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Correlation of prolactin levels and PRL-receptor expression with Stat and Mapk cell signaling in the prostate of long-term sexually active rats

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HIGHLIGHTS

- The long-term execution of sexual behavior produces an increased level of serum PRL.
- PRL must be constantly elevated for copulation to occur in the long time.
- The mRNA for PRL receptors at the prostate decreases in the long-term.
- Activation of PRL receptors only activates the Stat3 pathway in copulating males.

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ABSTRACT

Prolactin (PRL) is a key hormone for prostate function, with a basal level in serum and associated with two characteristic circadian peaks. In the male rat, the execution of one bout of sexual behavior with consecutive ejaculations produces a significant transient increase in PRL. However, the impact of a constant sexual life on both PRL levels and prostate function is unknown. Thus, by using constantly copulating males we analyzed the levels of serum PRL, the effect on prostate PRL receptors, and activation of pStat3, pStat5 and Mapk signaling pathways. Sexually experienced Wistar male rats were used, which underwent periodic sessions of sexual behavior tests. Males were subjected to a session of sexual behavior to achieve at least one and up to four ejaculations. Of these, a blood sample was collected from randomly selected males and the ventral prostate was removed for analysis. Serum PRL was quantified, the mRNA for PRL receptors was determined, and signaling pathways were analyzed. Data show that a constant sexual life produced a constant elevation of PRL in serum during four consecutive ejaculations. The ventral prostate showed a different mRNA expression profile for the long and short isoform of the PRL receptor, and both mRNA levels increased. Although the gland did not show modification of the activation of the pStat5 signaling pathway, the levels of pStat3 increased, and the Mapk pathway showed one significant elevation after the third ejaculation. Thus, we showed that an active and constant sexual life produces a sustained increase in serum PRL, its receptors, and the pStat3 signaling pathway. These responses seem to underlie the required physiological need to produce the quantity and quality of prostatic semen to ensure the appropriate environment for sperm to reach and fertilize the ovum.

1. Introduction

Prolactin (PRL) is one of the hormones underlying the physiology of the prostate gland. It shows a daily basal level with two peaks at light–dark–light transitions [1], which serves among other things to potentiate the effect of androgens on sexual accessory glands and increase luteinizing hormone receptor levels in Leydig cells, which in turn increases testosterone synthesis [2]. Thus, although these are some functions of PRL, we have shown that PRL by itself is also important for other prostate functions, where it controls zinc uptake, citrate synthesis, and the expression of androgen receptors and cathepsin D [1,3]. Although basal levels of PRL are extremely important for maintenance of the prostate, there are some behavioral situations that produce a significant increase in serum PRL levels. In the adult male rat, the execution of sexual behavior with consecutive ejaculations produces a significant transient increase in PRL, four times above basal levels, that we suggested serves to trigger the synthesis and release of the contents of the gland in order to produce enough semen to expel per ejaculation

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[1]. Thus, in sexually active males PRL plays a significant role in ensuring that the prostate produces an appropriate semen content for reproduction. However, our data was derived from males engaged in a single bout of sexual behavior. In nature, however, a male rat is involved in periodic sexual behavior during adulthood, with several consecutive ejaculations, and the impact of this activity on both PRL levels and prostate function is unknown. Thus, the first task of this study was to address this question.

Investigating the effect of long-term sexual behavior on prostate PRL receptors (PRL-R) was the second task of this study. PRL has three receptor isoforms, the short (291 aa), the intermediate (393 aa) and the long (591 aa), that belong to the cytokine class-1 receptor superfamily [4–6]. PRL-Rs are transmembrane proteins that possess the same extracellular domain, but different intracellular domain in terms of length [7]. However, the first 27 amino acids of the intracellular segment is the same in all three isoforms [8], and comprises a proline-rich region that is involved in interaction with tyrosine kinase JAK2, which is required for triggering cell signaling pathways [9]. It has also been shown that the short isoform of the receptor is involved in the negative regulation of the long isoform, and the latter becomes inhibited when both are active at the same time [10]. In the prostate, only the short and long isoform of the PRL-R are found in epithelial cells at the alveoli [11–13], and following activation by PRL they trigger the Stat and Mapk pathways [14, 15]. In the prepuberal rat, expression of PRL-R in the prostate occurs concurrently with the systemic increase in PRL [16]. Furthermore, attachment of PRL to its receptor is dependent on age, where attachment is greater in younger rather than in older subjects [17].

Following attachment of PRL to its prostate receptors, activation of the Stat5 signaling pathway occurs [3]. The importance of this activation is observed when it is blocked, since epithelial disorganization is observed at the ventral prostate [18]. In the same way, PRL triggers a transitory activation of the Stat3 pathway that lasts some minutes. This effect is clearly observed in males engaged in sexual behavior, where transitory activation of Stat1 and Stat3 was previously observed [19]. In addition to the activation of Stat pathways, it is also known that PRL triggers the Mapk pathway as an additional process in the control of prostate function. Although the precise role of this pathway is unknown, it seems to be associated with the induction of cell proliferation [20]. Thus, another aim of this work was to determine whether both Stat and Mapk signaling pathways become active following the engagement of long-term sexual behavior.

2. Materials and methods

2.1. Subjects and sexual behavior

Sexually naïve Wistar male rats between 300 and 350 g/bw and ovariectomized females were used. Sexual receptivity of females was induced using steroids dissolved in sesame oil. Accordingly, subcutaneous injections of estradiol benzoate (10 μg) and then progesterone (2 mg) were administered 48 and 4 h, respectively, before tests.

Males were trained to acquire sexual experience by subjecting them to a sexual behavior test twice a week for two weeks. The training was carried out during the last third of the dark phase of the light–dark cycle, being an optimum phase for the execution of sexual behavior in this species. Briefly, males were placed in the training arena for 5 min to adapt and then a receptive female was introduced. From this moment, sexual behavior was recorded until ejaculation occurred. After the 4 training sessions, males whose ejaculation latency was less than 10 min were selected for the experiment. Selected subjects were submitted to another 15 days of sexual behavior every other day. In the last test of this long-term period of sexual behavior, males were allowed to achieve at least one and up to four ejaculations. Immediately after each ejaculation, some males were randomly selected and a blood sample was collected as described below, then the animal was deeply anesthetized and the ventral prostate was removed, placed in physiological saline, frozen in liquid nitrogen and stored at −70 °C until use.

Males were cannulated for blood collection as previously described [1]. Selected subjects after the first training session were anesthetized with sodium pentobarbital (Smith Kline Mexico; 30 mg/kg bw, i.p.), and the right jugular vein was exposed. The vein was half-cut and 2.5 cm of a cannula (Dow Corning, USA, 0.30 mm ID and 0.64 mm OD) introduced to leave the tip in the right atrium. The cannula and vein were fixed and a small loop of the cannula was positioned in the ventral neck area to avoid displacement during head movements. The other side of the cannula was guided subcutaneously to the back and exposed through a hole in the skin. The skin in the neck was sutured, covering the cannula and loop. Therefore, only the end of the cannula in the back was exposed. It was filled with saline solution using a Hamilton syringe; the tip was closed with a small metal plug, and covered with a belt (1.5 cm wide) that surrounded the thoracic area. To collect blood, a small drop of heparin solution was placed in a Hamilton syringe which was then connected to the tip of the cannula and the blood extracted. Blood samples were centrifuged, and the plasma obtained stored at −20 °C until assayed.

2.2. Quantification of prolactin

To determine the blood concentration of PRL, the ALCO kit was used (cat. 12-MKVRP1), as established by Beach and col. 1985. The standard curve range was from 5 to 80 ng/ml. Briefly, 25 μl of each sample and standard curve solution was loaded and to each was immediately added 50 μl of rat PRL sample buffer before being incubated with constant agitation for 2 h at room temperature. The plate contents were decanted, washed four times, and 200 μl of tetramethyl benzidine-coupled secondary antibody was then added for 30 min. The reaction was carried out at dark room temperature. The reaction was stopped by adding 50 μl of hydrochloric acid and the plate was read using an ELISA reader at 450 nm. Absorbances were analyzed using linear regression and the data obtained are reported in ng/ml.

2.3. Western blot

Tissue sample from the ventral prostate (100 mg) was homogenized with 200 μl of Lysis buffer containing Solulyse-M (cat. L-30012, Genlantis Inc.) and protease inhibitor cocktail tablets (cat. No. 11836153001, ROCHE), and then incubated with constant agitation at 4 °C for 1 h. Samples were then centrifuged for 30 min at 13000 rpm at 4 °C and supernatants (total extract) were recovered. Aliquots were obtained and frozen at −70 °C. Protein concentration was determined by the Bradford method. Protein samples (100 μg) were subjected to 7.5% SDS-PAGE under reducing conditions at 200 V (Mini-Protean III, Bio-Rad) and then transferred onto a nitrocellulose membrane at 100 V for 1.30 h.

The nitrocellulose membrane 0.45 μm (162-0115, Bio-Rad) was blocked with TBS containing Tween-20 (0.1%) and milk (5%) for 1 h. The membrane was washed 3 times with a solution of TBS-Tween-20 (0.1%) for 5 min each. The membrane was then incubated overnight at 4 °C with the first antibody against pStat3 (H-190, 1:250), pStat5 a/b (H-134, 1:250), and Mapk (sc-252, 1:500) from Santa Cruz Biotechnology. The following day, the membrane was washed 3 times and incubated with secondary antibody (Goat anti-mouse 1/1000, Santa Cruz Biotechnology) for 1 h at room temperature. Bands were revealed using the HRP kit (170-6465, Bio-Rad) and densitometric analysis of the bands was performed using the Kodak image station 440-CF with Kodak 1D 3.6 software. B-actin (44 kDa) was used as a loading control.

2.4. RT-PCR

Tissue from the ventral prostate (100 mg) was homogenized in a Politron in 1 ml of TRIzol, incubated for 5 min at room temperature,