Domestic Animal Endocrinology 45 (2013) 171–179

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Expression of the cannabinoid receptor type 1 in the pituitary of rabbits and its role in the control of LH secretion

C. Dall'Aglio [a], P. Millán [b], M. Maranesi [c], P.G. Rebollar [d], G. Brecchia [c], M. Zerani [e,*], A. Gobbetti [f], G. Gonzalez-Mariscal [g], C. Boiti [c,d]

[a] Sezione di Anatomia, Dipartimento di Scienze biopatologiche veterinarie, Università di Perugia, Via S. Costanzo 4, 06126 Perugia, Italy
[b] Departamento Fisiología Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Ciudad Universitaria, s/n, 28040 Madrid, Spain
[c] Laboratorio di biotecnologie fisiologiche, Sezione di Fisiologia Veterinaria, Dipartimento di Scienze biopatologiche veterinarie, Università di Perugia, Via S. Costanzo 4, 06126 Perugia, Italy
[d] Departamento Producción Animal, ETSI Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria, s/n, 28040 Madrid, Spain
[e] Scuola di Scienze mediche veterinarie, Università di Camerino, Via Circonvallazione 93, 62024 Matelica, Italy
[f] Scuola di Bioscienze e biotecnologie, Università di Camerino, Via Gentile III da Varano, 62032 Camerino, Italy
[g] Centro de Investigación en Reproducción Animal, CINVESTAV-Universidad Autónoma de Tlaxcala, Mexico

ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form 20 August 2013
Accepted 21 August 2013

Keywords:
CB1
Endocannabinoids
Rimonabant
LH
Rabbit

ABSTRACT

The aim of this study was to elucidate the possible direct regulatory role of the endocannabinoids in the modulation of LH secretion in rabbits, a reflex ovulator species. The cannabinoid receptor type 1 (CB1) was characterized by RT-PCR techniques in the anterior pituitary of intact and ovariectomized does treated with GnRH and primed with estrogen and CB1 antagonist, rimonabant. Cannabinoid receptor type 1 immune reaction was evidenced by immunohistochemistry in the cytoplasm of approximately 10% of the pituitary cells with a density of 8.5 ± 1.9 (per 0.01 mm^2), both periodic acid–Schiff positive (30%) and negative (70%). All CB1-immunoreactive cells were also immune reactive for estrogen receptor type 1. Ovariectomy, either alone or combined with estrogen priming, did not modify the relative abundances of pituitary *CB1* mRNA, but decreased ($P < 0.01$) the expression of estrogen receptor type 1 mRNA. Treatment with CB1 antagonist (rimonabant) inhibited ($P < 0.01$) LH secretory capacity by the pituitary after GnRH injection, and estrogen priming had no effect. The present findings indicate that the endocannabinoid system is a potential candidate for the regulation of the hypothalamic-pituitary-ovarian axis in reflex ovulatory species.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

The endocannabinoids, arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl-glycerol, together with the 7 transmembrane G protein–coupled cannabinoid receptor subtypes 1 (CB1) and CB2, form an endogenous signaling system involved in a large array of physiological functions [1–4]. Besides controlling food intake and energy balance [5], the endocannabinoid system modulates several neuroendocrine functions, including the hypothalamic-pituitary gonadal and adrenal axes [6–9]. For instance, injections of delta-9-tetrahydrocannabinol (delta-9-THC) to rhesus monkeys [10] or rabbits [11], as well as administration of delta-1-THC to proestrus rats [12], inhibit gonadotropin release and ovulation, an effect that apparently occurs at the level of the hypothalamus. Moreover, anandamide and 2-arachidonoyl-glycerol inhibit LH and prolactin secretion in vivo and in vitro, although with different efficacy [13,14]. Both endocannabinoids have been identified in the anterior pituitary and the hypothalamus of rats, suggesting that these endogenous compounds may be synthesized also locally [15,16]. However, CB1 is widely

* Corresponding author. Tel.: +39 737 403463; fax: +39 737 403402.
E-mail address: massimo.zerani@unicam.it (M. Zerani).

0739-7240/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.domaniend.2013.08.004

distributed in several structures of the brain, including the hypothalamus [17], and it has also been localized in the anterior pituitary within the gonadotroph and lactotroph cells of adult male rats [18]. All these lines of evidences indicate that the endocannabinoids may control anterior pituitary hormone secretion not only indirectly, by modulating the release of neurotransmitters or releasing hormones in the hypothalamus or both, but also by acting directly on the pituitary. Indeed, the amount of *CB1* transcript fluctuates across the estrous cycle in the anterior pituitary of female rats, the highest values occurring during estrus and the lowest ones on the first day of diestrus and proestrus [16]. Moreover, estradiol-17β replacement downregulates *CB1* receptor gene expression in ovariectomized (OVX) females [16]. These findings show that estradiol inhibits the expression levels for *CB1* in the anterior pituitary, but they do not rule out the participation of additional hormones that fluctuate across the estrous cycle of rats and play a crucial role in determining the magnitude and timing of the pulsatile release of LH. By contrast, in rabbits (which are reflex ovulators) the peripheral concentrations of estradiol, progesterone, and gonadotropins remain at basal levels in unmated does [19,20]. Consequently, this species is a better model for investigating the specific action of estradiol on the cannabinoid system in the anterior pituitary and its potential role in the regulation of LH release.

Therefore, to investigate a possible direct regulatory role of endocannabinoids on the pituitary function of a reflex ovulator we determined a) the expression of CB1 in the anterior pituitary of rabbits (by immunohistochemistry, IHC), b) its coexpression with estradiol-17β receptor type 1 (ESR1), and c) the gene expression for *CB1* (by RT-PCR) and its regulation by estrogens in OVX rabbits (implanted or not with estradiol benzoate). To begin investigating whether endocannabinoids may play a role in the regulation of gonadotropin release in vivo we evaluated the dynamic secretion of LH after GnRH challenge to induce ovulation in control and estrogen-primed does pretreated with the CB1 antagonist, rimonabant (SR141716). The results of this study will enrich our understanding of how endocannabinoids contribute to the regulation of LH secretion in mammals because the model used has the advantage that variations in gonadal steroids, as triggers of changes in the pituitary gland, can be safely excluded.

2. Materials and methods

2.1. Animals and hormones

Sexually mature New Zealand White female rabbits (n = 27), weighing 3.5 to 4 kg, were housed individually in an indoor facility under controlled conditions of light (16 h light/8 h darkness) and temperature (18°C–24°C). All does were fed a standard commercial pellet diet and were provided free access to water throughout the experiment.

For estrogen priming, the Silastic capsules (13-mm length, sealed with adhesive silicone at their ends) contained 20 mg of estradiol-benzoate per kilogram of BW [21]. For treatment with CB1 antagonist, the Silastic capsules (13-mm length sealed as above) contained 1.0 mg of rimonabant per kilogram of BW. The estradiol-filled and rimonabant-filled Silastic capsules were implanted subcutaneously in the suprascapular area of treated does 2 d before the experiment, whereas corresponding controls were sham implanted with empty Silastic capsules.

To verify the effects of estrogens on the expression of *CB1* receptors in the anterior pituitary of rabbit does, as well as the role of the endocannabinoid system on the dynamic response of circulating LH to exogenous GnRH challenge, 3 experimental protocols were designed:

Experiment 1 was performed to localize the cell type distribution of CB1 and its coexpression with ESR1 in the anterior pituitary; 3 rabbits were euthanized to collect their pituitaries.

Experiment 2 examined the effect of ovariectomy and estrogen priming on the gene expression for *CB1* in the pituitary of rabbit does. Eight does underwent ovariectomy via laparotomic approach under general anesthesia with Sedator (medetomidine) (0.05 mg/kg) and Lobotor (ketamine) (35 mg/kg). The rabbits, after 15 d of recovery, were randomly assigned to 1 of the following 3 groups (4 animals/group): control (sham-operated does), OVX, and OVX-primed with estrogen. All does were euthanized 48 h after Silastic implant (h = 0) to collect the pituitary gland.

Experiment 3 evaluated the role of the endocannabinoid system on the dynamic response of LH to exogenous GnRH, through inhibition of the CB1 via treatment with antagonist rimonabant. Two days before intramuscular injection of 0.8 μg of GnRH analogue to induce ovulation (time 0), rabbits were randomly assigned to 1 of the following 3 groups (4 animals/group): control (implanted with empty capsules), implanted with rimonabant, and estrogen-primed implanted with rimonabant.

The animals were sacrificed in accordance to the guidelines and principles for the care and use of research animals. All efforts were made to minimize the number of animals used and their suffering. The protocols for the experiments were approved by the Bioethics Committee of the University of Perugia.

2.2. Tissue collection and blood sampling

On sacrifice, the pituitary gland of each doe belonging to experiment 1 and experiment 2 were promptly removed. The pituitary glands from experiment 1 were bisected through the medial sagittal plane into 2 symmetrical parts. One-half of the pituitary intended for IHC was immediately fixed by immersion in 4% paraformaldehyde solution in 0.1 *M* PBS, pH 7.4, for 24 h and subsequently processed for embedding in paraffin, by following routine tissue preparation procedures. The other half pituitary from experiment 1 and tissue samples from experiment 2 intended for RT-PCR evaluation of gene expression were immediately frozen at −80°C, after rinsing with RNAse-free PBS.

The day before induction of ovulation (experiment 3), a catheter was inserted into the central ear vein of each rabbit. Blood samples (1 mL) were collected from unrestrained catheterized animals, which were free to move in

Download English Version:

https://daneshyari.com/en/article/2393649

Download Persian Version:

https://daneshyari.com/article/2393649

Daneshyari.com