Behavioural Brain Research 226 (2012) 335–339

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Short communication

Rimonabant attenuates amphetamine sensitisation in a CCK2 receptor-dependent manner

Kertu Rünkorg*, Laura Orav, Sulev Kõks, Toshimitsu Matsui[1], Vallo Volke, Eero Vasar

Department of Physiology, University of Tartu, Ravila 19, Tartu 50411, Estonia

ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 2 September 2011
Accepted 4 September 2011
Available online 12 September 2011

Keywords:
CCK2 receptors
CB1 receptors
Rimonabant
Amphetamine
Motor sensitisation
Knockout mice

ABSTRACT

In this behavioural and pharmacological study in male CCK2 receptor-deficient mice ($CCK2^{-/-}$), we evaluated the role of the interaction of endocannabinoids (eCBs) and cholecystokinin (CCK) on the regulation of anxiety-related and motor behaviours. Repeated treatment with amphetamine (2 mg/kg daily for four days) induced slightly weaker motor sensitisation in $CCK2^{-/-}$ mice compared to their wild-type ($CCK2^{+/+}$) littermates. Co-administration of rimonabant (1 mg/kg) with amphetamine antagonised the development of motor sensitisation in $CCK2^{+/+}$ mice. However, we did not find a similar effect of rimonabant in $CCK2^{-/-}$ mice. We did not find any differences between the behaviour of $CCK2^{+/+}$ and $CCK2^{-/-}$ mice in models designed to assess emotional behaviours (dark/light exploration, marble burying and conditioned place aversion). This study supports the hypothesis that eCBs play a role in the development of amphetamine-induced sensitisation. Moreover, we have demonstrated that intact CCK2 receptors are necessary for the development of eCB-mediated sensitisation to amphetamine.

1. Introduction

Both cholecystokinin (CCK) and endocannabinoids (eCBs) are involved in the modulation of dopamine, serotonin, and opioid systems and play a role in the regulation of feeding behaviour, anxiety-related behaviours, pain, learning, and memory [1,2]. In various forebrain areas (the amygdala, hippocampus, and cerebral cortex), CCK is present in GABA- and glutamatergic neurons containing CB1 receptors [3]. The functional interaction between CCK and eCBs seems in most cases to be antagonistic, and it is possible that eCBs modulate the release of CCK. Indeed, the activation of CB1 receptors has been shown to inhibit potassium-evoked CCK release in the hippocampus [4]. In the present study, CCK2 receptor-deficient mice ($CCK2^{-/-}$) were used to further examine the interaction between CCK and eCBs. Our group has shown previously that $CCK2^{-/-}$ animals have a defect in the eCB-sensitive component of stress-induced analgesia [5]. Therefore, based on those findings, we aimed to determine if other eCBs-mediated effects are perturbed in $CCK2^{-/-}$ mice.

2. Materials and methods

2.1. Animals

The present study was performed in male $CCK2^{-/-}$ mice, with the original background of 129 Sv/C57Bl/6, and their $CCK2^{+/+}$ littermates. The $CCK2^{-/-}$ mice were generated by replacing a part of exon 2 and exons 3, 4 and 5 [6]. Breeding and genotype analysis were performed at the Department of Physiology, University of Tartu, as described in earlier studies [7]. The mice were backcrossed twelve times to the C57Bl/6 background. In total, 78 $CCK2^{-/-}$ and 81 $CCK2^{+/+}$ adult mice (3–5 months old) were used in the behavioural experiments. Mice were kept in the animal house at 20 ± 2 °C under a 12 h light/dark cycle (lights on at 0700). Tap water and food pellets were available ad libitum. Permission (No. 39, 7 October 2005) for the present study was given by the Estonian National Board of Animal Experiments in accordance with the European Communities Directive of 24 November 1986 (86/609/EEC). Mice were brought into the experimental room 1 h before the behavioural test. All experiments were performed between 11:00 and 19:00. The light-dark, motor activity and marble burying tests were performed in the first group of animals, the conditioned place aversion test was performed in the second group of animals, and the amphetamine-induced sensitisation test was performed in the third group of animals. Wild-type mice were always used in parallel with mutant animals, and the experiments were always performed in randomised order.

2.2. Drugs

In the behavioural experiments, the drug solutions were administered intraperitoneally in a volume of 10 ml/kg. Rimonabant (Sanofi-Aventis) was dissolved in vehicle (0.9% sodium chloride, 5% dimethyl sulphoxide, and a few drops of Tween-80). Amphetamine sulphate (Sigma–Aldrich) was dissolved in saline.

2.3. Light-dark test

The light-dark exploratory test was performed in an apparatus that consisted of a smaller, darkly painted and covered (illumination ~2 lux) compartment

* Corresponding author. Tel.: +372 7374338; fax: +372 7374332.
E-mail address: kertu.runkorg@ut.ee (K. Rünkorg).
[1] Division of Hematology/Oncology, Department of Medicine, Kobe University School of Medicine, Kobe 650-0017, Japan.

0166-4328/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2011.09.005

(20 cm × 15 cm × 20 cm) and a brightly illuminated (~700 lux) compartment (20 cm × 30 cm × 20 cm) that was not covered. These two compartments were connected by a 7.5 cm × 7.5 cm opening in the wall. Animals were treated with vehicle or rimonabant (1 or 3 mg/kg) and placed back into their home cage. In the saline-treated group, there were 12 CCK2$^{+/+}$ and 11 CCK2$^{-/-}$ mice; in the group treated with 1 mg/kg rimonabant, there were 8 CCK2$^{+/+}$ and 8 CCK2$^{-/-}$ mice; in the group treated with 3 mg/kg rimonabant, there were 4 CCK2$^{+/+}$ and 4 CCK2$^{-/-}$ mice. After 15 min, the animals were placed in the dark compartment of the apparatus. The number of transitions and time spent in the light compartment were recorded during a 5 min observation period. An increase in the time spent in the light compartment of the test box, as well as an increase in the number of transitions between the compartments, without an increase in motor activity, are considered to reflect reduced anxiety in mice.

2.4. Motor activity test

Immediately following the light-dark test, motor activity was measured using automated photoelectric motility boxes (44.8 cm × 44.8 cm × 45 cm) (MOTI, Technical & Scientific Equipment GMBH, Germany). The illumination level in the motility boxes was ~400 lux. The apparatus-naive mice were placed individually in the chamber, and vertical and horizontal activity was registered during a 10 min observation period.

2.5. Marble-burying test

One week following the locomotor activity test, the marble-burying test was performed with twenty-four glass marbles (1.5 cm in diameter) placed on fresh sawdust bedding (the thickness of the bedding was approximately 5 cm) along the perimeter of a transparent plastic box (44 cm × 22 cm × 20 cm). The illumination level of the test boxes was ~200 lux. Animals were treated with vehicle or rimonabant (1 or 3 mg/kg) and placed in their home cage for 15 min before the test. The mice were then placed in the test box individually for 30 min. After 10, 20 and 30 min the number of marbles covered in bedding up to at least two-thirds of their diameter was counted. In saline-treated group, there were 12 CCK2$^{+/+}$ and 11 CCK2$^{-/-}$ mice; in the group treated with 1 mg/kg rimonabant, there were 8 CCK2$^{+/+}$ and 8 CCK2$^{-/-}$ mice; in the group treated with 3 mg/kg rimonabant, there were 4 CCK2$^{+/+}$ and 4 CCK2$^{-/-}$ mice.

2.6. Conditioned place aversion test

The place aversion conditioning experiment with rimonabant was performed in shuttle boxes according to the method described previously [8]. The illumination level in the experimental room was ~200 lux. The conditioning period consisted of a 30 min experimental session performed once a day for six consecutive days (days 3–8). During the conditioning sessions, the door between the pale and dark green compartment of the shuttle box was closed, and injections of rimonabant were paired with the dark green side of the shuttle box, which was clearly preferred by the animals in the pre-conditioning sessions (days 1–2). Injections were given immediately before the beginning of the conditioning sessions. For the conditioning of place aversion, rimonabant-treated animals received a saline injection before being placed into the pale green compartment on the first day, and they were injected with rimonabant (1 or 3 mg/kg) and placed into the dark green compartment on the second day. The control group animals received an injection of vehicle before being placed into both compartments. A 15 min post-conditioning test was performed on day 9, and time spent in the pale green side of the shuttle box, which the animals avoided in the pre-conditioning sessions, was recorded. In each treatment group, there were 8 CCK2$^{+/+}$ and 8 CCK2$^{-/-}$ mice.

2.7. Behavioural sensitisation test

The behavioural sensitisation experiment was performed in automated photoelectric motility boxes (44.8 cm × 44.8 cm × 45 cm). The illumination level of the transparent test boxes was ~400 lux. All animals were pre-adapted to the motility boxes for three 15 min sessions on three consecutive days preceding the sensitisation procedure. Animals from both genotypes were randomly divided into four treatment groups. Animals were administered vehicle or rimonabant (1 mg/kg) and then placed into their home cages. Thirty minutes later, animals were administered saline or amphetamine (2 mg/kg) and placed into the motility box; motor activity was recorded for the subsequent 30 min. All animals were treated for four consecutive days. The motor activity of mice on the first and fourth day of the experiment was compared to evaluate motor sensitisation to amphetamine (2 mg/kg). After 21 days of withdrawal, all mice were treated with amphetamine (2 mg/kg), and motor activity was measured for the subsequent 30 min. Eight CCK2$^{+/+}$ and 8 CCK2$^{-/-}$ mice were administered vehicle and saline, vehicle and amphetamine (2 mg/kg), and rimonabant (1 mg/kg) and amphetamine (2 mg/kg); 9 CCK2$^{+/+}$ and 7 CCK2$^{-/-}$ mice were adminstered rimonabant (1 mg/kg) and saline.

2.8. Statistical analysis

The results are expressed as the mean values ± SEM and were analysed using multivariate analysis of variance (MANOVA) of repeated measures. Post hoc comparisons between the means of individual groups were performed using the Newman–Keuls procedure with Statistica for Windows software.

3. Results

3.1. Light-dark test

In the light-dark exploration test, male CCK2$^{-/-}$ mice did not display increased exploratory behaviour compared to their CCK2$^{+/+}$ littermates. We did not find any differences between these two genotypes in the number of transitions between the compartments or in the time spent in the light compartment (Fig. 1A). Pre-treatment with rimonabant (1 or 3 mg/kg) did not change the exploratory activity of CCK2$^{-/-}$ or CCK2$^{+/+}$ mice.

3.2. Locomotor activity test

The administration of rimonabant (1 or 3 mg/kg) did not cause any marked changes in the locomotor activity in CCK2$^{-/-}$ or CCK2$^{+/+}$ mice, and there was no difference due to genotype (Fig. 1B).

3.3. Marble-burying test

CCK2 receptors deficiency and treatment with rimonabant (1 or 3 mg/kg) did not affect marble-burying behaviour (Fig. 1C).

3.4. Conditioned place aversion test

In the conditioned place aversion test, repeated treatment with rimonabant (1 or 3 mg/kg) did not cause aversion to the drug-paired environment in CCK2$^{-/-}$ and CCK2$^{+/+}$ mice (Fig. 1D).

3.5. Behavioural sensitisation test

The ANOVA results are given in the legend of Fig. 2. In the behavioural sensitisation test, the first administration of amphetamine (2 mg/kg) suppressed motor activity in CCK2$^{-/-}$ mice but not in CCK2$^{+/+}$ mice (Fig. 2). Four subsequent amphetamine treatments induced significant motor sensitisation in both CCK2$^{-/-}$ and CCK2$^{+/+}$ mice. However, in CCK2$^{-/-}$ mice receiving amphetamine, the motor activity measured on day 4 was less pronounced. Nevertheless, the effect of amphetamine was significantly stronger on the fourth day in these mice when compared to their first treatment with amphetamine ($p = 0.0001$) and treatment with saline ($p = 0.0129$) on the fourth day in CCK2$^{-/-}$ mice (Fig. 2). Sensitisation to amphetamine was confirmed by injecting amphetamine 21 days after the last experiment. Both CCK2$^{-/-}$ and CCK2$^{+/+}$ animals that had received 4 days of amphetamine treatment displayed significant motor sensitisation compared to their first treatment session and compared to the animals that were acutely treated with amphetamin after having previously received saline. Similar to day 4, the amphetamine-induced motor effect was weaker in CCK2$^{-/-}$ mice (Fig. 2). Pre-treatment with the CB1 receptor antagonist rimonabant at a dose of 1 mg/kg did not affect locomotion when given alone but inhibited the effect of amphetamine. Co-administration of rimonabant and amphetamine for 4 days completely blocked the stimulant effect of the dopamine agonist (Fig. 2). When amphetamine was injected 21 days later, CCK2$^{+/+}$ animals that were previously administered rimonabant and amphetamine did display motor sensitisation, but this sensitisation was significantly less pronounced than in the group receiving vehicle and amphetamine. Remarkably, in the case of