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Gender differences in the anxiolytic action of benzodiazepines: role of CB1 and $GABA_A$ receptors

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Cannabinoid receptors play an important role in the regulation of anxiety. A large body of evidence suggests a differential response to anxiety between males and females that may depend on the presence of gonadal steroid hormones. Indeed, alterations in levels of testosterone, estradiol and/or endogenous cannabinoid CB1 receptor function may affect the anxiolytic actions of benzodiazepines.

Previously (Urigüen L et al, Neuropharmacology 46:966-973, 2004), we reported that CB1 deletion in male mice resulted in pronounced alterations in the hypothalamic-pituitary-adrenal axis in the response to stress and that cannabinoid receptors are necessary for bromazepam to achieve complete anxiolytic action.

In this context, the aim of this study was to examine the role of gonadal hormones in anxiety regulation, in the presence or absence of CB1 receptors. To this purpose, anxiety-like behaviour of intact and 2-week gonadectomized male and female cannabinoid knockout (CB1ko) and wild-type (WT) mice was compared. The anxiolytic efficacy of bromazepam as well as the expression of $GABA_A$ receptor subunits were also evaluated in these mice.

Anxiety was determined by using the light-dark box test (Urigüen L et al, Neuropharmacology 46:966-973, 2004). Ovariectomy in females and orchidectomy in males were conducted as described previously elsewhere (Stoffel EC et al, Pain 103:285-302, 2003). QRT-PCR was performed using TaqMan™ assays as described earlier (Urigüen L et al, Transl Psychiatry 3, e221, 2013).

Gonadectomy significantly decreased the percentage of time spent in the light box in both CB1ko (n=6) (Δ= -61.44%; SEM=13.40; $p<0.05$, Student´s *t*-test) and WT male mice (n=7) (Δ= -58%; SEM=8.88; $p<0.05$, Student´s *t*-test) compared to their respective intact mice. No differences were found in the anxiety response between gonadectomized and intact mutant and WT female mice. To explore whether the high states of anxiety induced by deletion of CB1 receptors in males or removal of testosterone may affect the anxiolytic action of benzodiazepines, the effects of bromazepam (50 µg/kg, p.o; 30 min) were examined in the light-dark box. Bromazepam was innefective in both CB1ko males and in 2-week gonadectomized WT males.

Under basal conditions, CB1ko males exhibited low basal corticosterone plasma concentrations. When males were submitted to 10 minutes of restraint stress a hypersensitive response was detected in mutants compared to WT mice. However, this did not occur in females, since no differences were found in plasmatic corticosterone levels between WT and CB1ko mice submitted to restraint stress.

Quantitative RT-PCR assays revealed that males, but not females, exhibited an increased expression of $GABA_A\gamma2$ (n=5) (Δ= +50.69%; SEM=9.2; $p<0.05$, Student´s *t*-test)

and decreased $GABA_A\beta2$ (n=5) (Δ= -30 %; SEM=4; $p<0.05$, Student´s *t*-test) in the paraventricular nucleus.

These findings revealed gender differences in the emotional responses between CB1ko and WT mice. In males, deletion of CB1 receptors and/or removal of testosterone, produced a high level of anxiety that bromazepam (50 µg/kg) was unable to reduce. These results suggest that endogenous cannabinoid receptor function together with the levels of circulating testosterone and the regulation of $GABA_A$ receptor subunits in males, play a key role in the anxiolytic actions of benzodiazepines.