The role of the cannabinoid CB2 receptor in depression-like behaviours

The purpose of this study was to examine the role of the cannabinoid CB2 receptor in the regulation of depressive-like behaviours and in the actions of antidepressant drugs such as selective serotonin-reuptake inhibitors. To this aim, transgenic mice overexpressing the cannabinoid CB2 receptor (CB2xP: developed in our laboratory) were challenged against different types of experimental paradigms to evaluate its response to depression-like behaviours.

**Materials:** The role of the cannabinoid CB2 receptor in depression-like behaviours (tail-suspension test, novelty suppressed feeding test) was examined in CB2xP and WT mice. The CB2xP and WT mice were exposed to the chronic mild stress (CMS) regime for 8 weeks. The CMS procedure consisted of a variety of unpredictable mild stressors including periods of food deprivation (12h), noise (2h), restraint stress (1h), intermittent lighting (24h), continuous light (36h), and unpredictable chronic mild stress (UCMS). The CMS stressed and non-stressed groups were evaluated. The sucrose intake was measured at the end of the CMS.

In addition, the behavioral effects of the antidepressant fluoxetine (10 mg/kg or 30 mg/kg) to study its antidepressant effects in mice exposed to food deprivation. The time of latency to initiate consumption and the total amount of consumption is significantly higher in CB2xP compared to WT mice in the novelty-suppressed feeding test (p<0.01). Fluoxetine decreased the time of latency to initiate consumption and the total amount of consumption in WT mice (p<0.05, One Way ANOVA followed by Student Newman Keul`s test) but did not change in CB2xP mice.

**MATERIALS AND METHODS**

**Animals**

Male Swiss Transgenic Albino ICR mice that overexpress the cannabinoid CB2 receptor (developed in our laboratory) and Swiss Albino ICR mice (age 2-3 months, 25-35 g) obtained from Harlan (Barcelona, Spain) were used for all behavioral experiments.

**Drugs**

Fluoxetine was dissolved in water and administered orally at the appropriated dose (5 mg/kg or 10 mg/kg) to study its antidepressant effects in mice exposed to chronic mild stress.

**Behavioral analyses**

- **Tail Suspension Test:** Mice were suspended, 30 cm above the floor, on the edge of a lever above the table top by using adhesive tape placed approximately 1 cm from the tip of the tail. The time of immobility was measured during a period of 6 min.

- **Novelty-Suppressed Feeding Test:** Mice tested for a period of 24 hours were exposed to food deprivation. The time of latency to initiate consumption and the total amount of consumption were measured for a period of 10 min.

- **Unpredictable Chronic Mild Stress:** This test was developed by Paul Wither, the effects of unpredictable mild stressors (food deprivation (14 h), noise (2h), restraint stress (1h), intermittent lighting (24h), continuous light (36h), and unpredictable chronic mild stress (UCMS)) on the depressive-like index were assessed. At different time points we evaluated light-dark box test, elevated plus maze test, tail-suspension test, and the antidepressant fluoxetine were challenged in a bottle containing sucrose 1%. Stressed mice are expected to show a decreased consumption of sucrose.

- **Sucrose Intake:** Mice were given a free choice between two bottles, one with 1% sucrose solution and another with water, during 24 hours. The sucrose intake was expressed in relation to the animal’s body weight (mg/kg).

- **Light-Dark box:** This test uses the rodent natural aversion to bright areas compared with darker areas. In a two-compartment box, rodents will prefer dark areas, whereas anxiety should increase the time spent in the light compartment. The box is divided into two equal opaque compartments, one transparent and one black and opaque separated by an opaque barrier (15 cm). Mice were introduced into the box from a 30 cm height and tested for 15 min. The light box provides intense illumination. Mice were individually tested in 5 min sessions. At the beginning of each session, mice were placed in the tunnel facing the dark box. The time spent by mice in the light area was recorded for a period of 15 min. A mouse whose four paws were in the dark box was considered as having changed boxes.

- **Elevated Plus Maze:** The elevated plus maze consisted of two open arms and two enclosed horizontal perpendicular arms 50 cm above the floor. The portion of four arms formed a central square platform (5X5 cm). The test began with the animal being placed in the centre of the apparatus facing one of the enclosed arms and allowing the mouse to choose between the open and the closed arms. Each mouse was allowed to explore the maze for 5 min.

**Statistical Analyses**

Statistical analyses were performed using the Student t-test when comparing two groups. ANOVA followed by Student Newman Keul test when comparing three or four groups. Differences were considered significant if the probability of error was less than 0.05. Data are presented as mean ± S.E.M.

**CONCLUSIONS**

- The results revealed that overexpression of cannabinoid CB2 receptors induced a phenotype resistant to depression-like behaviour and a lack of antidepressant activity after the administration of fluoxetine.

- These results point out the cannabinoid CB2 receptor as a potential target in the treatment of depression-related disorders.