Purpose of the study: The distribution of CB2r in brain areas of the rat involved in the response to stress and anxiety points the relevance of this receptor in the regulation of emotional behavior. Recent studies revealed that interoceptive/ventromedial administration of an antiepileptic molecule sequence directed against CB2 receptor modulated anxiety-like behaviors in mice. In addition, an association between CB2 cannabinoid receptor (CB2r) was also detected in Japanese depressed submucosal (2). The purpose of this study was to evaluate the effects of CB2r on anxiety-like behaviors and in the anxiolytic action of benzodiazepines. To this end, transgenic mice overexpressing CB2r (CB2xP) were challenged against different behavioral paradigms to evaluate its response to anxiety and stress.

Methods: Behavioral responses of CB2xP mice and its corresponding wild type littermates (WT) were evaluated in the open field (OF), light-dark box test (LDB) and the elevated plus maze (EPM) test. Furthermore, the effect of restraint stress was evaluated by measuring corticosterone-releasing factor (CRF) and prolactin-releasing factor (PONC) gene expressions in the paraventricular nucleus (PVN) and anterior hypothalamus. In addition, the anxiolytic effects of alprazolam (5 or 7mg/kg, i.p.) were evaluated in CB2xP and WT mice in LDB test. GABA and GABA receptor subunits gene expressions were measured in hippocampus and amygdala of CB2xP and WT mice by real time PCR.

Results of results: In the OF test, no differences were found between CB2xP and WT mice in the total distance travelled (Student t-test, p>0.05). In the light-dark box test, CB2xP mice spent significantly more time in the bright box (Student t-test, p<0.05) and in the open arms (Student t-test, p<0.05) than WT mice, respectively. Furthermore, restraint stress markedly increased CRF mRNA (25% in CB2xP mice and 55% in WT mice) and PONC mRNA (22% in CB2xP mice and 21% in WT mice). In contrast, restraint stress failed to produce any alteration in CB2 mRNA in PVN nucleus and in PONC mRNA in anterior hypothalamus of control mice (25% in CB2xP mice and 10% in WT mice), respectively. Furthermore, the evaluation of the anxiolytic-like effects of alprazolam (45 or 70mg/kg, i.p.) in the LDB significantly increased the time spent in the light box in WT mice (one way ANOVA followed by Student Newman-Keul’s test, p<0.05). Interestingly, the administration of alprazolam was without effects in CB2xP mice (one way ANOVA followed by Student Newman-Keul’s test, p>0.05).

Conclusions: The results obtained in this study provide evidence to support the relevance of CB2r in the regulation of anxiety-like behaviors and in the anxiolytic action of benzodiazepines. The findings suggest that CB2r could be a potential target for the development of new anxiolytic drugs.

Title: Overexpression of CB2r results in decreased response to acute anxiogenic-like stimuli and impaired anxiolytic action of alprazolam

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Materials and Methods

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

Doses: Alprazolam was dissolved in water and administered i.p. at the desired concentrations (45 or 70mg/Kg) or its saline vehicle (0.3ml/mice, i.p.) 30 minutes before the exposure to light-dark box test. Time in the light box and number of transitions in each test were evaluated for a period of 5 minutes. Columns represent the mean ± S.E.M. *Values from alprazolam-treated mice that are significantly different (One Way ANOVA, followed by Student Newman-Keul’s test, p<0.05) from the control group.

Figure 1: Water activity of CB2xP mice in open field test. Total distance covered (cm), central distance covered (cm), central speed (cm/s) and the time spent (s) in the centre were evaluated during a period of 30 minutes. Columns represent the mean ± S.E.M. *Values from alprazolam-treated mice that are significantly different (Student Newman-Keul’s test, p<0.05) from control.

Figure 2: Assessment of anxiety-like behaviors on elevated plus maze test. The time in the light box and the number of transitions were evaluated for a period of 5 minutes in control and restraint-stressed CB2xP mice. Columns represent the mean ± S.E.M. *Values from restraint-stressed mice that are significantly different (Student Newman-Keul’s test, p<0.05) from non-stressed mice.

Table 1: GABAα2 and GABAα5 gene expressions in hippocampus and amygdala of CB2xP and WT mice. Gene expressions of both GABAα2 and GABAα5 were measured in hippocampus and amygdala of CB2xP and WT mice by real time PCR.

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