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The role of endocannabinoids in the regulation of luteinizing hormone and prolactin release. Differences between the effects of AEA and 2AG

Key words: endocannabinoids, prolactin, luteinizing hormone, CB₁ KO mouse

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Summary

It has been shown that the endocannabinoids inhibit luteinizing hormone (LH) and prolactin (PRL) secretion. When the effects of the two well known endocannabinoids arachidonylethanolamide (AEA, anandamide) and 2-arachidonoyl-glycerol (2AG) have been compared it became evident that AEA caused inhibition was higher than that one of 2AG. AEA diminished the two investigated hormonal levels in CB₁ receptor inactivated mice, too. AEA, being an endogenous ligand of vanilloid type 1 (TRPV1) receptor, while activating TRPV1 receptor has an effect on both LH and PRL levels decrease because these later were abolished when capsazepin, antagonist of TRPV1 receptor was previously administered to the CB₁ KO animals. We postulate that the difference between the effects of AEA and 2AG on the serum levels of LH and PRL is due to the difference in receptor activation of these two compounds, namely AEA can activate both CB₁ and TRPV1 receptor but 2AG acts only on CB₁ receptor.

Introduction

The neuroendocrine homeostasis is regulated by the hypothalamo-pituitary-endocrine gland axis. Different factors (releasing or inhibiting) produced by the hypothalamus influence the anterior pituitary hormone secretion, including cells involved in reproduction. Of particular interest is the effect of cannabinoids on reproductive regulatory mechanisms because usually young people start to use cannabinoids (marihuana) while in puberty, during which the neuroendocrine regulation of reproduction is very vulnerable.

In the 70's and 80's a great number of studies dealt with the effects of tetrahydrocannabinol (THC), the main psychoactive component of marihuana, on reproduction (Wenger et al., 1992). It was postulated that THC acted primarily through central neuroendocrine mechanisms.

Since the early studies (Marks, 1973) number of studies reported the inhibitory effects of THC on pituitary luteinizing hormone (LH) secretion (Wenger et al., 1987, Maccarrone

and Wenger, 2005). THC also suppressed the tonic circulating level in male rats (Chakravarty et al., 1975) and the episodic LH secretion in female animals (Tyrey, 1980). In laboratory animals THC inhibited the prolactin (PRL) secretion as well (Murphy, 2004). Anandamide (AEA) has similar effects on both LH and PRL secretion. AEA temporarily decreases serum LH level, and this effect lasts up to 2-3 h (Gonzalez et al., 2000). PRL levels can also be decreased by endocannabinoid treatments (Wenger et al., 1999).

The presence of endocannabinoids has been shown in the hypothalamus (Herkenham, 1995) and in the anterior pituitary (Gonzalez et al., 1999). The central cannabinoid receptor (CB₁ receptor) is also present in these structures (Moldrich and Wenger, 2000). The hypothalamus contains fewer cannabinoid binding sites than other areas of the CNS. Nevertheless, the effects caused by the activation of CB₁ receptors in the hypothalamus may be important because the receptors are more or less concentrated within specific hypothalamic nuclei-areas (Fig. 1). CB₁ receptors in the hypothalamus seem to be activated by endogenous cannabinoids present in hypothalamus because after hypothalamic deafferentation the number of binding sites did not vary (Romero et al., 1998).

It became evident that AEA effects on the control of the regulation of reproduction are mediated by CB₁ receptors located in the hypothalamus and anterior pituitary (Fernandez-Ruiz et al., 1997, Romero et al., 1998). The central cannabinoid receptor inactivation suppresses gonadotropic functions (Wenger et al., 2001). It has been demonstrated that AEA changes dopaminergic turnover, thus altering inhibitory dopaminergic effects on PRL secretion (Scorticati et al., 2003). The question arises that is there a dual mechanism at both pituitary and hypothalamic levels which acts on the regulation of hormone secretion. It has been demonstrated that AEA inhibits pituitary gonadotropic secretion in vitro, too (Wenger et al., 2000). According to the above mentioned data we concluded that endocannabinoids by both, central and pituitary mechanisms have to be taken into consideration when looking at the regulatory effects of endo- (and exo-) cannabinoids on reproductive functions (Maccarone and Wenger, 2005).

In search for endogenous ligand of transient receptor potential vanilloid type 1 (TRPV1 receptor) it has been suggested that AEA may be one of such compound (Di Marzo et al., 2002). Indeed, it is known that AEA can bind and activate both CB₁ and TRPV1 receptors and AEA could trigger vanilloid-receptor mediated effects (Ross, 2003, Di Marzo et al., 2005). The potency of AEA for inducing TRPV1- mediated effects is much less than the average potency at CB₁ receptors.

TRPV1 receptors are present in hypothalamic regulatory centers (Mezey et al., 2000). The question arises does the TRPV1 receptor activation influence reproductive functions? In 2000 it was described (Gonzalez et al., 2000) that AEA and 2AG levels did not change at the same manner when different endocrine parameters were measured (e.g. in estrous cycle or endocannabinoid contents in male vs. female).

In this pilot study we have observed if the changes of plasma PRL concentrations differ after AEA or 2AG administration. The aim of our further experiences was to evaluate the possible role of TRPV1 in the regulation of LH and PRL release.

Materials and methods.

Male, adult homozygote CB₁^{+/+} (wild type) and CB₁^{-/-} (mutant) mice were used. The mutant animals were generated as previously described (Ledent et al., 1999). The CB₁ gene was cloned from a 129/SV mouse genomic library. The housing, experimental procedures and immunohistochemical methods were performed as described before (Moldrich and Wenger, 2000, Wenger et al., 2001, Wenger et al., 2003). All housing and experiment were conducted according to European standard rules (directive 86/609/EEC). Statistical analysis was performed using one-way ANOVA and Tukey's post-hoc tests (StatSoft Statistica 6.0).

Result and Discussion

As it has been mentioned above, endocannabinoids decrease serum LH and PRL levels in both male and female rats. Recently we have found that in CB₁ receptor inactivated mouse (CB₁ KO) AEA has a tendency to decrease LH level unlike 2AG (Fig. 2.B). The same has been obtained when PRL serum levels were measured although the diminution was more pronounced in this later (Fig. 3.B). In wild type animals both endocannabinoids significantly decrease the serum hormonal levels with a higher degree as far as the AEA concerns (Fig. 2.A and 3.A).

There are differences between AEA and 2-AG while acting on cannabinoid receptors since AEA was reported to be a weaker agonist for CB₁ receptors and not to significantly binds to CB₂ receptors (Mechoulam and Hanus, 2000, Sugiura et al., 2006).

TRPV1-expressing neurons were observed in the hypothalamus (Mezey et al. 2000), this should also be considered as a mechanism to explain the differences between the effects of AEA and 2AG. We investigated AEA effects when TRPV1 channels were blocked. We have administered CB₁ receptor antagonist, rimonabant and inhibited TRPV1 receptors with capsazepine, the selective TRPV1 receptor inhibitor (Szallasi and Blumberg, 1999) simultaneously to see if the activation of the vanilloid receptor is responsible for such an effect. Fig. 2.A. and 3.A show that rimonabant reverses the effects of both AEA and 2AG on LH and PRL secretion in different manner. The effect of 2AG is fully antagonized while that of AEA only partially. If capsazepine and rimonabant are applied together (Fig. 2.C and 3.C) no effects are recorded.

When the same experiments have been repeated on CB₁ KO mice, only AEA decreased both LH and PRL (with a lower degree of this later) (Figs. 2.D. and 3.D.) which indicates that the effect is not only CB₁ receptor mediated. Indeed capsazepine antagonises AEA produced activation on PRL and LH secretion, meaning that AEA acts on TRPV1 receptors, as it has been postulated by several authors, (Pertwee, 2005), and as such TRPV1 activation may modulate gonadotropin secretion although with a less extent than CB₁ receptors.

According to literary data and our experiences we may conclude that the action of endocannabinoids on reproductive regulatory mechanisms occurs through both pituitary and hypothalamic sites. According to our knowledge no TRPV1 receptors are present in the anterior pituitary thus the effects of AEA on TRPV1 are localized only in hypothalamus unlike that of 2-AG.

This also happens in the case of endocannabinoid ligands. In some pathological conditions, it has been suggested that 2-AG is the true, if not only the endogenous agonist for CB₂ receptors (Eljaschewitsch et al., 2006, Sugiura et al., 2006).

It is concluded that both AEA and 2AG play a role in the neuroendocrine regulatory mechanism of gonadotropin secretion. The endocannabinoids inhibit LH and PRL release and/or secretion through the activation of CB₁ receptor. AEA caused higher decrease of both investigated hormone levels than 2AG. Because AEA has a modulator effect on hormone secretion in CB₁ KO animals, too it is postulated that TRPV1 receptor, of which AEA is an endogenous ligand, is also involved in the reproductive regulatory mechanisms.

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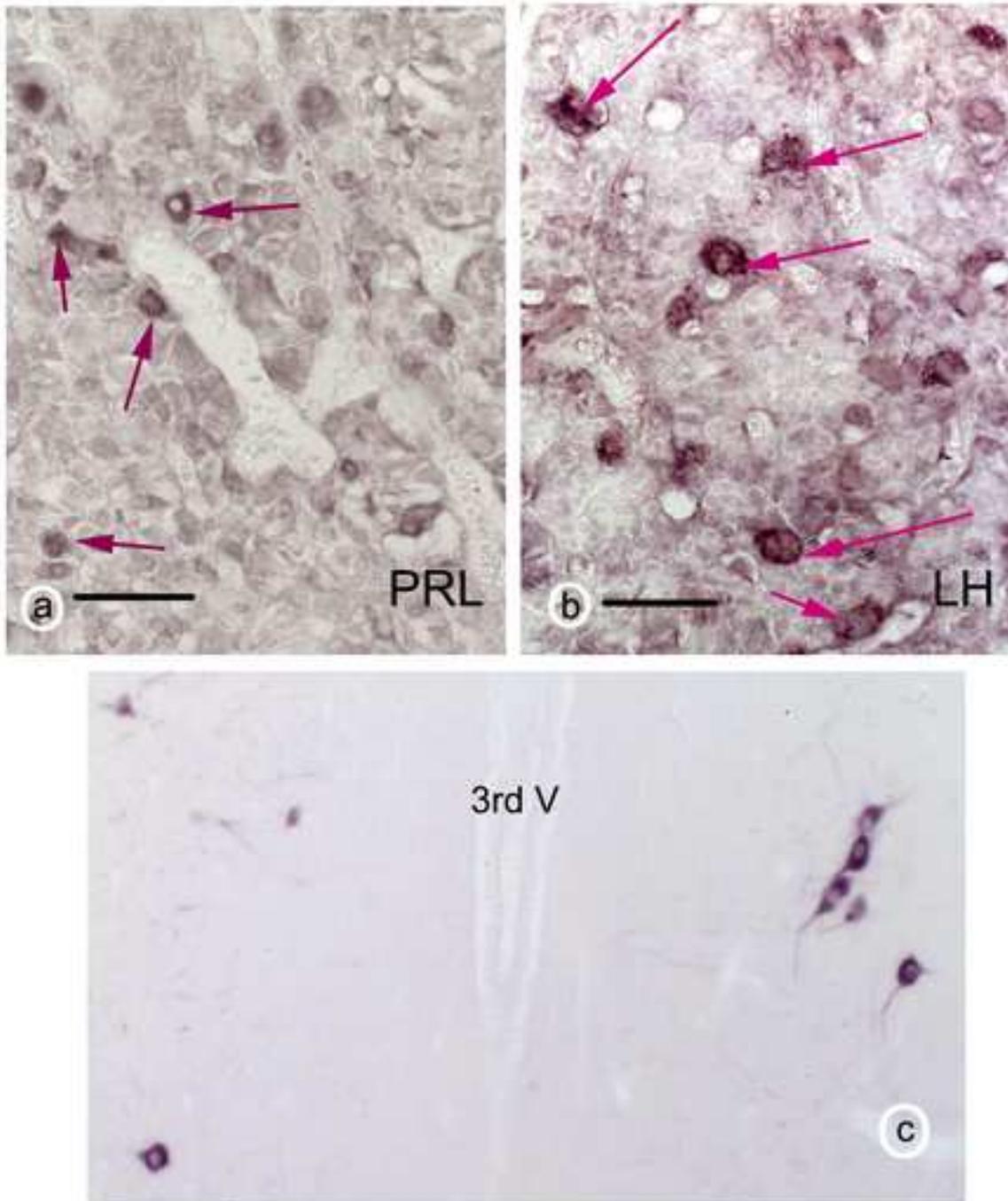
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Figure legend

Figure 1: CB₁ receptor expression by immunohistochemistry in wild-type (WT) mouse: in pituitary gonadotroph (A), in pituitary lactotroph (B) cells and in the hypothalamus (C). The arrows in figures A and B show those cells which expressed LH and PRL positivity on consecutive slides. Scale bars: 20 μm

Figure 2: Effect of AEA, 2AG and RIM on serum LH concentration in WT (A) and CB₁ knock-out (B) mice. Pre-treatment with Capsazepine (Caz) and/or with RIM is followed by cannabinoid agonists treatment on WT (C) and on CB₁ knock-out (D) mice. Each value represents the mean±S.E.M. at least of 6 animals. *= $p < 0,05$ **= $p < 0,001$ compared with control values.

Figure 3: Effect of AEA, 2AG and RIM on serum PRL concentration in WT (A) and CB₁ knock-out (B) mice. Pre-treatment with Capsazepine (Caz) and/or with RIM is followed by cannabinoid agonists treatment on WT (C) and on CB₁ knock-out (D) mice. Each value represents the mean±S.E.M. at least of 6 animals. *= $p < 0,05$ **= $p < 0,001$ compared with control values.



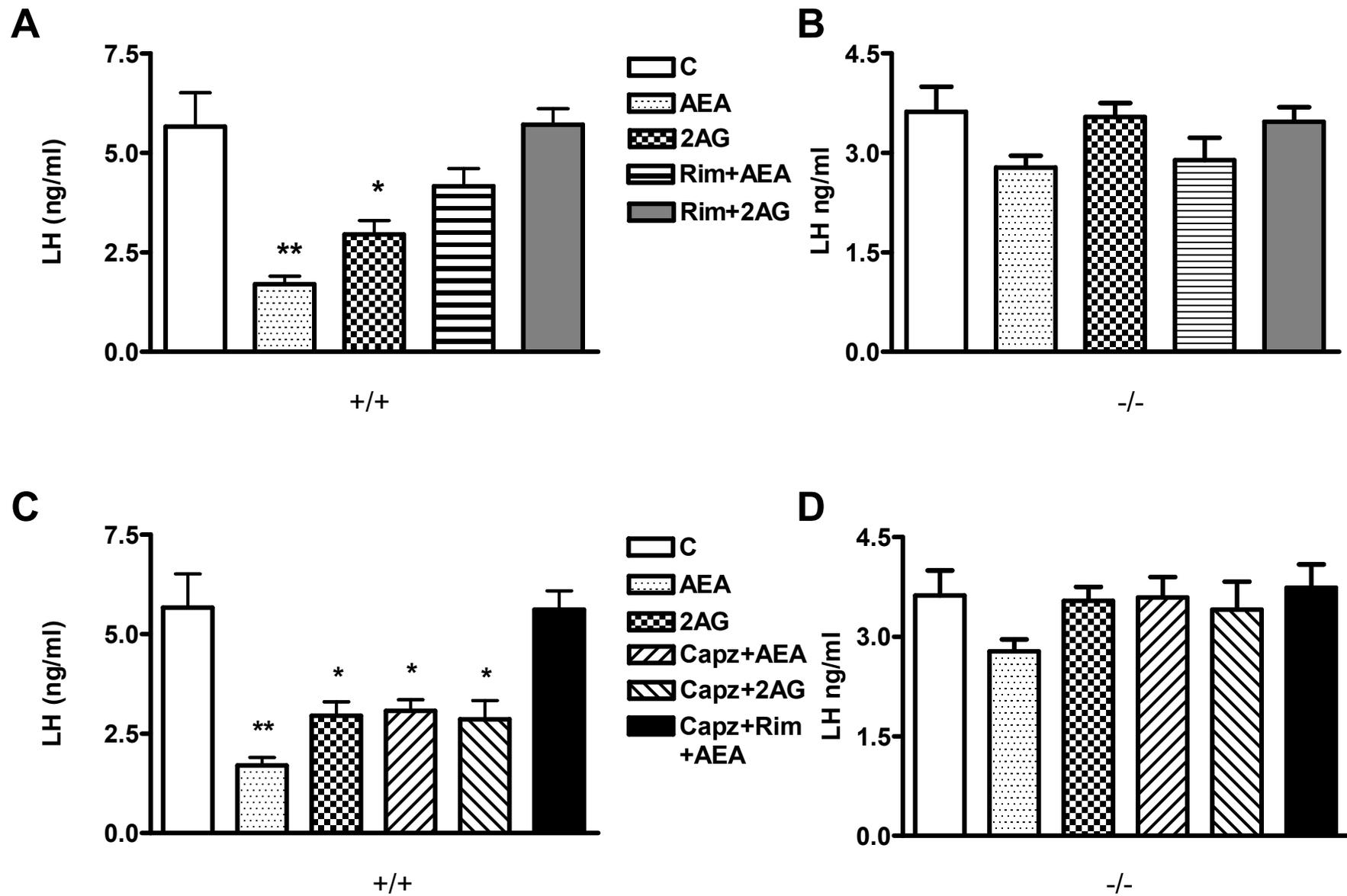


Figure 2.

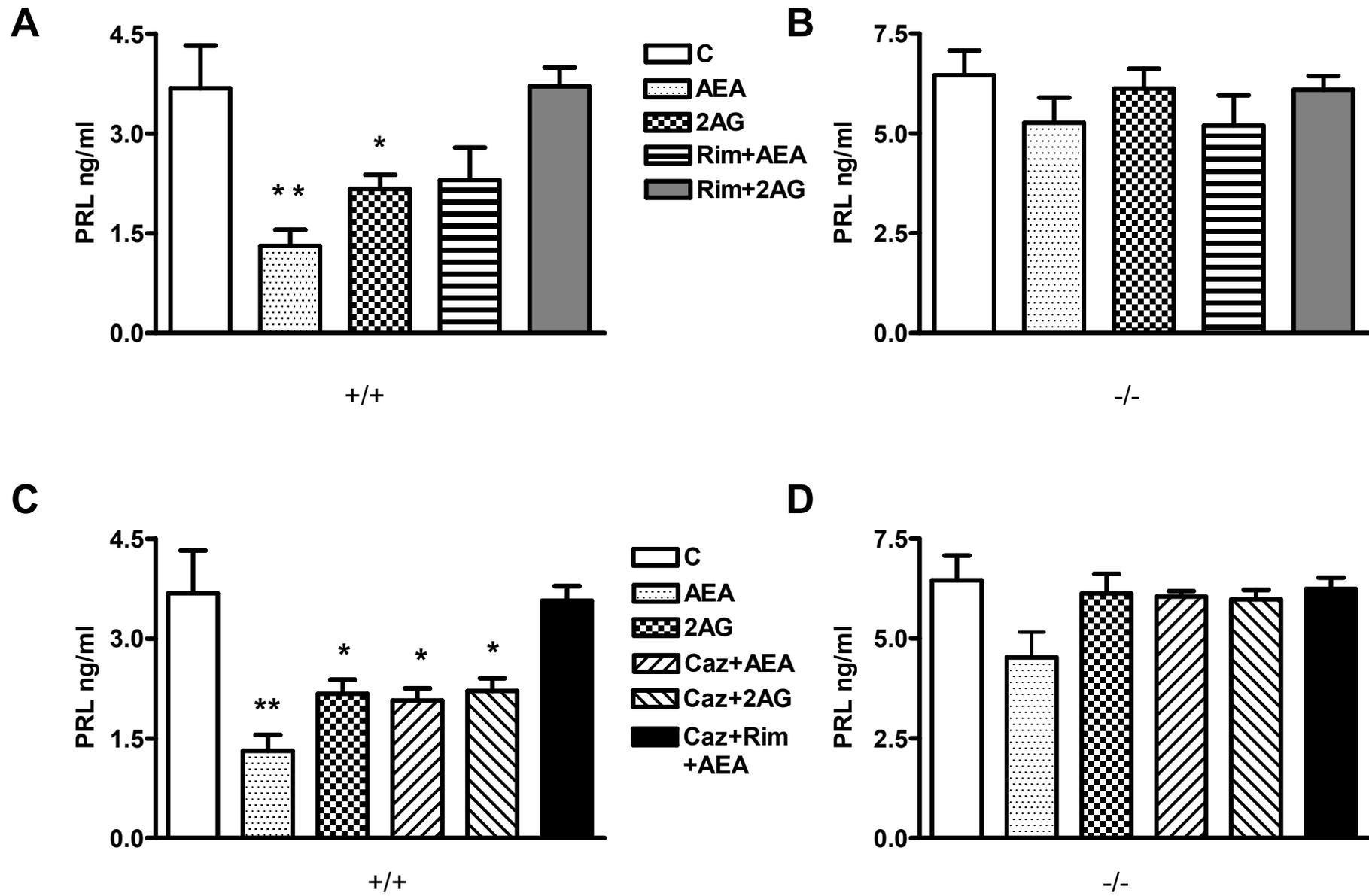


Figure 3.