INTRODUCTION

Cocaine is one of the most addictive psychostimulants and its abuse has been linked with many psychosocial and psychiatric problems. The pharmacological mechanism of cocaine’s action is directly connected with inhibition of monoamine reuptake transporters leading to augmented synaptic noradrenaline (NA), dopamine (DA) and serotonin (5-HT) (1).

Despite knowledge of the neurobiological actions of cocaine, no effective therapy for cocaine addiction has yet been introduced (2). However, one of the key challenges in discovering novel therapies for psychostimulant addiction will need to focus on anti-relapse treatments.

Some empirical evidence suggests that the brain endocannabinoid (eCB) (e.g. anandamide) may play an important role in cocaine addiction. The eCB act as a retrograde messengers activating CB receptors at the presynaptic membrane and are degraded by enzymatic actions of fatty acid amide hydrolase (FAAH). The present study aimed to examine the effect of the FAAH inhibitors, phenylmethylsulphonyl fluoride (PMSF; i.p.) or cyclohexylcarbamic acid 3-carbamoyl biphenyl-3-yl ester (URB597; i.p.) on the cocaine- or food-maintained self-administration as well as on the cocaine-seeking or food-taking behaviors in rats. Male Wistar rats were implanted with a catheter (iv.) and trained to self-administer cocaine (0.5 mg/kg/infusion) on a fixed ratio 5 schedule of reinforcement with a conditioned stimulus (tone+light). After self-administration stabilized, extinction/reinstatement procedures were carried out during which the rats were tested for the response reinstatement induced by cocaine (10 mg/kg, i.p.) or a cue (light+tone). The food (sweetened milk) self-administration and extinction/reinstatement procedures were conducted in a manner resembling cocaine self-administration. Neither PMSF (30–120 mg/kg) nor URB597 (0.1–3 mg/kg) affected cocaine self-administration. PMSF, 60 mg/kg, significantly reduced cocaine-induced reinstatement and at 120 mg/kg (combined with the challenge dose of cocaine) it evoked behavioral disruption. PMSF (60-120 mg/kg) dose-dependently inhibited cue-induced reinstatement. URB597 (1-3 mg/kg) attenuated both cocaine- and cue-induced drug-seeking behaviors. PMSF (60 mg/kg) decreased food self-administration. Toward reinstatement of food-taking behavior PMSF (60-120 mg/kg) and URB597 (3 mg/kg) showed inhibitory effects. Our results indicate that FAAH inhibitors could be potent modulators of motivational and conditioned aspects of goal-directed behaviors with less prominent effects on consumatory behaviors.

Key words: cocaine self-administration, endocannabinoid system, fatty acid amide hydrolase inhibitors, food self-administration, rats, seeking behaviors
Some recent behavioral findings indicate that CB₁ receptors are not involved in cocaine reinforcing effects in rodents (15-16), but their stimulation seems to potentiate cocaine reinforcement in self-administration procedures (17, but see 18). Furthermore, the role of tonic activation of CB₁ in cocaine seeking behaviors has been demonstrated (19-22).

To further examine the role of eCB system in cocaine rewarding and seeking behaviors, the present investigation evaluated the effects of the FAAH inhibitors phenylethylisulsphonyl fluoride (PMSF) or cyclohexylicarbamic acid 3-carbamoyl biphenyl-3-yl ester (URB597) were assessed on cocaine self-administration and extinction/reinstatement behavior in rats. Moreover, in order to ascertain the specificity of the FAAH inhibitors on cocaine-induced behaviors we evaluated effects of these drugs on food self-administration and on reinstatement of food-taking behaviors. Additionally, we studied the effects of the FAAH inhibitors on basal locomotor activity.

MATERIALS AND METHODS

Animals
Male Wistar rats (Warszawa, Poland) weighing 280-300 g were used in this study. Animals were housed individually (self-administration procedures) or 6-8/cage (locomotor activity studies) in standard rodent cages in a colony room at a room temperature of 20±1°C and at 40-50% humidity under 12-h light-dark cycle (lights on at 06:00). They had free access to water and rodent food (locomotor activities studies), or free access to water and limited access to food (cocaine and food self-administration procedures). In cocaine self-administration procedures the amount of food each animal received was restricted to 20 g after session, while in food self-administration procedures animal received food that given in daily session (sweetened milk) and 20 g rodent chow after session. On the next day, they began lever pressing for cocaine reinforcement during 2-h daily sessions performed 6 days/week. The house light was on throughout each session. Each completion of a FR 5 schedule on the “active” lever resulted in a 5-s injection of cocaine (0.5 mg/kg per infusion) and a 5-s presentation of a stimulus complex, consisting of activation of the white stimulus light directly above the “active” lever and tone from a 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 10 days until subjects met the following criteria: minimum requirement of 24 reinforcements and active lever presses with average of 6 consecutive days and a standard across those 6 days to vary by 10%; this criterion was selected based on our prior experiments (24). After the acquisition criterion (see above) was met, the separate groups of rats (n=6-8 rats/group) were pretreated with either vehicle, PMSF (30-120 mg/kg) or URB597 (0.1-3 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.

Extinction/reinstatement
Following acquisition of cocaine (0.5 mg/kg/infusion) self-administration test naive rats were used for extinction/reinstatement tests. During extinctions sessions subjects had 2-h daily training sessions with no delivery of cocaine or the presentation of the conditioned stimulus complex. Once they reached the extinction criteria (a minimum of 10 extinction days with the responding on the active lever below...
15% of the level observed during maintenance during at least 3 consecutive days), the rats were tested for response reinstatement induced by either a noncontingent presentation of the self-administered reinforcer (10 mg/kg cocaine, i.p.) or discrete contextual cue (tone + light previously paired with cocaine self-administration). During the reinstatement tests (2-h sessions), active lever presses on the FR 5 schedule resulted only in an intravenous injection of saline. The separate groups of rats (n=6-8 rats/group) were pretreated with either vehicle, PMSF (60-120 mg/kg) or URB597 (0.3-3 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 extinction sessions.

Food self-administration procedures

Maintenance

Food self-administration was conducted in a manner parallel to cocaine self-administration. Food-restricted rats (20 g/rat/day) were trained to press the lever in a standard operant chambers (Med-Associates, USA) under a FR 5 schedule of reinforcement in daily 2-h sessions. Each completion of a FR 5 schedule on the "active" lever resulted in a delivery of the portion of sweetened milk (0.1 ml). Following each reward, 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 food delivery. The house light was on throughout each session. Rats remained in maintenance training until lever pressing stabilized (the number of active lever presses varied by 10% or less over the course of 3 consecutive maintenance days). Once stable rates of responding were established, subjects were divided into the separate groups (n=6-7 rats/group) to investigate the effects of the FAAH inhibitors PMSF (30-60 mg/kg) or URB597 (0.3-3 mg/kg) on food self-administration. 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 food self-administration.

Extinction/reinstatement

Following acquisition of food self-administration test naive rats were used for extinction/reinstatement tests. During extinctions sessions subjects had 2-h daily training sessions with no delivery of food. Once they reached the extinction criteria (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 tested for response reinstatement induced by a contingent presentation of food. Each completion of a FR 5 schedule on the active lever resulted in a delivery of a portion of sweetened milk (0.1 ml) in daily 2-h sessions. Following each reward, 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 food delivery. The separate groups of rats (n=6-8 rats/group) were pretreated with either appropriate vehicle, PMSF (60-120 mg/kg) or URB597 (1-3 mg/kg). The order of injections was counterbalanced according to a Latin square design, and test sessions were separated by at least two-three extinction sessions.

Locomotor activity procedures

The locomotor activity of rats was recorded for each animal as described previously by Frankowska et al. (25). Briefly, the locomotor activity was measured in Opto-Varimex cages surrounded with a 15 x 15 array of photocell beams located 3 cm from the floor surface (Columbus Instruments, Columbus, USA). Interruptions of these photobeams resulted in horizontal activity defined as distance traveled and expressed in cm. Animals were placed individually into the locomotor activity cages for 2 h, their locomotion was recorded during 2-h test in 15-min interval sessions and analyzed using Auto-track software (Columbus Instruments, USA). Before locomotor activity was recorded, rats were injected with PMSF (30-120 mg/kg) or URB597 (0.3-3 mg/kg) in their home cages and at the appropriate time were transferred to the experimental cages. The animals were drug- and test-naive, n=6-8 rats/group.

Statistical analyses

In cocaine and food self-administration procedures, the number of responses on the active and inactive lever (including time-out responding) for each group pretreated with the FAAH inhibitors were analyzed by two-way analyses of variance (ANOVA) for repeated measures. Post-hoc Bonferroni test was used to analyze differences between group means.

In locomotor activity studies, data are expressed as the mean horizontal distance traveled in cm (+ S.E.M.) for the 2-h test session. Comparisons between groups were carried out by one-way ANOVA, followed by intergroup comparisons using the Dunnett’s test.

RESULTS

Effects of the FAAH inhibitors on cocaine (0.5 mg/kg/infusion) self-administration

Rats showed stable responding on levers during the last 6 self-administration maintenance sessions with an acquisition criterion requiring that the rate of active lever presses varied by less than 10%. The animals had self-administered 25-33 infusions of cocaine with the daily mean cocaine intake between 12.5-16.5 mg/kg. Rats responded significantly more frequently on the active lever than on the inactive lever (P<0.05), independently of self-administration day.

PMSF (30-120 mg/kg) did not change the number of active and inactive lever presses (F(3,36)=0.31 (Fig. 1), URB597 (0.1-3 mg/kg) failed to alter the number of active and inactive lever presses (F(4,48)=1.49 (Fig. 1).

Effects of the FAAH inhibitors on cocaine- or cue-induced reinstatement of cocaine-seeking behaviour

After 10 days of extinction trials during which active lever presses resulted in the i.v. delivery of saline without the presentation of the conditioned stimulus (cue), the rats were tested for response reinstatement induced by cocaine (10 mg/kg, i.p.) or by the presentation of the cue paired previously with cocaine infusions. During cocaine - or cue- induced reinstatement test, rats responded more often on the active lever in comparison to the inactive lever (P<0.05) and to the extinction (P<0.05). The responses on the inactive lever were not different across the days.

Cocaine-induced reinstatement

The ANOVA indicated a significant PMSF (60 mg/kg) pretreatment × lever responding interaction effect (F(3,54)=7.27, P<0.0003). Post-hoc Bonferroni test revealed that PMSF (60 mg/kg) significantly decreased active lever presses (P<0.001) without effect on inactive lever presses (Fig. 2). At the same
time PMSF at the higher dose of 120 mg/kg (results not showed) combined with cocaine (10 mg/kg) produced behavioral disruption (0 responses emitted on either lever during the 120-min test session).

The ANOVA indicated a significant URB597 (1-3 mg/kg) pretreatment × lever responding interaction effect (F(3,30)=6.77, P<0.001). Post-hoc Bonferroni test revealed that URB597 dose-dependently decreased active lever presses, at doses of 1 and 3 mg/kg a reduction by 48% (P<0.01) and 80% (P<0.001), respectively, was seen. URB597 did not change inactive lever presses (Fig. 2).

Cue-induced reinstatement

The ANOVA indicated a significant PMSF (30-120 mg/kg) pretreatment × lever responding interaction effect (F(3,66)=36.06, P<0.0001). Post-hoc Bonferroni test revealed that PMSF dose-dependently reduced active lever presses, at doses of 60 and 120 mg/kg a reduction by 24% (P<0.01) and 87% (P<0.001), respectively, was found (Fig. 3). PMSF did not alter inactive lever presses (Fig. 3).

The ANOVA indicated a significant URB597 (0.3-3 mg/kg) pretreatment × lever responding interaction effect (F(4,48)=6.77, P<0.0005). Post-hoc Bonferroni test revealed that URB597 dose-dependently decreased active lever presses, at doses of 0.3, 1 and 3 mg/kg a reduction by 30% (P<0.05), 36% (P<0.01) and 60% (P<0.001), respectively, was seen. URB597 did not change inactive lever presses (Fig. 3).

Effects of the FAAH inhibitors on food self-administration

Rats showed stable responding on levers during the last 3 self-administration maintenance sessions with an acquisition criterion requiring that the rate of active lever presses varied by less than 10%. Rats responded significantly more frequently on the active lever than on the inactive lever (P<0.05), independently of food self-administration day.

Pretreatment with PMSF (30-60 mg/kg) caused reduction in the number of lever presses (F(2,30)=6.53, P<0.01). Post-hoc analysis showed that administration of PMSF in a dose of 60 mg/kg significantly (P<0.001) reduced the number of active lever presses (Fig. 4).
URB597 (0.3-3 mg/kg) did not change the number of active and inactive lever presses (F(3,30)=0.30) (Fig. 4).

**Effects of the FAAH inhibitors on reinstatement of food-taking behaviours**

After 10 days of extinction trials during which active lever presses resulted in no food delivery, the rats were tested for response reinstatement induced by food (sweetened milk). During food-induced reinstatement test, rats responded more often on the active lever in comparison to the inactive lever (P<0.05) and to the extinction division (P<0.05). The responses on the inactive lever were not different across the days.

The ANOVA indicated a significant PMSF (60-120 mg/kg) pretreatment × lever responding interaction effect (F(3,24)=44.36, P<0.0001). Post-hoc Bonferroni test revealed that PMSF dose-dependently decreased active lever presses, at doses of 60 and 120 mg/kg a reduction by 19% (P<0.05) and 98% (P<0.001), respectively, was seen. PMSF did not change inactive lever presses (Fig. 5).

The ANOVA indicated a significant URB597 (1-3 mg/kg) pretreatment × lever responding interaction effect (F(3,24)=35.14, P<0.0001). Post-hoc Bonferroni test revealed that URB597 in a dose of 3 mg/kg decreased active lever presses (P<0.001). URB597 did not change inactive lever presses (Fig. 5).

**Locomotor activity**

PMSF (30-120 mg/kg) and URB597 (0.3-3 mg/kg) did not significantly alter the rats basal locomotor activity expressed as a distance traveled in cm F(3,24)=1.76 and F(3,24)=1.67, respectively; (Fig. 6).
DISCUSSION

The present findings demonstrate that presumed eCB activation (by the inhibition of FAAH) does not lead to alterations in the rewarding effects of cocaine, but appears to result in suppression of cocaine-seeking behavior and well as food-taking reinstatement in rats.

In our experiment neither PMSF (30-120 mg/kg) nor URB597 (0.1-3 mg/kg), the FAAH inhibitors, altered cocaine self-administration (i.e. no changes in the number of “active” lever responding and cocaine injections were observed) in rats. Lack of the effects of the FAAH inhibitors in the present study is in line with the findings that the direct pharmacological activation of CB1 receptors by CP55,940 (18), but not by WIN55,212-2 (17) did not alter cocaine intake in a self-administration procedures in rats. Furthermore, an endogenous tonic activation of CB1 receptors is not required for cocaine reinforcement since the CB1 receptor antagonist SR141716A was inactive in cocaine self-administration procedures in rats (20) and mice (16) and is commensurate with the normal cocaine self-administration in mice lacking CB1 receptors vs wild type control (15). Altogether, our present and other above-cited studies emphasize that eCB system does not mediate cocaine-controlled (15). Altogether, our present and other above-cited studies emphasize that eCB system does not mediate cocaine-relapse behavior and reduced food-taking behavior, what implicate that eCBs directly activate presynaptic CB receptors that in turn decrease extrasynaptic levels of both anandamide and 2-AG. These eCBs directly activate presynaptic CB receptors in turn decrease extrasynaptic levels of several neurotransmitters, including glutamate (Glut) (30). The latter neurotransmitter is postulated to play a major role in cocaine-seeking behavior. Thus, an increase in extracellular Glut in the core of the nucleus accumbens - the structure responsible for production of procognitive and effects, emotional state). However, inhibitory responses of either FAAH inhibitors could be potent modulators of motivational and conditioned aspects of goal-directed behaviors with less prominent effects on consumatory behaviors.

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