Interactions between serotonin 5-HT3 and cannabinoid CB1 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 behaviors.
- 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 to 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 (Sulzfeld, Germany). The CB1 knockout and wild-type mice derived from a genetically modified stock obtained from IRIBHN, Université libre de Bruxelles (Ledent et al., 2001). The CB1 null mutation was generated in vivo by targeted deletion of the CB1 receptor gene and was confirmed by genotyping. Male mice were used in all experiments. Each group consisted of 10 mice. All experiments were conducted in the light phase of the day. All experiments and animal care 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.

Elevated plus-maze test
The elevated plus-maze test consisted of four open arms (60x30 cm) and two enclosed arms (60x30 cm) separated by a central area (20x20 cm). Anxiety was induced by placing the mice at the edge of the central area and recording the time spent in the enclosed arms versus the open arms. The central area was considered an additive measure of anxiety.

Open field test
The open field was a plastic box of 40x40x40 cm 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 number of line crossings (each square was 10x10 cm). Anxiety in this test was measured as percentage total time spent in the central area (20x20 cm) of the box. Sample size was 20 per group.

Statistical analysis
Data are expressed as mean±SEM. Changes in behavior were assessed by Kruskal-Wallis ANOVA and post-hoc Mann-Whitney U tests where appropriate.

Results

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. mCPBG 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. mCPBG treatment did not induce any significant changes in locomotion and anxiety.

Figure 4. The effects of 1 mg/kg mCPBG on behavior 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.

Figure 5. The effects of 3 mg/kg mCPBG on behavior 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 anxiety in knock-out but not in wild-type mice, without affecting locomotion. # significantly different from wild-type control, *p<0.01.

Figure 6. The effects of 10 mg/kg mCPBG on behavior 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 mg/kg mCPBG induced anxiety in knock-out but not in wild-type mice, without affecting locomotion. * significantly different from wild-type control, #p<0.01.

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 influence 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 mCPBG, 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.