Interactions between serotonin 5-HT3 and cannabinoid CB-1 receptor function in the control of anxiety in mice

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Background

❖ The serotonergic and cannabinoid neurotransmitter systems are considered to play significant roles in the control of anxiety-related behaviours.

❖ Both cannabinoid CB1 receptors and serotonin 5-HT3 receptors are expressed in cholecystokinin-containing GABAergic interneurons in cortical regions innervating pyramidal cells, creating a potentially important regulatory system of mood and affective states. While postsynaptic 5-HT3 receptors mediate the input from serotonergic raphe afferents in these interneurons, presynaptic CB1 cannabinoid receptors are involved in the control of inhibitory input to pyramidal cells.

❖ It has been hypothesized that the two neurotransmitter systems converging on this interneuron population may interact in the regulation of anxiety (Figure 1).

Aims

The aim of the present study was to investigate the interaction between 5-HT3- and CB1 receptor-mediated effects on anxiety. We studied the effects of the selective 5-HT3 agonist m-chlorophenylbiguanide (mCPBG) on anxiety and locomotion in wild-type and cannabinoid CB1 receptor knock-out mice.

Methods

Animals and housing

NMRI mice were obtained from Charles-River Laboratories (Budapest, Hungary).
The CB1-knock-out and wild-type mice derived from a genotyped stock obtained from IRIBHN, Universite libre de Bruxelles (Ledent et al., 1999), and were bred in the Institute of Experimental Medicine. The subjects of the present experiments were obtained from heterozygous parents of the 5th generation. Each offspring of this parent stock was genotyped by real-time PCR. Only homozygotes were studied (wild-types and CB1-knock-outs).
Two-month-old, male mice weighing approximately 35 g were used in all experiments. Food and water were freely available. Experiments were conducted in the light phase of the day. To avoid confounds from social status, subjects were kept in individual cages for 2 weeks prior to experimentation. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine.

Drug administration

The selective 5-HT3 agonist m-chlorophenylbiguanide hydrochloride (mCPBG, Tocris) was dissolved in saline and injected intraperitoneally 30 min before testing.

Elevated plus-maze test

The elvated plus-maze comprised two opposite open arms (30x7 cm) and two enclosed arms (30x7x30 cm) connected by a central area (7x7 cm); the platfrom height was 70 cm. Animals were placed on the central area facing an open arm and allowed to explore the maze for 5 min. Behaviour was recorded by a video camera and analysed by a computer based event-recorder (H77). Closed arm entries were considered indicators of locomotor activity whereas open arm exploration was used as a measure of anxiety. Open arm exploration was characterized by two variables: percentage time spent in the open arm, and percentage open arm entries (100 x open arm entries/total arm entries). Sample size was 10 per group in the experiment using NMRI mice and 18-20 per group in experiments using CB1 knock-out and wild-type animals.

Open field test

The open field was a plastic box of 40x40x30 (height) cm. Mice were placed near the wall of the box and allowed to explore the apparatus for 10 min. Locomotion was assessed by counting the crossing of the lines of a 4x4 grid that divided the open field into 16 small squares (each square was 10x10 cm). Anxiety in this test was measures as percentage time spent in the central area (20x20 cm) of the box. Sample size was 8 per group.

Statistical analysis

Data are expressed as means+SE. Changes in behaviour were assessed by Kruskal-Wallis ANOVA and post-hoc Mann-Whitney U-tests where appropriate.

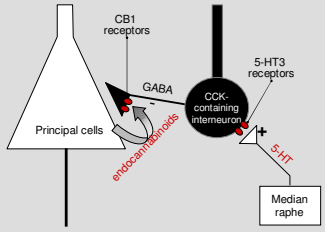

Figure 1. Brain mechanism underlying the hypothesis that the effects of 5-HT3 ligands on anxiety depend on endocannabinoid signaling mediated by CB1 receptors.

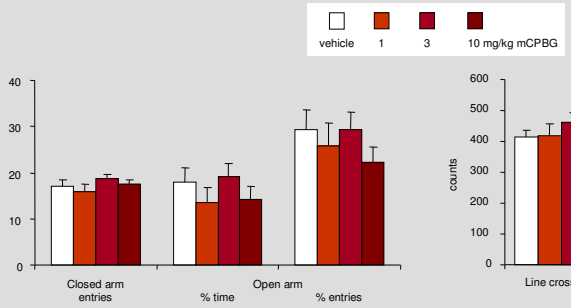

Figure 2. The effects of mCPBG on behaviour of NMRI mice in the elevated plus-maze test. mCPMG treatment did not induce any significant changes in locomotion and anxiety.

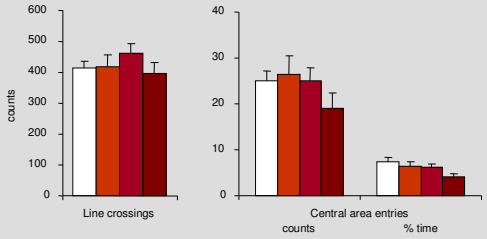

Figure 3. The effects of mCPBG on behaviour of NMRI mice in the open field test. mCPMG treatment did not induce any significant changes in locomotion and anxiety.

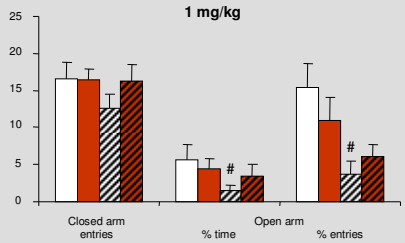

Figure 4. The effects of 1 mg/kg mCPBG on behaviour of CB1-receptor knock-out and wild-type mice in the elevated plus-maze test. Knock-out mice were significantly more anxious than wild-type mice. mCPBG treatment at this dose did not induce any significant changes in locomotion and anxiety. #, significantly different from wild-type controls. #p<0.01

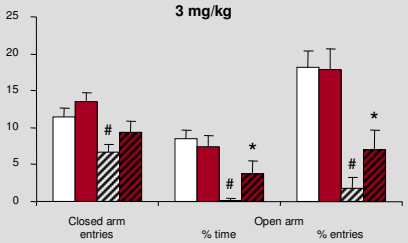

Figure 5. The effects of 3 mg/kg mCPBG on behaviour of CB1-receptor knock-out and wild-type mice in the elevated plus-maze test. Vehicle-injected knock-out mice showed significantly more anxiety and less locomotion than wild-type mice. 3 mg/kg mCPBG induced anxiolysis in knock-out but not in wild-type mice, without affecting locomotion. #: significantly different from wild-type controls, #p<0.01. *: significantly different from vehicle-treated knock-out, *p<0.03.

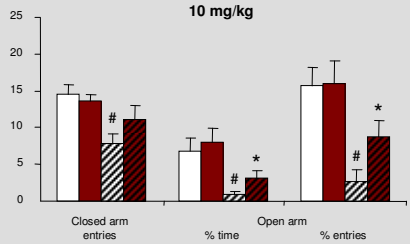

Figure 6. The effects of 10 mg/kg mCPBG on behaviour of CB1-receptor knock-out and wild-type mice in the elevated plus-maze test. Vehicle-injected knock-out mice showed significantly more anxiety and less locomotion than wild-type mice. 10 mh/kg mCPBG induced anxiolysis in knock-out but not in wild-type mice, without affecting locomotion. #: significantly different from wild-type controls, #p<0.02. *: significantly different from vehicle-treated knock-out, *p<0.02.

Summary

❖ The 5-HT3 receptor agonist mCPBG did not influence any parameter of anxiety at any dose in NMRI mice tested on the elevated plus-maze.

❖ mCPBG did not influence locomotion or anxiety in NMRI mice in the open field test.

❖ Cannabinoid CB1 receptor knock-out mice showed significantly increased anxiety compared to wild-type mice in the elevated plus-maze test.

❖ mCPBG did not infleunce anxiety in wild-type mice but induced a dose-dependent anxiolysis in CB1 knock-out animals.

Conclusions

❖ Our data suggest that the 5-HT3 agonist mCPBG per se does not influence anxiety in the elevated plus-maze test, confirming earlier data with different anxiety tests

❖ Genetic disruption of CB1 receptors results in a significant increase in anxiety that can be counteracted by the administration of mCBPG, suggesting that CB1 receptors may be involved in controlling 5-HT3-mediated effects on anxiety

❖ Understanding this complex mechanism may open up new and effective treatment strategies in emotion-related disorders.

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The authors declare that there is no conflict of interest.