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The Mutual Modulation of Endocannabinoid and Kisspeptin Systems in Rat Testis

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Abstract

Background: The endocannabinoid system (ECS) and the Kisspeptin system (KS) play crucial roles in the central and peripheral regulation of male reproduction. The KS comprises Kisspeptins, the cleavage product of the Kiss1 protein, and its receptor Kiss1R; it is a critical central regulatory factor of the Gonadotropin Releasing Hormone (GnRH), but its role in the testis in sustaining spermatogenesis is not fully understood. Similarly, in addition to the brain, the ECS is widely expressed in the testis, where it regulates spermatogenesis, steroidogenesis, and the production of high-quality gametes. Since the possible crosstalk between KS and ECS at the gonadal level is poorly understood, this study investigates the possible mutual modulation between ECS and KS in rat testis. Methods: Experiment 1: Testis pieces collected from adult rats were treated ex vivo for 1 h with the endocannabinoid anandamide (AEA, 10^{-8} M) ± SR141716A (10^{-7} M, a cannabinoid receptor (CB) 1 antagonist), or with SR141716A alone. Experiment 2: Testis pieces were treated for 4 h with decreasing doses of Kisspeptin-10 (Kp10, 10^{-6}–10^{-9} M) ± Kp234 (a Kiss1R antagonist). Proteins extracted from the treated tissues were analyzed by Western blot for Kiss1R, Kiss1, CB1, CB2, AEA-hydrolyzing enzyme Fatty Acid Amide Hydrolase (FAAH), and AEA-biosynthetic enzyme *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) proteins. Results: AEA treatment, via CB1, reduced Kiss1R protein in testis. Kp10 treatment increased the expression of CBs and NAPE-PLD at all doses and increased FAAH at 10^{-9} M dose only. Pre-incubation with Kp234 abolished Kp10 effects on CB1, NAPE-PLD, and FAAH, suggesting a direct Kp10-dependent modulation; on the other hand, pre-incubation with Kp234 did not abolish Kp10's effects on CB2, suggesting an indirect action of Kp10 on CB2. Conclusions: Mutual modulation between ECS and KS exists in the testis: AEA, via CB1, suppresses Kisspeptin signaling, while Kisspeptin regulates the ECS through both Kiss1R-dependent and independent mechanisms. These local interactions identify new potential mechanisms in the intratesticular communications sustaining spermatogenesis via ECS and suggest that KS might be a new therapeutic target to rescue ECS impairment in male reproductive dysfunction.

Keywords: Kiss1; Kiss1R; CB1; CB2; FAAH; endocannabinoid system; spermatogenesis; testis

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1. Introduction

Spermatogenesis is a highly complex process requiring coordination among mitotic division, meiosis events, cell death and differentiation; it is finely modulated by endocrine, paracrine and autocrine communication to achieve the formation of high-quality gametes [1,2]. In recent decades, a sustained increase in male infertility among reproductive-aged men has been documented worldwide [3,4]. Environmental factors (e.g., unhealthy lifestyles, smoking, cannabis use, environmental pollutants and endocrine-disrupting chemicals) also contribute significantly to the threat, alongside genetic factors and pathological conditions such as obesity, hypogonadotropic hypogonadism, endocrine disorders, reproductive system infections, etc. [4–6]. Hence, the preservation of spermatogenesis and male fertility requires detailed knowledge of the molecular mechanisms and interplay regulating the hypothalamic–pituitary–gonadal (HPG) axis. In this respect, endocannabinoids and Kisspeptins are recognized modulators of the HPG in both sexes [7,8].

Endocannabinoids are lipid mediators capable of binding cannabinoid receptors (i.e., type 1 and type 2 cannabinoid receptors (CB1 and CB2 respectively), the ion channel transient receptor potential vanilloid 1 (TRPV1), and non-CB1 and non-CB2 receptors [9,10]. Anandamide (*N*-arachidonoylethanolamide, AEA) and arachidonoylglycerol (2-AG) are the main endocannabinoids in biological systems [9,10]. Endocannabinoid tone is governed by a combination of biosynthetic, inactivation and transport pathways. In this respect, *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and diacylglycerol lipase α and β (DAGLα and DAGLβ) are involved in the biosynthesis of AEA, and 2-AG respectively; the fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)/FAAH are critical to the hydrolysis of AEA and 2-AG, respectively; transporters like the endocannabinoid membrane transporter (EMT) and the fatty acid binding protein (FABP) act as intracellular carriers of AEA [9,10]. Ligands, receptors, biosynthetic/hydrolyzing enzymes, and even transporters, together form the endocannabinoid system (ECS) [9].

The Kisspeptin system (KS) comprises Kisspeptins and their receptor, originally known as Gpr54 (G protein-coupled receptor 54). This membrane receptor was initially identified as an orphan receptor (a receptor whose natural ligand was unknown) and was later found to be activated by Kisspeptins. Following this discovery, Gpr54 was renamed Kisspeptin receptor 1 (Kiss1R) [11]. Kisspeptins (e.g., Kp10, 13, 14 and 54) are produced by the cleavage of the Kiss1 precursor [12,13]. Through the activation of Kiss1R, they have acknowledged roles in the modulation of the HPG axis and sex steroid feedback [14]. Most Kisspeptin activity is primarily conducted within the hypothalamus, where distinct Kiss1 neuronal populations have been identified in the arcuate nucleus and the anteroperiventicular nucleus [7]. Kisspeptins stimulate the release of Gonadotropin-Releasing Hormone (GnRH), driving gonadotropin release and sex steroid production [15]. Consistently, loss and gain of function mutation in *KISS1/KISS1R* genes cause hypogonadotropic hypogonadism or precocious puberty, respectively [16–20].

Similarly, endocannabinoid signaling is also critical for the release of hypothalamic GnRH in the portal circulation, with opposite effects compared to KS [21]. Endocannabinoids reduce GnRH release [22] and GnRH neuron firing rate [10] ex vivo and in vivo via CB1, acting as retrograde signals that reduce GABAergic synaptic transmission to GnRH-secreting neurons. Consistently, the impairment in endocannabinoid signaling causes a reduction in *GnRH* mRNA in *CB1*$^{-/-}$ knockout animals [23].

In addition to the hypothalamus, CBs and KISS1R are widely distributed in testis and reproductive tissues [24,25]. Nevertheless, while endocannabinoid signaling, via different receptors, has a recognized role in spermatogenesis progression [25,26], Leydig cells' ontology [27] and sex steroid production [28], Sertoli cells' nursing activity [29], blood–testis

barrier remodeling [30], spermiogenesis, sperm maturation and sperm physiology [31,32], the real need for intratesticular kisspeptin signaling is still debated [33,34]. Nevertheless, KS functions related to sex steroid production, Leydig cell activity, spermatogenesis progression and sperm physiology have been reported in the testis of non-mammalian vertebrates [35–37]. Lastly, involvement in spermatogenesis progression [38,39], Leydig cells cytoarchitecture [40], but debated role on sex steroid production [33], have been reported in mammals.

Recent findings revealed that ECS and KS are interrelated because they both participate in the neuroendocrine control of reproduction, with the ECS acting as a modulatory pathway of kisspeptin–GnRH signaling [39]. Furthermore, mutual interplay between the two systems has been reported in the testis of adolescent rats following in vivo treatment with AEA or Kp10 [39], suggesting hypothalamus-mediated effects. In fact, while the endocannabinoid AEA administered in vivo reduced Kiss1 and Kiss1R proteins within the hypothalamus and testis respectively, the in vivo administration of Kp10 (the shorter active peptide cleaved by the Kiss1 precursor [41]) increased protein expression of both CB1 and FAAH in the testis [39]. Since previous observations came from in vivo treatments only, data on the direct intratesticular crosstalk between the two systems in mammals is still being obtained. In the present work, through the ex vivo treatments of rat testis pieces with AEA and Kp10, alone or in combination with specific antagonists, we investigated the direct intratesticular mutual regulation between the ECS and KS, providing evidence of KS modulation via CB1 and direct and indirect effects of Kp10 on the ECS.

2. Materials and Methods

2.1. Chemicals

Metastin 45–54 of human origin (Kp10, h-YNWNSFGLRF-NH_2) and Kisspeptin-234 trifluoroacetate salt (Kp234), a specific kisspeptin antagonist [42], were dissolved in saline; endocannabinoid *N*-Arachidonylethanolamine (Anandamide, AEA, sc-396321A) and SR141716A (rimonabant, 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-piperidino-1H-pyrazole-3-carboxamide, sc-205491A), a selective CB1 receptor antagonist [43], were dissolved in ethanol. All the aforementioned chemicals were obtained from SantaCruz, Biotechnology Inc., Dallas, TX, USA.

2.2. Ex Vivo Treatment

2.2.1. Animals

Adult Wistar male rats (n = 6, 90 post-natal days, pnd, *Rattus norvegicus*) (Harlan Laboratories, S.r.l., San Pietro al Natisone, Udine, Italy] were sacrificed by decapitation following deep anesthesia with intra-peritoneal injection of Urethane (2 g/kg bw, Merk Life Science S.r.l. Milano, Italy). After sacrifice, testes were removed, decapsulated, rinsed in PBS, cut into small pieces (70–100 mg), and used for ex vivo incubations. Treatments were carried out in triplicate/animal at 35 °C in Dulbecco's Modified Eagle Medium (DMEM, ThermoFisher Scientific, Waltham, MA, USA). In general, for each animal, one testis was used for control and treatment with agonists; the controlateral testis was used for the combined treatments.

2.2.2. Experiment 1: AEA and SR Treatments

Testis pieces (n = 6 animals/treatment) were incubated for 1 h in DMEM (Control, C), or DMEM + AEA 10^{-8} M, DMEM + SR141716A 10^{-7} M, or DMEM + SR141716A 10^{-7} M+ AEA 10^{-8} M, using doses previously reported to be effective [22,44]. In the combined treatment, testes were preliminarily incubated in DMEM+ SR141716A 10^{-7} M for 30′; then

AEA was added to the incubation medium. Testes were then stored at −80 °C and used in Western blot.

2.2.3. Experiment 2: Dose–Response Experiment with Kp10 ± Kp234

Testis pieces (n = 6 animals/treatment) were incubated with DMEM alone (control, C) or increasing doses of Kp10 (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) ± 10-fold higher Kp234 (10^{-8}, 10^{-7}, 10^{6}, 10^{-5} M) for 4 h. The range of Kp-10 doses and the incubation time were chosen on the basis of dose–response experiments previously carried out in mammals [40,45,46]. In the combined treatment, testes were preliminarily incubated in DMEM + Kp234 (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) for 30 min, and then Kp10 was added in the incubation medium. Testes were then stored at −80 °C and used for Western blot.

2.3. Total Protein Extraction and Western Blot

Tissues from the ex vivo experiments were processed for the extraction of total proteins by the RIPA Lysis Buffer System (SantaCruz, Biotechnology Inc., Dallas, TX, USA, sc-24948) supplied with a protease inhibitors cocktail (10 µL/mL RIPA), 200 mM phenylmethylsulfonyl fluoride (PMSF, 10 µL/mL RIPA), and 100 mM sodium orthovanadate (10 µL/mL RIPA), following the manufacturer's instructions. Cleared protein extracts were collected by centrifugation at 11,000× g for 30 min at 4 °C and quantified by Lowry assay [47].

Total proteins (40 µg) were resolved for SDS-PADE and processed for Western blot, as previously reported [39]. Details on primary and secondary antisera are summarized in Table 1. Signals were detected by Western blotting Luminol Reagent (SantaCruz, Biotechnology Inc, Dallas, TX, USA, sc-2048) following the purchaser's protocol.

Table 1. Primary and secondary antisera used for Western blot.

Antisera	Working Dilution	Predicted Size (KDa)
Anti-CB1 (C-11) mouse monoclonal IgG (sc-518035, Santa Cruz Biotechnology Inc., Dallas, TX, USA)	1:350	~54
Anti-CB2 (3C7) mouse monoclonal IgG (sc-293188, Santa Cruz Biotechnology Inc., Dallas, TX, USA)	1:3000	~45
Anti-FAAH rabbit polyclonal IgG (no. 101600, Cayman Chemical, Ann Arbor, MI, USA)	1:500	~63
Anti-Kiss1 (24-Q) mouse monoclonal IgG_2a (sc-101246, Santa Cruz Biotechnology Inc., Dallas, TX, USA)	1:350	~18
Anti-Kiss1R rabbit polyclonal IgG (E-AB-64300, Elabscience, Huston, TX, USA)	1:700	~43
Anti-NAPE-PLD (E-8) mouse monoclonal IgG_{1K} (sc-514372, Santa Cruz Biotechnology Inc., Dallas, TX, USA)	1:500	~46
Anti-α-Tubulin Monoclonal IgG (E-AB-20036 Elabscience, Huston, TX, USA)	1:5000	~52
HRP-conjugated Goat Anti-Mouse IgG (GtxMU-003-DHRPX, ImmunoReagents, Inc., Raleigh, NC, USA)	1:2000	-
HRP-conjugated Goat Anti-Rabbit IgG (GtxRb-003-DHRPX, ImmunoReagents, Inc., Raleigh, NC, USA)	1:2000	-
m-IgGk BP-HRP (sc-516102, Santa Cruz Biotechnology Inc. Dallas, TX, USA)	1:1000	-

For the quantification of the Western blot signals, membranes were stripped at RT for 30′ in stripping buffer (Santa Cruz Biotechnology Inc, Dallas, TX, USA, sc-281698), and then probed with the mouse monoclonal anti-α-tubulin. Autoradiography films were scanned; signals were plotted and quantified with ImageJ software (version 1.54p; National Institutes of Health, Bethesda, MD, USA). Western blot data are reported as specific protein Optic Density (O.D.)/housekeeping O.D. ± S.D. (n = 4 at least).

2.4. Statistics

Normality of data was verified by the Shapiro–Wilk test; homogeneity of variance was verified by the Levene's test. One-way ANOVA, followed by Duncan's test for multi-group comparison, was carried out to demonstrate significant differences (at $p < 0.05$) in protein expression between groups.

3. Results

3.1. Ex Vivo Effects of AEA on the KS

Direct or hypothalamus-mediated effects on the physiology of testis were evaluated by ex vivo incubations of testis pieces with AEA, alone or in combination with the CB1 antagonist SR141716A. Then, Kiss1R and Kiss1 were analyzed by Western blot (Figure 1A). AEA treatment significantly reduced Kiss1R protein (Figure 1B) but had no effect on Kiss1 (Figure 1C). The combined treatment with AEA and SR141716A fully reversed the AEA-dependent effects on Kiss1R. Since SR141716A may also function as a low-potency mixed agonist/antagonist of TRPV1, testis pieces were also incubated with SR141716A (10^{-7} M) alone; this treatment did not exert any effect on Kiss1R and Kiss1 proteins, thus confirming the involvement of the CB1 receptor.

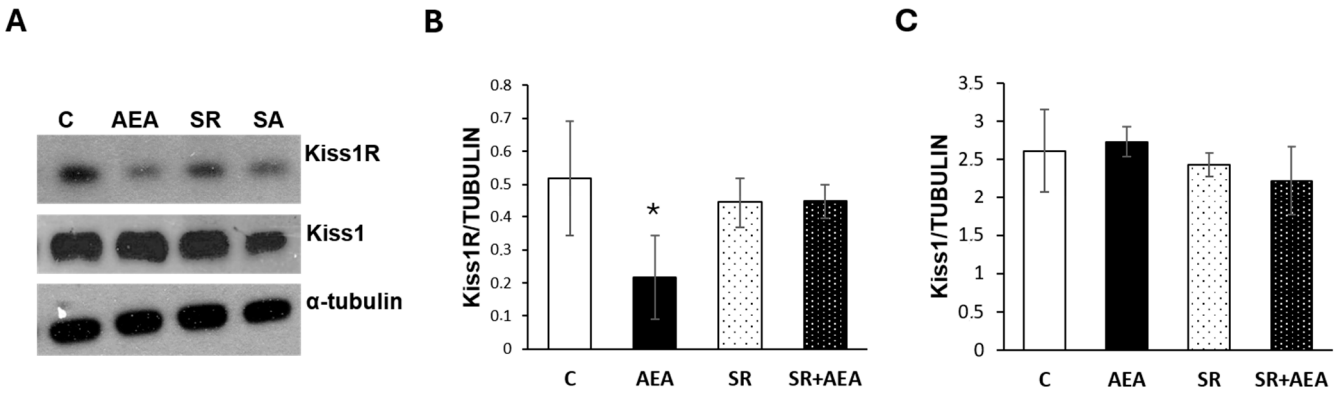

Figure 1. Modulation of the KS by AEA. Representative Western blots for Kiss1R and Kiss1 (**A**) and normalization of Kiss1R (**B**) and Kiss1 signals (**C**) carried out against α-tubulin. Data are representative of n = 4 different animals and are expressed as protein O.D./tubulin O.D. ± standard deviation. C, control group; AEA, testis pieces incubated with anandamide (AEA) 10^{-8} M; SR, testis pieces incubated with SR141716A 10^{-7} M; SR + AEA, testis pieces pre-incubated with SR141716A 10^{-7} M for 1 h and then incubated with anandamide (AEA) 10^{-8} M and SR141716A 10^{-7} M; Kiss1R, Kisspeptin receptor; Kiss1, Kisspeptin precursor; *, $p < 0.05$ at least.

3.2. Dose–Response Effects of Kp10 on the ECS

Dose–response experiments with increasing doses of Kp10 ± Kp234 were carried out to assess the direct effects of Kp10 on CB1, CB2, FAAH and NAPE-PLD.

At all the tested doses, Kp10 significantly increased both CB1 and CB2 proteins compared to control ($p < 0.05$, at least) (Figure 2A–C). Dose–response effects were reported for CB1 (10^{-6} M vs. 10^{-9} M, $p < 0.05$) (Figure 2B), but not for CB2 (Figure 2B). Pre-incubation of testis pieces with the Kiss1R antagonist Kp234 reverted all Kp10 effects on CB1 protein

(Figure 2D), but not on CB2 (Figure 2E) suggesting direct modulation on CB1 and indirect modulation on CB2.

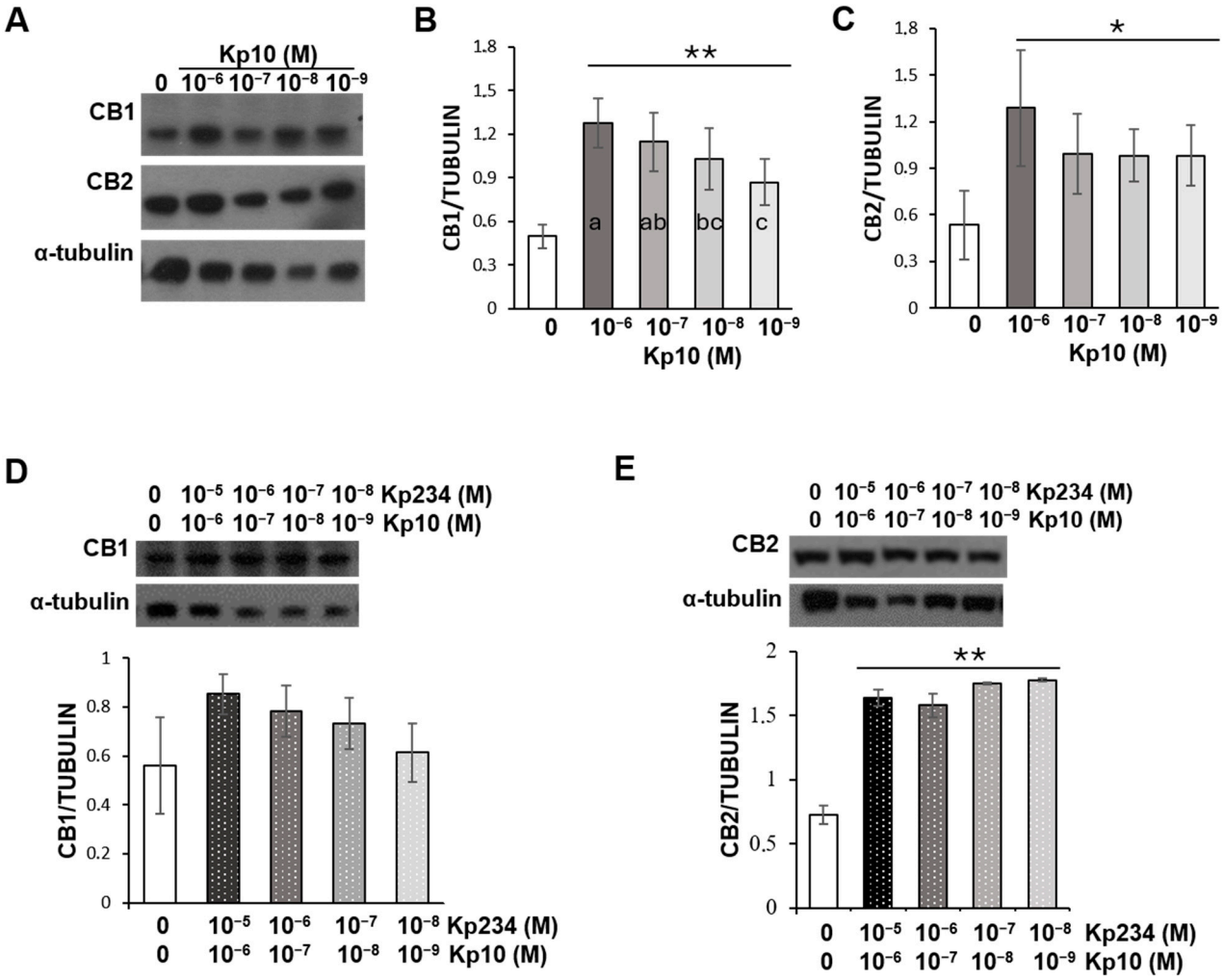

Figure 2. Ex vivo modulation of CB1 and CB2 by Kp10 in rat testis. Representative Western blots for CB1 and CB2 (**A**) following the ex vivo treatment with medium (0) or decreasing doses of Kp10 (10^{-6}–10^{-9} M) and normalization of the signals against α-tubulin (**B**,**C**). Pretreatments with medium (0) or 10-fold higher doses of Kp234 (10^{-5}–10^{-8} M) were carried out to assess the direct involvement of the Kiss1R (**D**,**E**). Testis pieces were incubated for 4 h in medium (0) or decreasing doses of Kp10 (10^{-6}–10^{-9} M). In the combined treatment, testis pieces were incubated for 30′ with medium (0) or the Kiss1R antagonist Kp234 (10^{-5}–10^{-8} M) and then incubated for 4 h with medium (0) or Kp10 (10^{-6}–10^{-9} M) ± 10-fold higher doses of Kp234 (10^{-5}–10^{-8} M). Data are representative of n = 4 different animals and are expressed as protein O.D./tubulin O.D. ± standard deviation. CB1, Cannabinoid receptor 1; CB2, Cannabinoid receptor 2; Kp10, Kisspeptin10; Kp234, Kisspeptin receptor antagonist; **, $p < 0.01$; *, $p < 0.05$ vs. control group; different letters indicate statistically significant differences ($p < 0.05$).

Kp10 significantly increased FAAH protein compared to control at 10^{-9} M dose only ($p < 0.05$) (Figure 3A) and this effect was reverted by pretreatment with Kp234 (Figure 3B).

Kp10 significantly increased NAPE-PLD protein compared to control at all the tested doses ($p < 0.05$, at least) (Figure 3C) and these effects were reverted by pretreatments with Kp234 (Figure 3D).

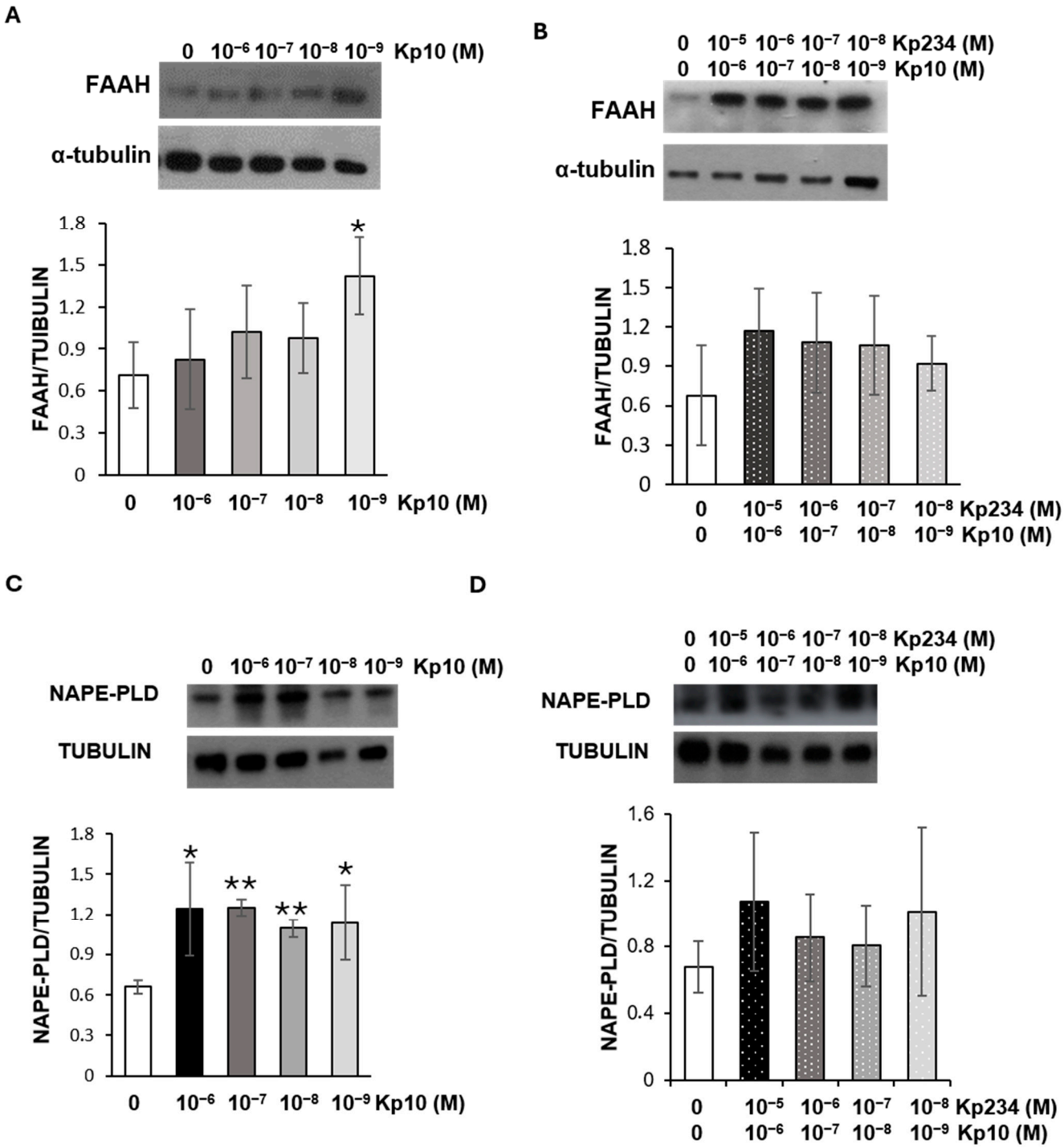

Figure 3. Ex vivo modulation of FAAH and NAPE-PLD by Kp10 in rat testis. Representative Western blots for FAAH (**A**,**B**) and NAPE-PLD (**C**,**D**) following the ex vivo treatment with medium (0) or decreasing doses of Kp10 ± 10-fold higher doses of Kp234 and normalization of the signals against α-tubulin. Testis pieces were incubated for 4 h with medium (0) or decreasing doses of Kp10 (10^{-6}–10^{-9} M). In the combined treatment, testis pieces were incubated for 30′ with medium (0) or Kiss1R antagonist Kp234 (10^{-5}–10^{-8} M) and then incubated for 4 h with medium (0) or Kp10 (10^{-6}–10^{-9} M) ± 10-fold higher doses of Kp234 (10^{-5}–10^{-8} M). FAAH, Fatty Acid Amide Hydrolase; Kp10, Kisspeptin-10; Kp234, Kisspeptin receptor antagonist; NAPE-PLD, *N*-acylphosphatidylethanolamine-specific phospholipase D. Data are representative of $n = 3$–4 different animals and are expressed as protein O.D./tubulin O.D. ± standard deviation. **, $p < 0.01$; *, $p < 0.05$.

4. Discussion

The ECS and the KS are two major signaling systems in the control of male and female reproduction, both centrally and peripherally. Their possible interplay along the HPG axis has been reported in non-mammalian vertebrates [35], but only partially investigated in mammals, particularly in the testis.

Marino et al. [39] recently reported that in vivo AEA treatment affected the protein expression of KS in both the brain and testis, whereas in vivo Kp10 affected the intragonadal levels of CB1 and FAAH [39]. Nevertheless, the observations were carried out only in vivo, were not supported by treatment with a kisspeptin antagonist and did not consider the possible effect of SR141716A as low-potency mixed agonist/antagonist of TRPV1 [48,49], thus raising the possibility of direct and also indirect interplay in the communications between the two systems. In this respect, the ex vivo treatment of testis pieces allows for focus on direct intratesticular mechanisms bypassing all the feedback routes occurring along the HPG axis and involving Kisspeptin, GnRH, gonadotropins and sex steroids.

The present data revealed that AEA, via CB1, reduces Kiss1R expression, without any effect on Kiss1 protein. This effect is fully reversed by cotreatments with SR141716A, while treatments with SR141716A alone did not affect Kiss1 or Kiss1R, thus confirming the involvement of CB1 signaling and excluding the possible involvement of TRPV1 in the decrease in Kiss1R. The present data are consistent with previous observation in vivo [39]. Of particular interest is the CB1-mediated effect on Kiss1R but not on the ligand. Kisspeptins are circulating hormones produced within the hypothalamus and in several peripheral organs, including the testis; they induce hypothalamic and intratesticular GnRH production and release [7,36,50] and connect metabolism and reproduction [5]. Hence, in the testis, kisspeptins are endocrine, paracrine and autocrine modulators of spermatogenesis progression [39], Leydig cells' physiology and sex steroid biosynthesis [37,40,50,51]. In this respect, present data demonstrate that AEA in testis directly reduces Kiss1's efficacy. Due to the localization of Kiss1R in both interstitial compartment and germ cells [33], present observations suggest possible modulatory effects on spermatogenesis and Leydig cells physiology, with specific cellular functions to be further investigated in future studies.

Present data reveal, for the first time in mammals, the direct and indirect effects of Kp10 on CBs and endocannabinoid biosynthetic and hydrolyzing enzymes. At present, the only data available in the literature reported a Kp10-dependent increase in intratesticular CB1 and FAAH following in vivo treatment with Kp10 [39]. In the same study, Marino et al. did not report any statistically significant effects on CB2 or NAPE-PLD [39]. Here, through ex vivo treatments with decreasing doses of Kp10 (10^{-6} to 10^{-9} M), we demonstrate that Kp10 increases CB1, CB2, FAAH and NAPE-PLD proteins at all the selected doses, except the effects on FAAH, which are statistically significant at the lowest dose (10^{-9} M) only. Interestingly, the effects on CB1/FAAH/NAPE-PLD are Kiss1R-dependent, while the effect on CB2 is Kiss1R-independent. In fact, when blocking Kiss1R by Kp234, CB1, FAAH, and NAPE-PLD proteins return to control levels; by contrast, Kp234 pre-treatment did not reduce CB2, suggesting an indirect mechanism of action of Kp10 on CB2. Among the large number of modulators of testis physiology, sex steroids (i.e., testosterone and estradiol) are critical to sustain spermatogenesis, from spermatogonia proliferation in the basal compartment of the seminiferous tubule to spermiogenesis and sperm maturation in the luminal compartment of the germinal epithelium [52,53].

While CB1 controls the homeostasis of the blood–testis barrier [30], spermiogenesis and sperm maturation [31,32], both CB2 and Kiss1R were critical for spermatogenesis progression in rodents [26,39]. Interestingly, exposure to bisphenol A, an estrogen-mimicking endocrine disruptor, increased the expression levels of CB2 in mouse Sertoli cells [54] and testis [55]. Nevertheless, in murine primary Leydig cells, in vitro treatment with

Kp10 results in an increase in Cyp19, the enzyme that irreversibly converts testosterone into estradiol [40]. Interestingly, in amphibians, AEA reduces intratesticular production of Kiss1/Kiss1R and modulates AEA tone through the Cyp19/estradiol/FAAH pathway [56]. Furthermore, in the same experimental model, KS has an acknowledged role in the progression of spermatogenesis and estrogen responsivity, and in the control of testosterone/estradiol balance, with outcomes affecting spermatogenesis progression and spermatozoa physiology [36,37]. Despite the differences may exist in spermatogenesis homeostasis between mammalian and non-mammalian species, a role for estradiol in the Kp10 modulation of CB2 cannot be excluded. This hypothesis needs further investigation in the near future and represents a limitation of the present study. In addition, testis is a metabolically active tissue; thus. prolonged incubation conditions may themselves influence protein expression patterns. However, previous ex vivo dose–response experiments with Kp10 revealed that, in the testis of both amphibians and rodents, Kp10 is able to differently affect biomarkers of steroidogenesis, endocrine signaling, proliferation and epigenetic signature [36,40,56]. Therefore, this experimental model is useful to ascertain testis' physiology and its biological effects beyond its hypothalamic influence.

Both ECS and KS have a significant role in spermatogenesis progression, sex steroid production and sperm maturation, and their impairment has an established role in male infertility. The increasing recreational use of cannabis, particularly in adolescent and young males, may severely adversely impact male reproduction with significant effects on sperm quality (i.e., reduced sperm count, concentration, motility, and viability, increased morphological abnormalities and inhibition of capacitation and fertilizing capacity) [57]. Possible effects on paternal epigenetic inheritance have been reported [58]. Delta-9-tetrahydrocannabinol (THC), the main phytocannabinoid in the marijuana plant, uses CB1 signaling to gain psychoactive effects [57]. Thus, present data may provide additional information on the signaling pathways involved in the interference in the endogenous ECS due to environmental factors and lifestyle. Similarly, kisspeptin administration may represent a possible therapeutic strategy to rescue the functionality of the ECS in reproductive dysfunction.

5. Conclusions

The present work demonstrates the reciprocal direct effects of ECS and KS in rat testis.

This result provide insight into the mutual communications between the ECS and the KP, revealing CB1-dependent effects on Kisspeptin efficacy and Kp10-dependent and independent modulation of the ECS. Taken together, these results reveal a local dialog in the testis involving reciprocal modulation between ECS and KS, which may have a critical effect on the physiology of male reproduction.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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