 1–5) animals (locomotor sensitization) (Fig. 4).

When the animals were given a challenge dose of cocaine in combination with rimonabant at 5 and 10 mg/kg, but not at 1.25 and 2.5 mg/kg, a significant decrease (p < 0.001) in locomotor activity compared

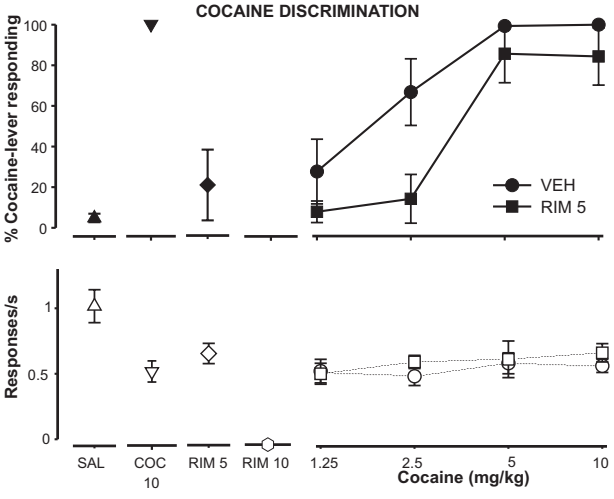

Fig. 3. Effects of rimonabant on the cocaine discrimination in rats trained to discriminate cocaine (10 mg/kg) from saline. Data represent the percentage of cocaine-appropriate lever responses (*upper*) and the response rate (*lower*). Performance was tested after injection of saline (SAL; 1 ml/kg; triangle), the training dose of cocaine (COC; 10 mg/kg; reversed triangle), rimonabant (RIM; 5 mg/kg; diamond), or rimonabant (10 mg/kg; hexagon) during a test session (left). Performance was tested after systemic administration of cocaine (1.25–10 mg/kg) preceded by the injection of vehicle (VEH; circles) or rimonabant (5 mg/kg; squares)

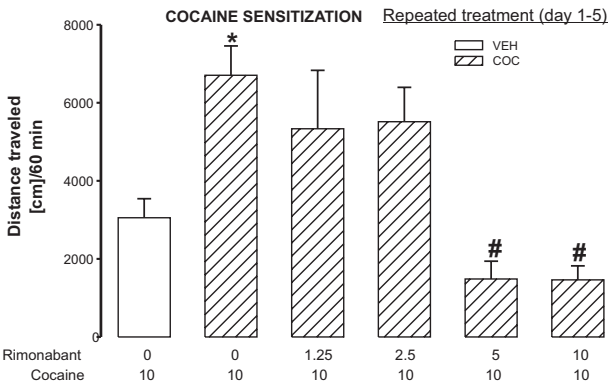

Fig. 4. Effects of rimonabant on the expression of cocaine sensitization. Rats were treated repeatedly with vehicle (VEH) or cocaine (COC; 10 mg/kg, *ip*) daily for 5 days. On day 10, they were challenged with vehicle + cocaine (10 mg/kg) or rimonabant (1.25–10 mg/kg) + cocaine (10 mg/kg). * p < 0.001 *vs.* vehicle-treated and vehicle + cocaine-challenged group, # p < 0.001 *vs.* cocaine-treated and vehicle+cocaine-challenged group

to cocaine-treated and cocaine-challenged animals was found on day 10 [$F(5,35) = 10.5$, $p < 0.001$] (Fig. 4).

Discussion

Our present findings reveal that cannabinoid CB_1 receptors play a role in cocaine sensitization and relapse while an endogenous tonic activation of cannabinoid CB_1 receptors is not required for cocaine reinforcement and discrimination. In fact, we found that pretreatment with the CB_1 receptor antagonist/partial agonist rimonabant did not modify cocaine intake or its subjective properties but it protected the animals against the expression of cocaine sensitization and the drug- or the drug-associated cue-induced reinstatement of cocaine seeking.

The lack of anti-cocaine effects of rimonabant (5–10 mg/kg) observed in cocaine self-administration (i.e. no changes in the number of "active" lever respondings and cocaine injections) are in agreement with the previous findings in rats [18, 23] and mice [48] that used different experimental approaches (e.g. nose-poke operanda, rimonabant dose-range, rat strains), and fully support the observation of normal cocaine self-administration in mice lacking cannabinoid CB_1 receptors [15, but see also 72]. The above findings emphasize that cannabinoid CB_1 receptors do not mediate cocaine-induced enhancement of reward system function and they are not necessary for support the drug-taking behavior. The above statement is strongly supported by the current findings that rimonabant did not reduce the discriminative stimulus effects for cocaine as it did not change significantly the cocaine dose-response effects in this model. In fact, the cocaine discriminative stimulus properties are thought to model the drug-induced subjective effects reported by humans [68] that comprise – similarly to cocaine self-administration – positive elements [22, 51].

The findings of the present study indicate also that rimonabant given before the priming dose of cocaine decreased the effectiveness of cocaine in reinstatement of lever responding in animals withdrawn from cocaine self-administration. These results extended previous observation of De Vries et al. [18] showing that rimonabant attenuated relapse induced by re-exposure to cocaine-associated cues or cocaine itself and brought in further explanation that the rimonabant-induced reduction of the reinstatement of cocaine seeking was not due to its blocking properties toward the cocaine-induced discriminative stimulus properties or locomotor depression. It is in line also with a recent report showing that the selective CB_1 receptor antagonist AM 251 inhibits cocaine-primed relapse in rats [84]. Since the reinstatement was initiated by *ip* cocaine administration by an experimenter, it was also interesting to investigate whether rimonabant directly influenced the motivational effects of cocaine or its other mechanisms (e.g. locomotor stimulant effects, emotional state). Cocaine priming could function as a discriminative stimulus and affect motivational aspects indirectly by being a cue of the cocaine availability [19, 67]. As shown in this study, rimonabant (5 mg/kg) neither substituted for or significantly altered the cocaine dose-response curve in the drug discrimination model in rats, while its higher dose (10 mg/kg) in substitution studies induced a behavioral disruption of animals that did not finish lever-selection, possibly due to rimonabant (10 mg/kg)-induced locomotor suppression. The statement that rimonabant directly influences cocaine seeking behaviors is further supported by another our result showing that both doses of rimonabant (5 and 10 mg/kg) effectively blocked the cue-induced reinstatement in the absence of cocaine. Such inhibitory responses of rimonabant were not due to its motor artifacts as we demonstrated the lack of its influence on the basal locomotor activity or on inactive lever presses in self-administration procedures (at least for the dose of 5 mg/kg of rimonabant). Regarding the involvement of emotional state in controlling the expression of cocaine reinstatement, rimonabant was shown to produce either anxiolytic or anxiogenic effects in several preclinical studies in rodents [e.g. 35, 56, 63, 64], and, therefore, the issue whether the anxiolytic activity of rimonabant alters the cocaine-induced relapse cannot be resolved in this paper and needs further studies.

We are the first to show that rimonabant co-administered with the challenge dose of cocaine potently reduces cocaine sensitization expressed after 5-day withdrawal. The maximum inhibitory effects of rimonabant were seen following its higher doses and these effects may be considered as a specific response, since the drug did not alter significantly basal locomotor activity (present study). However, it should be noticed that rimonabant (10 mg/kg) had some sedative effects of, what might alter (reduce) expres-

Tab. 1. Effects of cannabinoid CB_1 receptor blockade with rimonabant or CB_1 receptor deficiency (CB_1R KO) on the behavioral responses to drugs of abuse in preclinical studies. Abbreviations:↓ attenuation; + effect exists; --- lack of effect

Drug of abuse	Model	Rimonabant	CB_1R KO
Cocaine	Self-administration		
	– acquisition		+ [15] ↓ [72]
	– maintenance	--- [18, 23, 48, present study] ↓ [72]	
	– drug-induced relapse	↓ [18, present study]	
	– cue-induced relapse	↓ [18, present study]	
	Conditioned place preference		
	– acquisition	↓ [10]	+ [52]
	– expression	--- [10]	
	Locomotor sensitization		
	– acquisition	--- [48]	+ [52]
	– expression	↓ [present study]	
	Drug discrimination	– [present study]	
Methamphetamine	Self-administration		
	– acquisition		
	– maintenance		
	– drug-induced relapse	↓[1]	
	– cue-induced relapse	↓ [1]	
	Conditioned place preference		
	– acquisition		
	– expression		
	Locomotor sensitization		
	– acquisition		
	– expression		
	Drug discrimination		
Nicotine	Self-administration		
	– acquisition	↓ [13]	+ [15]
	– maintenance		
	– drug-induced relapse		
	– cue-induced relapse	↓ [12, 13]	
	Conditioned place preference		
	– acquisition	↓ [29]	– [9]
	– expression	↓ [29, 47]	
	Locomotor sensitization		
	– acquisition		
	– expression		
	Drug discrimination	--- [13, 47, 85]	
Alcohol	Self-administration		
	– acquisition	↓ [70]	↓ [40, 57, 77, 82]
	– maintenance	↓ [2, 14, 21, 30, 31, 57, 65]	
	– drug-induced relapse	↓ [69]	
	– cue-induced relapse	↓ [21]	
	Conditioned place preference		
	– acquisition		↓ [38, 77]
	– expression		
	Locomotor sensitization		
	– acquisition		
	– expression		
	Drug discrimination		

Tab. 1. Effects of cannabinoid CB_1 receptor blockade with rimonabant or CB_1 receptor deficiency (CB_1R KO) on the behavioral responses to drugs of abuse in preclinical studies – continued from the previous page. Abbreviations: ↓ attenuation; + effect exists; – lack of effect

Opiates	Self-administration – maintenance – drug-induced relapse – cue-induced relapse	↓[7, 17, 53, 55, 71] ↓ [17, 24, 73] ↓ [17]	+ [15, 45]
	Conditioned place preference – acquisition – expression	↓ [5, 10] ↓ [55]	+ [59] – [52]
	Locomotor sensitization – acquisition – expression		– [52]
	Drug discrimination		
Δ^9-THC	Self-administration – maintenance – drug-induced relapse – cue-induced relapse	↓ [4, 75]	
	Conditioned place preference – acquisition – expression	 ↓ [4]	
	Locomotor sensitization – acquisition – expression		
	Drug discrimination	↓ [41, 71, 83]	

sion of cocaine sensitization. Interestingly, rimonabant, when given repeatedly with cocaine during the development of sensitization, did not alter the locomotor stimulant effects of the cocaine challenge [48]. These findings together with the report of Martin et al. [52] that chronic cocaine treatment produced a similar behavioral sensitization in wild-type and cannabinoid CB_1 knockout mice indicate that CB_1 receptors are not involved in the development but are essential for expression of cocaine sensitization. Such inhibitory effects of rimonabant on the expression of cocaine sensitization may indicate its potential as a novel antipsychotic and anti-relapse medication for the treatment of cocaine dependence and psychosis.

As shown in the present study, cannabinoid CB_1 receptors play an important role in the consolidation of cocaine reinforcement. In the light of these results, it is important to consider whether rimonabant might alter the learning and/or extinction of the drug memory associated with repeated exposure to cocaine. In fact, cannabinoid CB_1 receptors [37] as well as the endocannabinoids [20] are present at high concentrations in the hippocampus and other forebrain areas associated with learning and memory. Second, at the behavioral level, the cannabinoid CB_1 receptor blockade with rimonabant impairs the extinction of contextual fear-memory [74] or induces memory-enhancing effects in rats [76], however, it does not change working or short-term memory in a variety of operant paradigms [6, 36, 50]. In the context of the above data, the issue whether the blocking effects of rimonabant on the cocaine-induced relapse were related to its actions on the learning and/or memory processes cannot be solved and needs further investigations.

A lot of evidence indicate that endocannabinoids are implicated in drug addiction. In the present study, we found that endocannabinoids are not involved in maintenance of cocaine reinforcement and its subjective effects since pharmacological blockade of cannabinoid CB_1 receptors by rimonabant altered neither self-administration nor discriminative stimulus effects of cocaine. On the other hand, withdrawal from repeated access or exposure to cocaine, and then reinstatement of cocaine-seeking behavior or sensitized locomotor response to a single cocaine challenge, respectively, was potently reduced by pretreatment with rimonabant. The latter observations may show that repeated cocaine treatment and the drug withdrawal

produce, apart from behavioral effects, also different neural consequences in the endocannabinoid systems in rats. A possible participation of the endogenous cannabinoid system in the regulation of relapsing phenomena to other drugs of abuse was demonstrated (Tab. 1) [25]. In fact, cannabinoid CB_1 receptor blockade reduces or even prevents the reinstatement of extinguished drug-seeking behavior produced by alcohol, cocaine, opioid, or methamphetamine as well as the expression of cocaine, opioid or THC sensitization. Similarly, when administered following withdrawal from alcohol, cocaine, methamphetamine, nicotine, or opioid self-administration, the cannabinoid CB_1 receptor antagonist rimonabant was effective in reducing drug-seeking behavior induced by re-exposure to drug-associated stimuli. The persistence of the drug-evoked conditioned behavior under cannabinoid CB_1 receptor control is supported by the results of nicotine- or opioid-induced expression of conditioned place preference. Interestingly, mechanisms of maintenance of self-administered alcohol, cocaine or opioids are different in terms of their impact on the endocannabinoid system. In fact, the specific blockade of endocannabinoids at the level of cannabinoid CB_1 receptors seems to be necessary to control the maintenance of alcohol, nicotine, opioid or THC, but not cocaine, reinforcement in self-administration models. In other words, findings from preclinical studies suggest that antagonists of cannabinoid CB_1 receptors that reduce the maintenance of drug self-administration and its relapse may be efficacious as an aid for drug cessation and in the prolongation of abstinence period from nicotine, opioid, THC, while the specific blockade of cannabinoid CB_1 receptors is a new therapeutic option to treat cocaine relapse. In support of preclinical research, rimonabant was shown to block the subjective effects of Δ^9-THC in humans and to prevent relapse to smoking [39].

Tab. 2. Effects of drugs of abuse on cannabinoid CB_1 receptor binding, mRNA levels and contents of endocannabinoids in the rodents' brain

Treatment	CB_1 receptor level		Endocannabinoids content	
	Protein	mRNA	Anandamide	2-Arachidonylglycerol
Cocaine: – repeated	--- [32]	--- [32]	↑ BST, LFB [32] ↓ CC, HIP, STR [32]	↑ BST [32] ↓ CC, HIP, STR [32]
Nicotine: – repeated	--- [32]	↓ CC, HYP [32] --- HIP, LFB [32]	--- BST, CC, CER, HIP, LFB, STR [32]	↓ LFB [32] --- BST, CC, CER, HIP, STR [32]
Alcohol – repeated	↓ CC, CER, HIP, STR [80] --- [32]		↑ CC [80] ↑ LFB [32, 34] ↑ CER [3] ↓ MDB [32, 34]	↑ LFB [34] --- MDB [34]
– deprivation (withdrawal) – relapse	--- [80]		↓ LFB [34] --- CC [80] ↓ MDB [34]	↓ LFB [34] ↓ LFB, MDB [34]
Morphine –- repeated	↑ C-P, NAC, SEP [32] ↓ AMY, CC, HIP, MDB [32, 33]	↓ C-P, CER, SEP [32]	--- [33]	
Δ^9-THC: – repeated	↓ BST, CC, CER, HIP, STR [20] ↓ AMY, CC, CER, C-P, HIP, NAC SEP, SN [66] --- GP, EPDN [66] --- LFB [20]	↑ STR [66] --- CER, HIP [66]	↑ LFB [20] ↓ STR [20] --- BST, CC, CER, HIP [20]	↓ STR [20] --- BST, CC, CER, HIP [20]

Abbreviations: AMY – amygdala, BST – brainstem, C-P – caudate-putamen, CC – cerebral cortex, CER – cerebellum, EPDN – entopeduncular nucleus, GP – globus pallidus, HIP – hippocampus, HYP – hypothalamus, LFB – limbic forebtain, MDB – midbrain, NAC – nucleus accumbens, SEP – septum, SN – substantia nigra, STR – striatum ↑ – increase, ↓ – decrease, --- lack of effect

The observation that repeated treatment with drugs of abuse produced different changes in the level of cannabinoid CB_1 receptor mRNA and protein as well as endocannabinoid transmission partly supports the differences in the effects of rimonabant on behavioral responses elicited by these drugs (Tab. 2). Thus, prolonged administration of alcohol, nicotine and THC (but not cocaine or morphine) induced an increase in anandamide contents in the limbic forebrain. In rat striatum, repeated cocaine led to an increased anandamide formation, while repeated nicotine or THC produced a decrease in anandamide and 2-arachidonylglycerol contents. In addition repeated treatment with various drugs of abuse elicited different alterations in cannabinoid CB_1 receptor binding and mRNA levels; they were limited only to transcript levels in selected brain regions following cocaine, without changes following nicotine, whereas morphine or THC produced bi-directorial and region-dependent effects.

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