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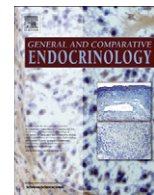

Effect of prolactin acting on the coeliac ganglion via the superior ovarian nerve on ovarian function in the postpartum lactating and non-lactating rat

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ABSTRACT

Whether prolactin (PRL) has a luteotrophic or luteolytic effect in the rat ovary depends on the nature of the corpora lutea present in the ovaries and the hormonal environment to which they are exposed. The aim was to investigate the effect of PRL acting on the coeliac ganglion (CG) on the function of the corpora lutea on day 4 postpartum under either lactating or non-lactating conditions, using the CG–superior ovarian nerve–ovary system. The ovarian release of progesterone (P), estradiol, PGF2α, and nitrites was assessed in the ovarian compartment at different incubation times. Luteal mRNA expression of 3β-HSD, 20α-HSD, aromatase, PGF2α receptor, iNOS, Bcl-2, Bax, Fas and FasL was analysed in the corpus luteum of pregnancy at the end of the experiments. Comparative analysis of control groups showed that the ovarian release of P, nitrites, and PGF2α, the expression of PGF2α receptor, and the Bcl-2/Bax ratio were lower in non-lactating rats, with increased release of estradiol, and higher expression of aromatase, Fas and FasL, demonstrating the higher luteal functionality in ovaries of lactating animals. PRL added to the CG compartment increased the ovarian release of P, estradiol, nitrites and PGF2α, and decreased the Bcl-2/Bax ratio in non-lactating rats; yet, with the exception of a reduction in the release of nitrites, such parameters were not modified in lactating animals. Together, these data suggest that the CG is able to respond to the effect of PRL and, via a neural pathway, fine-tune the physiology of the ovary under different hormonal conditions.

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1. Introduction

In rats ovulation occurs immediately following parturition; therefore, in the postpartum rat ovary, the corpora lutea of pregnancy that are undergoing regression coexist with the newly-formed corpora lutea after postpartum ovulation. In non-lactating rats, this new cohort of corpora lutea belong to a new estrual cycle and have very short life before regressing [13,43]. Postpartum luteal regression and apoptosis in non-lactating animals might be triggered by an increase in the circulating estradiol at the end of pregnancy [44], likely in response to pituitary PRL [21]. In this regard, Casais et al. [10] demonstrated that estradiol has a direct effect on the ovary favoring regression of the luteal tissue and can certainly mediate PRL-induced luteal regression.

If lactation is established after parturition, however, high and sustained concentrations of PRL are induced as a consequence of the suckling reflex; in this case PRL has a luteotrophic effect on the newly formed corpora lutea after postpartum ovulation – the corpora lutea of lactation – leading to the production of high levels of P, which prevents apoptosis in both the corpora lutea of pregnancy and the corpora lutea of lactation [20,43]. Overall, the previous findings suggest that the dual response (luteotrophic/luteolitic) of the luteal tissue to PRL is dependent on the nature of the corpora lutea and of the hormonal environment to which they are exposed.

Other agents involved in the process of luteal regression are prostaglandin F2α (PGF2α) and the gaseous neurotransmitter nitric oxide (NO). The existence of a feedback mechanism between PGF2α and NO has been shown in a model of rat pseudopregnancy. Administration of NO donors during luteal regression increases the synthesis of ovarian PGF2α and decreases serum P concentrations

Abbreviations: CG, coeliac ganglion; CLP, corpus luteum of pregnancy; iNOS, inducible nitric oxide syntase; NO, nitric oxide; P, progesterone; PGF2α, prostaglandin F2α; PGF2αR, prostaglandin F2α receptor; PRL, prolactin; RIA, radioimmunoassay; SON, superior ovarian nerve.

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[34]; furthermore, administration of a luteolytic dose of PGF2α increases the activity of NO synthase (NOS) in the ovary, while it reduces P production [35].

In mammals, the ovary is controlled by endocrine and neural factors. The latter come from two routes: the ovarian plexus and the superior ovarian nerve (SON). While the former is important for the innervation of the blood vessels of the ovary, the latter is critically relevant to regulate the overall physiology of the ovary, including ovulation and steroidogenesis. The SON is mainly constituted by adrenergic fibers, most of which originate in the coeliac ganglion (CG) [28,25,5–7]. The CG is included in the sympathetic pre-vertebral chain and it is constituted by principal neurons and interneurons [18,1,30,37]. Sympathetic ganglia possess a diversity of neurotransmitters, among them catecholamines [24], neuropeptides [14] and NO [33], receptors for hormones, neurotransmitters [48,49], and a profuse capillary plexus that constitutes a microcirculation among the different ganglionic structures [12]. This suggests that bioactive substances such as hormones can reach through this plexus and modulate the ganglionic activity. In addition, in rats, the muscarinic cholinergic neurotransmission of the superior cervical ganglion is affected by several hormones, including PRL [19].

The aim of this study was to investigate the effect of PRL acting on the CG in the *ex vivo* CG–SON–O system previously standardized in our laboratory [9] on the physiology of the corpora lutea found in the ovary on day 4 postpartum under two different endocrine environments: non-lactating and lactating conditions.

2. Materials and methods

2.1. Reagents

The following drugs: ovine PRL (L6520), dextrose (D9434), ascorbic acid (A5960), bovine serum albumin fraction V (BSA) (A2153), sulphanilamide (S9251) and *N*-1-naphthyl-ethylenediamine (N9125) were purchased from the Sigma Chemical Co. 1,2,6,7-[^{3}H] Progesterone (107.0 Ci/mmol) was provided by New England Nuclear Products (Boston, MA, USA). Other reagents and chemicals were of analytical grade.

2.2. Animals

To induce pregnancy, virgin Holtzman female rats weighing 250 ± 50 g were caged individually with fertile males beginning on the afternoon of proestrus. Positive mating was verified on the following morning by identifying sperm or copulation plugs in the vagina. This day was designated as day 0 of pregnancy. In our laboratory, rats usually give birth on day 22. Pregnant rats were divided into two groups at parturition: non-lactating rats encompassing mothers whose pups were removed immediately after delivery, and lactating rats, including mothers that were normally kept with their newborn pups (the number of pups was adjusted to 8). Animals had free access to food (Cargill SACI, Saladillo, Buenos Aires, Argentina) and water. They were kept in a light (lights on from 07:00 to 19:00 h) and temperature controlled room (24 ± 2 °C). Animals were handled according to the procedures approved in the UFAW Handbook on the Care and Management of Laboratory Animals. The experimental protocol was approved by the University of San Luis Animal Care and Use Committee (number protocol: B49/10).

2.3. Extraction of CG–SON–O system of rats

The extraction of CG–SON–O system was carried out at 9 am on day 4 postpartum. Thus, in non-lactating animals, the system is only influenced by the preovulatory PRL surge occurring at 3 h postpartum, but not by that of afternoon of proestrus (day 4 postpartum).

Groups of six animals were used for each experimental procedure. The surgical procedure to remove the CG–SON–O system and the incubation conditions were carried out according to Casais et al. [9]. Animals were anaesthetized with ketamine and xylazine (80 and 10 mg kg^{-1}, respectively, I.P.). The system was extracted and the mothers were sacrificed by decapitation. The complete system was removed by surgery, avoiding contact between the surgical instruments and the nerve fibres in order to prevent spontaneous depolarisation of the nerves. The piece of tissue removed consisted of the left ovary, the fibres that constituted the SON, inserted in the suspensory ligament, and the CG accompanied by some small ganglia surrounding it. The total surgical procedure was completed in 1–2 min.

2.4. Experimental procedures

The system was washed with incubation medium and placed in a cuvette with two compartments, one for the CG and the other for the ovary, both joined by the SON. The incubation medium used was Krebs–Ringer bicarbonate buffer, pH 7.4 with the addition of 0.1 mg glucose/ml and 0.1 mg albumin/ml at 37 °C in an atmosphere composed by 95% of O_2 and 5% of CO_2.

The system was preincubated for 30 min, and the end of this period was considered incubation time 0. After this time, buffer was changed in both compartments and 0.1 mg of ascorbic acid/ml was added to the ganglion compartment as antioxidant agent. At time zero of incubation, 10^{-7} M PRL was added to the ganglionic compartment [$(PRL)_g$]. Control groups consisted of untreated CG–SON–O systems. Periodic extractions (250 μl) of the ovary incubation liquid were carried out at 30, 60, 120, 180 and 240 min and kept at −20 °C until dosage of P, estradiol and PGF2α (only at 240 min) by radioimmunoassay (RIA) and determination by Griess technique of nitrite, the soluble metabolite of NO. The corresponding corrections were made in all cases, taking into consideration the volume extracted in each tested period. After incubation (240 min), whole ovaries were weighed, the CLP were separated and stored at −80 °C. From the CLP of both groups on day 4 postpartum, total RNA was extracted for analysis of mRNA expression of 3β-hydroxysteroid-dehydrogenase (3β-HSD), 20α-hydroxysteroid-dehydrogenase (20α-HSD), aromatase (P synthesis and degradation and estradiol synthesis enzymes, respectively), PGF2α receptor (PGF2αR), inducible nitric oxide synthase enzyme (iNOS), Bcl-2, Bax, Fas and FasL by RT-PCR.

2.5. Progesterone and estradiol radioimmunoassay

Steroids were measured in duplicate by RIA in the ovary incubation liquid. The progesterone antiserum, provided by Dr. R. Deis (IMBECU, Mendoza, Argentina), was produced in rabbits against progesterone conjugated to bovine serum albumin at the 11 position. The antiserum was highly specific for progesterone with low cross-reactivities, <2.0% for 20α-dihidro-progesterone and deoxycorticosterone and 1.0% for other steroids. The sensitivity was less than 5 ng/ml and the inter and intraassay coefficients of variation were less than 10%. This assay has been validated previously [4]. Progesterone concentration was expressed as nanogram per milligram ovary (ng/mg ovary), all against incubation time. The estradiol levels were determined using a double antibody RIA Diasource kit (DiagnosMed SRL) following the manufacturer's instructions. Estradiol was expressed as picograms per milligram of ovarian tissue (pg/mg ovary), all against incubation time. The percentages of cross-reactions were 1.8% for estrone, 1.2% estriol, 0.0011% androstenedione and 0.0002% for progesterone. The assay

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