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Signal transducers and activators of transcription 1 and 3 in prostate: Effect of sexual activity

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Abstract

The signal transducers and activators of transcription (Stat) are effector molecules downstream of cytokine receptors. Ligand occupancy of these receptors results in the tyrosine phosphorylation, dimerization and nuclear translocation of the Stat family of transcription factors and by these means regulate gene expression. Prolactin receptors as members of the cytokine-hematopoietin receptor superfamily, are linked to Stat activation. Sexual stimulation leads to an increase in prolactin secretion that might be involved in long-term changes in the protein repertoire associated to prostate hyperplasia. In order to gain insight into this phenomenon, we analyzed the tyrosine phosphorylation and DNA binding activity of two members of the Stat family in the prostate of sexual experienced rats after different number of ejaculations. A significant increase in Stat-1 and Stat-3 tyrosine phosphorylation was found after three ejaculations. Concomitantly an increase in Stat-1 and Stat-3 DNA-binding activity is detected after two and three ejaculation series. These results, favor the notion that ejaculation-induced prolactin secretion activates its prostate receptors resulting in Stat-1 and Stat-3 nuclear translocation, event likely to be associated to the so-called benign prostate hyperplasia.

Keywords: Signal transducers and activators of transcription (Stat); Sexual activity; Prolactin receptors; Prostate hyperplasia

Introduction

Prolactin (PRL) is a pituitary polypeptide hormone that regulates several physiological functions such as reproduction, differentiation and sexual activity. PRL plasma concentrations are substantially elevated over 1 h following orgasm in both men and women (Kruger et al., 2002). In male rats, serum PRL levels are elevated 45 min following both mount and ejaculation in young (85 days) animals (Balin and Schwartz, 1976). Moreover, chronic hyperprolactinemia severely impairs sexual behavior and leads to dramatic enlargement of the prostate gland (Doherty et al., 1990; Clark, 1995; Wennbo et al., 1997; Van Coppenholle et al., 2001). PRL interacts with specific receptors expressed on different tissues, including prostate (Kelly et al., 1991). PRL receptors (PRLRs) belong to the cytokine/growth hormone/PRL receptors superfamily, characterized by the presence of two disulfide loops and an intracellular proline-rich Box1 homology domain. These receptors do not display any intrinsic tyrosine kinase activity, but signal through cytoplasmic protein tyrosine kinases of the Janus kinase family (Jak/Tyr kinases) and the Src-kinase family. Ligand binding induces receptor dimerization and activation of the associated kinases. This leads to the tyrosine phosphorylation of multiple cellular proteins including the receptors themselves (Ilhe et al., 1995).

Several Src homology 2 domain (SH2)-containing proteins interact with the activated PRLRs, e.g. phospholipase C-γ, the regulatory subunit of phosphatidyl inositol 3-kinase, the protein tyrosine phosphatases SHP1 and SHP2 and adapter proteins such as Grb2, Shc, and others. Interestingly, the family of SH2 containing transcription factors called signal transduction and activator of transcription (Stat) 1, 3 and 5 are activated after PRL

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stimulation (Das and Vonderhaar, 1995). Rapidly, upon PRLRs activation, tyrosine phosphorylation of Stats occurs leading to their homo- or heterodimerization and translocation to the nucleus where they induce transcription through the recognition of *cis* regulatory elements within the promoter regions of cytokine responsive genes (Shuai et al., 1994). It is now clear that tyrosine-phosphorylated PRLRs act as docking sites for the SH2 domains of Stats, allowing them to be phosphorylated by the Jak tyrosine kinases, although the contribution of other signaling pathways such as the mitogen-activated protein kinase cascade that leads to Stats serine phosphorylation is also required for nuclear translocation (Zhang et al., 1995).

In reproduction, PRL has been shown to have luteotropic actions and to regulate testicular function by increasing the binding of luteinizing hormone to Leydig cells. PRL has also been shown to have a role in the regulation of prostatic development, growth, and differentiation. In fact, PRL has a direct effect on morphology, on DNA labeling with [^{3}H] thymidine, and obviously on the expression of prostatic tissue-specific genes, like M-40 and RWB encoding secretory proteins (Nevalainen et al., 1997). PRL has also been postulated to regulate abnormal prostatic function. As mentioned above, hyperprolactinemia leads to biphasic effect, first a reduction in animal sexual activity and lately to an increase in the size of the prostate (Doherty et al., 1990; Clark, 1995; Wennbo et al., 1997; Van Coppenholle et al., 2001). Taken together, these results suggest that PRL secreted after sexual activity is involved in short-term as well as long-term modifications in the prostate physiology. In the short term, PRL may represent a regulatory factor for reproductive function whereas in the long-term it might be an important factor in the transformation of prostatic cells. In this context, in the present contribution, we decided to evaluate if sexual activity results in an increase in Stat tyrosine phosphorylation and DNA binding activity.

Materials and methods

Materials

Protease inhibitors were purchased from Roche Molecular Biochemicals (Indianapolis, IN). The antibodies used were: rabbit polyclonal anti-Stat-1 sc-592, mouse monoclonal anti-phospho Stat-1 sc-8394, rabbit polyclonal anti-Stat-3 sc-482, mouse monoclonal anti-phospho Stat-3 sc-8059 (Santa Cruz Biotechnology, Santa Cruz, CA). Horseradish peroxidase-linked anti-mouse or anti-rabbit immunoglobulins and the enhanced chemiluminescence reagent were obtained from Amersham (Little Chalfot, Buckinghamshire, UK). All other chemicals were from Sigma (St.Louis, MO).

Subjects and housing

Sexually experienced male Wistar rats, weighing 250–350 g were used. Ovariectomized receptive females were used for mating tests. Receptivity was induced by the subcutaneous injection of 10 μg of estradiol benzoate and 2 mg of progesterone, 48 and 4 h before tests respectively. Rats maintained in controlled lightening (12 h) and temperature conditions (22 °C) and housed in accordance with the NIH guidelines for the care and use of laboratory animals.

Mating tests and copulatory parameters

Four copulatory series per animal were observed twice a week with a three-day interval. Tests were conducted during the last third of the dark period. After a 5 min adaptation of the male in a plexiglass arena, a receptive female was introduced beginning the test, whereas the ejaculatory pattern (1, 2, 3 or 4 ejaculations) ended the test. The observational study of sexual behavior in males allows the identification of three copulatory movements known as mounts, intromissions and ejaculation. The performance of these movements is quantified by means of the number of mounts (NM) and the number of intromissions (NI) that represent the frequency of each movement before ejaculation. The time that takes the male to mount the female represents the latency of mount (LM). Similarly, the latency of intromission (LI) is the time between the introduction of the female and the first intromission. The ratio between the NI and NM+NI is known as the hit rate parameter of copulatory behavior and represents the capacity of the male to have a penile erection. Sexually experienced animals were defined as those with a latency of ejaculation (LE)=10 min and were divided into five groups, one control and four experimental groups (Larsson, 1979). The sexual activity parameters were recorded using the SBR software (Claro et al., 1990). The experimental groups were allowed to copulate and were killed after one, two, three or four consecutive ejaculations.

Preparation of nuclear extracts

Seven minutes after ejaculation, the animals were deeply anesthetized with 25 mg/kg weight of pentobarbital and killed by cervical dislocation. The prostate was dissected and nuclear extracts were prepared by homogenization of 150 mg of tissue in 400 μl of buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 1 mg/ml aprotinin, 1 mg/ml leupeptin, pH 7.9). The cells were allowed to swell on ice for 20 min after which 25 μl of 10% NP-40 was added and the tube vigorously vortexed for 10 s. The homogenate was centrifuged for 50 s at top speed. The supernatant containing the cytosolic fraction was transferred to a fresh tube, whereas the nuclear pellet was resuspended in 50 μl of ice-cold buffer C (20 mM HEPES, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1 mg/ml aprotinin, 1 mg/ml leupeptin, pH 7.9) and the tube was vigorously rocked for 15 min and centrifuged for 5 min at top speed. The supernatant containing the nuclear proteins was stored at −70 °C until use.

Gel electrophoresis and DNA binding

Nuclear extracts were prepared as outlined above from prostates dissected from the experimental animals, and approximately 15 μg were incubated on ice with 1 μg of poly (dI–dC) as non-specific competitor and 1 ng of [^{32}P] end-labeled double-stranded