

Reproductive role of prolactin

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Abstract

The biological actions of prolactin (PRL), a polypeptide hormone, are mostly related to lactation and reproduction. These actions have been clarified by studies of PRL and PRL-deficient receptor mice, which have a clear phenotype of reproductive failure at multiple sites. This review aims to summarize current knowledge about PRL and its receptor, role in reproductive axis and presents information of hyperprolactinemia in reproductive medicine. Our understanding of the physiology and transduction pathway of PRL has largely increased in the past 20 years with the cloning of PRL and its receptor gene.

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Prolactin

Prolactin (PRL) is mainly synthesized and secreted by the lactotrop cells of the pituitary (Freeman *et al.* 2000), but also by extrapituitary sites such as mammary gland, placenta, uterus and T lymphocytes (Ben-Jonathan *et al.* 1996). Its amino acid sequence is similar to that of growth hormone (GH) and placenta lactogen (PL) sharing genomic, structural and biological features and belonging to the same PRL/GH/PL protein family. It is now considered that PRL is a cytokine, based on both molecular and functional evidence (Horseman & Yu-Lee 1994).

The gene encoding PRL is unique, in humans it is located on chromosome 6 (Horseman & Yu-Lee 1994), and was initially described as containing five exons and four introns for an overall length of 10 kb, since then, an additional exon 1a has been described. After removal of the signal peptide (28 residues), the mature form of the protein contains 199 residues (23 kDa). Several variants of PRL resulting from posttranslational modifications have been identified (Sinha 1995). Moreover, it has been described some proteolytic cleavage of PRL via cathepsin D, resulting into 16 and 6 kDa fragments (Baldocchi *et al.* 1993) or via kallikrein giving rise to a 22 kDa fragment. Very recently, it has been shown that cathepsin D, secreted from various tissues, is able to process PRL into anti-angiogenic 16 kDa PRL outside the cell. The data support the concept that secreted lysosomal enzymes could be involved in the maintenance of angiogenesis dormancy via the generation of

active anti-angiogenic peptides in nonpathological contexts (Piwnica *et al.* 2006). Phosphorylation of PRL constitutes 5–30% of the PRL released by the pituitary, but the function of PRL phosphorylation is widely debated. In fact, it has been shown that phosphorylated PRL can have agonistic and antagonistic properties (Bernichtein *et al.* 2001, Wu *et al.* 2003). The function of some other modifications of PRL (by glycosylation, deamination, sulfonation and polymerization; Sinha 1995) are not well studied.

Prolactin receptor and signaling

PRL, as well as PL and primate GH, binds the same PRL receptor (PRLR). Multiple isoforms of membrane-bound PRLR resulting from alternative splicing of the primary transcript have been identified in several species (Bole-Feysot *et al.* 1998). These different PRLR isoforms (short and long) differ in the length and composition of their cytoplasmic tail.

The understanding of how PRL transmits diverse signals to target cells is facilitated by the elucidation of the JAK/Stat pathway as the prototype signaling pathway used by all cytokine receptors (Fig. 1). PRL is believed to activate sequentially the receptor by dimerizing two identical receptor subunits, leading to activation of Jak2 kinase associated with the cytoplasmic domain. Recently, it has been reported for the growth hormone receptor (GHR) that dimerization alone is insufficient to activate full-length receptor; then it has been proposed for the GHR, an activation mechanism involving a

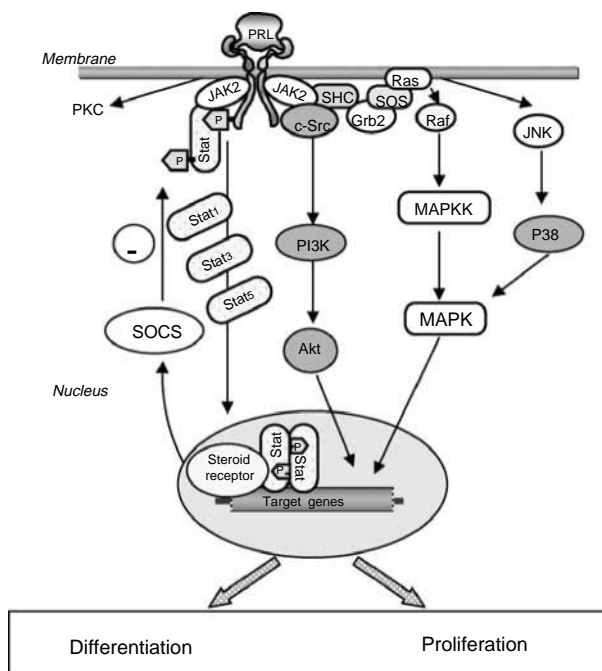


Figure 1 Signal transduction pathways of PRL. PRL-induced receptor dimerization induces the association of the Jak2 kinase, resulting in the activation of Jak2, PRLR phosphorylation, and the association and phosphorylation of Stat1, 3 or 5 proteins. This triggers Stat dimerization and nuclear translocation and events necessary for PRL-triggered differentiation. Signaling through the SHC/GRB2/Ras/Raf/MEK/MAPK pathway also directly stimulates proliferation and modulates Stat activity. Activation of the kinase Akt is directly tied to the PRL-induced activation of PI3K. Members of the CIS family, also known as SOCS, also modulate cytokine signaling. These proteins are rapidly induced after prolactin stimulation with distinct time courses depending on the CIS/SOCS family member. They interact with the receptors and/or Jak2, and either inhibit signaling or relieve suppression.

relative rotation of subunits within a dimeric receptor as a result of asymmetric placement of the receptor-binding sites on the ligand (Brown *et al.* 2005). Very recent studies have suggested that long and intermediate PRLR receptor isoforms could also be dimerized irrespective of the ligand binding (Tan *et al.* 2005, Gadd & Clevenger 2006, Qazi *et al.* 2006). Hormonal stimulation of PRLR leads to tyrosine phosphorylation of several cellular proteins, including the receptor itself. The PRLR–Jak2 interaction involves the membrane-proximal region of the PRLR cytoplasmic domain, in agreement with the ability of the short PRLR isoform to associate with the kinase (Binart *et al.* 2003a). It is assumed that activation of Janus kinases occurs by trans-phosphorylation of tyrosines upon ligand-induced oligomerization of cytokine receptors, which brings two JAK molecules close to each other (Ihle & Kerr 1995). Jak2 Phosphorylates tyrosine residues on different target proteins, the best identified of which is the receptor itself and a family of transcription factors termed signal transducers and activators of transcription (Stats). These factors are signaling the molecules containing consensus domains involved in protein–protein interactions, such as SH2 or PTB domains recruited by phosphorylated tyrosines of the PRLR–Jak2 complex. Stat proteins exist within the cytoplasm in a latent or inactive state; they are recruited by cytokine receptor complexes through an interaction

involving a phosphotyrosine (on the cytokine receptor and/or the associated JAK) and the SH2 of the Stat protein. Three members of the Stat family have been thus far identified as transducer molecules of the PRLR: Stat1, Stat3 and, mainly, Stat5 (both a and b isoforms). Stat5 was originally identified as mammary gland factor (MGF; Wakao *et al.* 1995) and is the major Stat activated by the PRLR. It is preferentially recruited by the C-terminal tyrosine of the receptor. Both Stat1 and Stat3 also have been reported to be activated by the PRLR. After activation, Stats dimerize and migrate to the nucleus, where they specifically interact with DNA sequences (a palindromic consensus sequence TTC xxx GAA (Ihle 1996)) within the promoters of target genes. Then they bind to their cognate DNA-binding sequence resulting in promoter transactivation under appropriate conditions. Once bound, Stats engage several elements of the transcriptional machinery, stimulating gene expression. Very recently, a novel mechanism of PRL signaling has been identified in mammary epithelial cells demonstrating that nuclear Jak2 regulates the amount of active nuclear transcription factor through tyrosine phosphorylation and proteasomal degradation (Nilsson *et al.* 2006).

Although the Jak2–Stat cascade is the major signaling pathway used by the PRL receptor, other transducing pathways are also involved in signal transduction by this receptor. Signaling through MAP kinases (MAPK)

involves the Shc/Grb2/SOS/Ras/Raf/MAPK cascade. Activation of the MAPK pathway has been reported in different cellular systems under PRL stimulation (see (Bole-Feysot *et al.* 1998)). Activation of the nucleotide exchange protein Vav has also been reported (Clevenger *et al.* 1995). It has also been shown that PRL induces a rapid tyrosine phosphorylation of the insulin receptor substrate 1 (IRS-1) and of the 85 kDa subunit of the phosphatidylinositol (PI)-3' kinase (Berlanga *et al.* 1997). Both PI-3' kinase and IRS-1 appear to associate with the PRLR in a PRL-dependent manner. The existence of two PRL-dependent signaling cascades has been initiated by the c-Src-mediated activation of Fak/Erk1/2 and PI3K pathways that control the expression of c-Myc and cyclin D1 and the proliferation. In agreement with the fact that most transducer molecules are activated by tyrosine phosphorylation (JAKs, Stats, Src, etc.), involvement of tyrosine phosphatases to modulate or down-regulate the signaling cascades is expected. It has been reported that the phosphatase SHP-2 is activated by Jak2 and acts as a positive regulator of PRLR-dependent induction of β -casein gene transcription (Ali *et al.* 1996). Moreover, a group of molecules whose expression is induced by PRL feedbacks to inhibit further signaling from the PRLR. These include suppressors of cytokine signaling (SOCS), which bind to and inhibit JAK; and PIAS (protein inactivator of activated Stat), which bind to and inhibit Stats. These data would indicate that JAK inhibition by SOCS proteins occurs either as a binary complex between JAK–SOCS or as a ternary complex constituted of JAK–SOCS–receptor.

PRL and reproduction

PRL exerts multiple effects, regulating both differentiation and proliferation in diverse tissues (Bole-Feysot *et al.* 1998). A large body of literature attests that lactogenic hormones play a role in reproductive function. A number of PRL key functions have been clarified from studies of transgenic and knockout model mice. Accordingly, PRL $^{-/-}$ female mice are completely infertile. After mating with males of established fertility, no litters were produced following several matings. Each female mated repeatedly at irregular intervals, without entering a state of pseudopregnancy. Estrous cycles were irregular, and individual females failed to establish any consistent pattern of cycling. All these observations led to the conclusion that PRL is essential to female reproduction (Horseman *et al.* 1997).

Function of the ovarian corpus luteum

PRL plays a critical role in corpus luteum maintenance and progesterone production in rodents (Risk & Gibori 2001), but not in other mammals; while synthesis of PRL by the decidua is unique to humans. PRLR $^{-/-}$ female

mice showed an absence of pseudopregnancy and an arrest of egg development immediately after fertilization, with only a few reaching the stage of blastocysts (Ormandy *et al.* 1997). The outcome is a complete sterility. Uterine preparation for embryo implantation is dependent upon continued estrogen and progesterone secretion by the corpus luteum, which is supported by a functional pituitary during the first half of pregnancy in rodents. Thus, whereas PRLR $^{-/-}$ females cannot implant blastocysts, the defect of the preimplantation egg development can be rescued by exogenous progesterone, indicating that one of the actions of PRL is to stimulate ovarian production of progesterone. However, although implantation occurs, full term pregnancy is not achieved (Binart *et al.* 2000), most probably because of the absence of decidual PRLR. Indeed, the rodent decidua was shown to express both PRL (Prigent-Tessier *et al.* 1999) and its receptor (Gu *et al.* 1996). The role of decidual PRL appears to involve the local stimulation of the estradiol receptor and the inhibition of decidual IL-6 and 20 α -HSD (hydroxysteroid dehydrogenase), both of which are detrimental to fetal life. Cells of PRLR $^{-/-}$ corpus luteum failed to organize appropriately and underwent dramatic apoptosis, clearly demonstrating the anti-apoptotic role of PRL (Grosdemouge *et al.* 2003; Fig. 2). Proliferation of endothelial cells is required for the neovascularization during luteal development, which results in the corpus luteum's extensive capillary network. It is known, in rats, that PRL secretion induced by mating leads to the increased endothelial cell proliferation in the corpus luteum of pregnancy. Its survival beyond diestrus depends on PRL stimulation and it is associated with an increase in luteal size and vascularization. In the PRLR $^{-/-}$ corpus luteum, such a vascularization failed to develop and thus the presence of the PRLR appears to be crucial for the induction of vascular factors. Indeed, low levels of progesterone in PRLR $^{-/-}$ are independent of the proliferative state and the accumulation of corpora, undergoing regression expressing high levels of 20 α -HSD, would further contribute to a decrease in the levels of progesterone. The ovulation rate is not different between PRLR $+/+$ and PRLR $^{-/-}$ mice, and the corpus luteum is formed but an elevated level of apoptosis and extensive inhibition of angiogenesis occur during the luteal transition in the absence of PRL signaling. These modifications lead to the decrease of luteinizing hormone (LH) receptor expression and consequently to a loss of the enzymatic cascades necessary to produce adequate levels of progesterone, which are required for the maintenance of pregnancy. In the reproductive system, PRL induces transcription of the estrogen receptor (Frasor & Gibori 2003) and the enzyme 3 β -hydroxysteroid dehydrogenase involved in progesterone synthesis (Feltus *et al.* 1999).

The mechanism controlling luteal function, principally in ruminants, has been largely reviewed

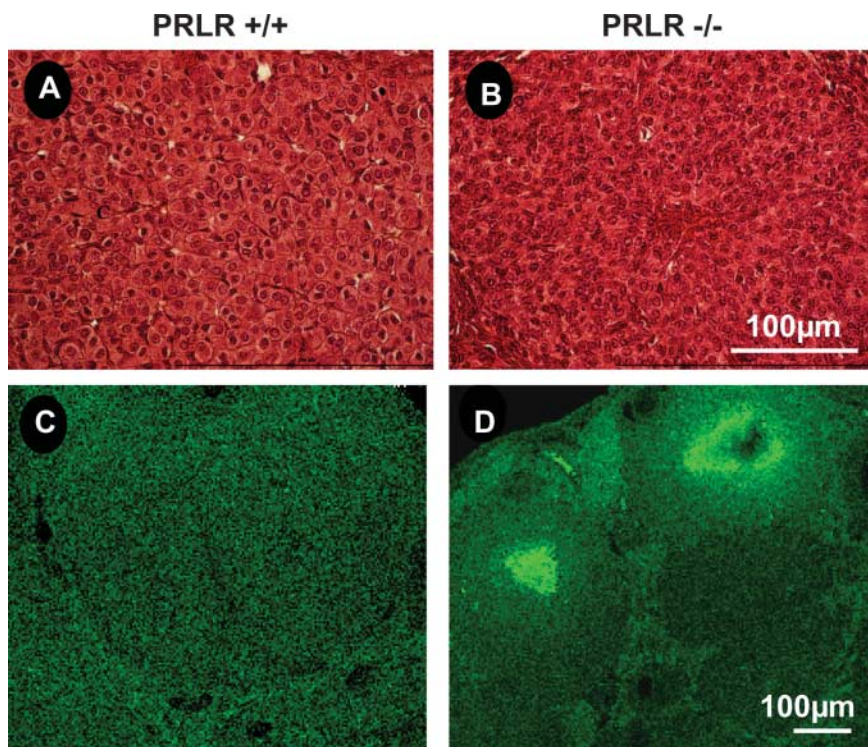


Figure 2 Histological analysis of ovary from wild-type and PRLR^{-/-} mice. Representative view of corpus luteum sections of ovaries from PRLR^{+/+} (A) and PRLR^{-/-} (B) from 8-week-old female mice. Detection of apoptosis in control ^{+/+} (C) and PRLR^{-/-} (D) corpora lutea by the TUNEL method at day 2.5 after fertilization. Apoptotic nuclei are labeled green by TUNEL with fluorescein detection. Analysis of sections by confocal microscopy exhibits fluorescence only in PRLR^{-/-} ovaries, where several apoptotic nuclei are present in PRLR^{-/-}. Scale bar is 100 μ m.

(Niswender *et al.* 2000, Webb *et al.* 2002), other aspects of luteal function such as angiogenesis and overall role of the luteal microvasculature have also been revisited (Reynolds & Redmer 1999, Davis *et al.* 2003, Tamanini & De Ambrogi 2004).

Development of the mammary gland

Continued proliferation and branching lead to the formation of a small ductal tree at birth, whereas the fat pad forms the stroma of the adult mammary gland. Mammary glands develop from the surface epithelium and underlying mesenchyme; however, the molecular controls of embryonic mammary development are largely unknown. The essential hormonal factors, regulating the later phases of mammary gland development in mice, have been established to be estrogen, glucocorticoids and GH during puberty, and estrogen, progesterone and placental lactogen and/or PRL during pregnancy (Nandi 1958, Neville & Daniel 1987). Functional development of mammary epithelium during pregnancy depends mostly on PRL signaling. Proliferation and differentiation of secretory mammary epithelium is dependent on the presence of PRL receptor as well as its downstream Jak2–Stat5 pathway. However, the underlying molecular and cellular events are not completely understood. The specific contributions of the PRL receptor and the transcriptional factors Stat5a and 5b, in the formation and differentiation of mammary alveolar epithelium, have been explored (Miyoshi *et al.* 2001). By transplantation of PRLR- and Stat5-null

mammary epithelia into wild-type hosts, the pregnancy-mediated development was investigated at both histological and molecular level. Stat5-null mammary epithelium developed ducts but failed to form alveoli, and no milk protein gene expression was observed. In contrast, PRLR-null epithelium formed alveoli-like structures with small open lumina. Electron microscopy revealed a perturbation of cell–cell contacts in PRLR- and Stat5-null epithelia. Expression of NKCC1, an Na–K–Cl cotransporter characteristic for ductal epithelia, and a protein associated with tight junction (ZO-1), were maintained in the alveoli-like structures of PRLR- and Stat5-null epithelia. In contrast, the Na–Pi cotransporter Npt2b, and the gap junction component connexin 32, usually expressed in secretory epithelia, was undetectable in PRLR- and Stat5-null mice. Collectively, these data demonstrate that signaling via the PRLR and Stat5 is critical for the proliferation and differentiation of mammary alveoli during pregnancy.

Mammary development in PRL (Horseman *et al.* 1997) and PRL receptor (Ormandy *et al.* 1997) null mice is essentially blocked at the stage of extended ductal outgrowths, which is reasonable since most of the development of the mammary gland in mice occurs during pregnancy and since these female are sterile. Transplantation experiments of PRLR^{-/-} epithelium to the mammary fat pad of an immunocompromised animal allows examination of PRLR^{-/-} mammary gland development in a normal endocrine environment, allowing development during pregnancy to be assessed. The transplanted PRLR^{-/-} epithelium develops side

branches in virgin animals, however during pregnancy a complete failure of lobulo-alveolar development is observed and only alveolar buds are produced (Briskin *et al.* 1999). In agreement, the mammary glands of PRLR^{-/-} animals that could maintain pregnancy, following progesterone treatment, also experience failure of lobulo-alveolar development (Binart *et al.* 2000; Fig. 3). PRL receptors are present in the stroma (Camarillo *et al.* 2001, Hovey *et al.* 2001), but play no direct role in mammary gland development. In fact in mammary glands, constituted of PRLR^{-/-} stroma and PRLR^{+/+} epithelium (Ormandy *et al.* 2003), a normal development occurs but it is not known whether these receptors are essential for the lactation function.

The impaired mammary development and alveolar differentiation seen during pregnancy in the hemizygous PRLR mice suggests that the level of signaling flux initiated by PRL can modulate mammary gland development. It corresponds to a reduction in Stat5 phosphorylation levels, and markedly reduced expression of milk protein genes. Development of the glands in these mice is arrested at around mid pregnancy. While PRL activates Stat5 only in the mammary epithelium, GH activates Stat5 preferentially in the stroma. Ductal development in GHR-null mice is impaired; these observations support

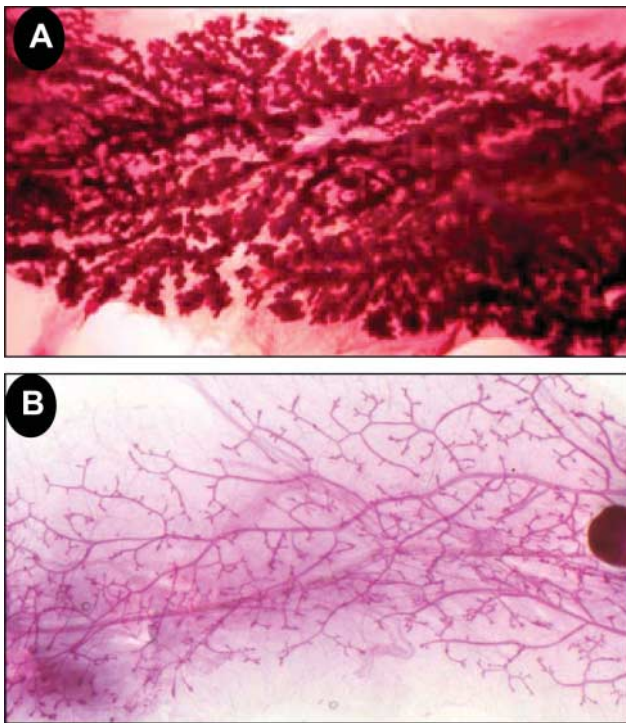


Figure 3 Histological analysis of mammary gland from wild-type and PRLR^{-/-} mice. Representative ductal branching and lobulo-alveolar development of pregnant wild type, and knockout mice. The fourth inguinal mammary gland was analyzed using whole-mount histology at day 18.5 of pregnancy in wild-type (A) and in progesterone treated PRLR^{-/-} mouse (B).

the notion that GH signals through the stromal compartment. Taken overall, it has been demonstrated that GH and PRL activate Stat5 in separate compartments, which in turn reflects their specific role in ductal and alveolar development and differentiation (Gallego *et al.* 2001).

Mice in which either one or both *Stat5a* and *Stat5b* genes were inactivated have revealed unique and redundant roles of the two Stat5 isoforms. *Stat5a* deficiency results in the loss of PRL-dependent mammary gland development (Liu *et al.* 1997), in contrast, inactivation of *Stat5b* does not adversely affect mammary development and function but leads to severe growth retardation (Udy *et al.* 1997).

In mice, alternative splicing of the *PRLR* gene results in four different forms, each having a different cytoplasmic length. The function of the long form is now clearly established, however the functions of the three short forms of the receptor are not well characterized. Transgenic mice expressing the rat short form (F3-SPRLR) of the receptor in the mammary glands results in reduced alveolar development, Stat5 phosphorylation and milk protein expression but no change in proliferation (Saunier *et al.* 2003), suggesting that this form may act as a dominant negative to control the rate of differentiation occurring in the mammary gland. Transgenic expression of another short form (S1) in PRLR^{+/-} mice, normally unable to lactate following their first pregnancy, restores mammary gland development at the level of proliferation and differentiation, indicating that this form acts in a similar way to the long form (Binart *et al.* 2003a). The function of the short S2 form has not been evaluated to date.

A number of transcript profiling studies have recently been undertaken using these mice models; they should reveal molecular candidates regulated by PRL at the transcriptional level. These candidates include cytokeratins, cell adhesion molecules, transcription factors and a number of growth factors already shown important for mammary development. Among them, IGF2 and RankL have been demonstrated crucial. These data highlight a new role of IGF2 as a mediator of PRL-induced morphogenesis in breast (Briskin *et al.* 2002, Hovey *et al.* 2003) and indicate that IGF2 acts downstream of PRL and upstream of cyclin D1.

Maternal behavior

Maternal behavior in PRL^{-/-} mice is normal (Horseman *et al.* 1997), however pup-induced maternal behavior in both virgin and pregnant PRLR^{-/-} or PRLR^{+/-} mice is significantly reduced, independent of other behavioral activities and olfactory function, implicating the receptor as an important regulator of maternal behavior (Lucas *et al.* 1998). Moreover, the decrease in maternal behavior is associated with failure of lactation in PRLR^{+/-} females (Lucas *et al.* 1998).

In addition, the molecular mimic of phosphorylated PRL, S179D, is able to delay the onset of normal maternal behavior in rats (Bridges *et al.* 2001). During pregnancy and lactation, it has been recently reported that new neurons produced in the forebrain migrate to the olfactory bulb where they likely participate in processing olfactory cues received by the new mother as they adapt to the needs and challenges of raising pup (Shingo *et al.* 2003). These new neurons established functional connections on day 7 of pregnancy as well as day 7 of lactation, it was also apparent in pseudopregnant females and that the response could be generated solely by changes associated with mating. The authors demonstrated that neurogenesis was inducible by either systemic or central administration of PRL. This constitutes the first demonstration that a hormone can stimulate the genesis, migration and differentiation of neurons in the adult mammalian brain and the first evidence that rates of neurogenesis can be influenced by alterations in the endocrine state.

PRL and male function

A preliminary analysis of male fertility described a proportion of 20% of PRLR^{-/-} infertile males, however this was not due to a defect in mating behavior (Grosdemouge *et al.* 2003). Two subsequent studies using separate PRLR^{-/-} mouse lines that originated from this initial study population, but which have since diverged, have re-examined this phenotype. One study found no effect on fertility (Binart *et al.* 2003b), whereas the other showed that a proportion of 10% are infertile and those that are fertile only have a 40% chance of producing a successful first pregnancy (Robertson *et al.* 2003). These contrasting data may be due to the effect of PRL on male reproduction being modified by divergence in the genetic background. Moreover, PRL^{-/-} males are fertile, although PRL does play a role in reproductive neuroendocrine function by control of LH release (Steger *et al.* 1998). Then, it appears that PRL plays a modest role on male fertility contrary to that on female fertility.

PRL and its receptor are expressed in human and rat prostate epithelial cells, where their level is increased by androgen treatment (Nevalainen *et al.* 1997a), it has been also viewed as an autocrine/paracrine growth factor (Nevalainen *et al.* 1997b) or a survival factor (Ahonen *et al.* 1999) for the prostate epithelial cells *in vitro*. Prostate weight is slightly increased in young PRLR^{-/-} animals and some changes in epithelial and stromal content of the dorsal lobe occur probably regulated by cooperation between PRL and androgens (Robertson *et al.* 2003). PRL has a subtle role in the prostate, whereas hyperprolactinemia has a direct effect on hyperplasia of the prostate. This is in accordance with the role of PRL on cellular proliferation.

Prolactin and reproductive medicine

Against a background of substantial knowledge of the biology of PRL, a large amount of clinical experience has been gathered about hyperprolactinemia in humans and its best treatment. Circulating serum PRL in normal individuals is thought to reflect almost entirely pituitary PRL secretion: other extra pituitary sources probably contribute significant amounts mainly to the local tissue environment. In contrast to what is observed for other pituitary hormones, no mutation of the *PRL* gene or of the *PRLR* gene has been described yet, so that there is no clinical model to clearly evaluate the consequences of the absence of PRL actions in humans.

Clinically, high PRL levels have been associated with certain autoimmune diseases (Walker *et al.* 1998). There are also several intersections between PRL, psychological stress and chronic hyperprolactinemia reviewed in (Sobrinho 2003). Either higher or lower than normal PRL levels have been shown to compromise the immune responses in animal models (Elbourne *et al.* 1998). *In vitro* PRL has been shown to modulate the proliferation and effector function of T and B cells, macrophages and natural killer cells. Similarly, *in vivo* thymocytes and splenocytes synthesize and secrete PRL acting as autocrine or paracrine growth factor (Yu-Lee 1997).

PRL secretion is under dual regulation by hypothalamic hormones via the pituitary portal circulation. The predominant regulatory signal is the inhibition of PRL secretion by the neurotransmitter, dopamine, from neurons in the hypothalamus. The stimulatory signal for PRL secretion can be mediated by other factors including thyrotropin releasing hormone (TRH). Except for high levels of PRL observed during pregnancy and lactation, hyperprolactinemia is a pathological condition at any age. Any process (i.e. some medications) that disrupts dopamine secretion or interferes with the delivery of dopamine to the portal vessels may cause hyperprolactinemia (Table 1). Psychotropic agents causing hyperprolactinemia include the neuroleptics, tricyclic anti-depressants, monoamine oxidase inhibitors, serotonin reuptake inhibitors, opiates and cocaine, as well as several anti-hypertensive medications cause hyperprolactinemia, including α -methyl dopa, reserpine and verapamil. About 40% of all pituitary adenomas are prolactinomas. Micro- (<1 cm) or macroprolactinomas (>1 cm) contain lactotrops that secrete PRL. Age prevalence varies widely, and prolactinomas have been reported in patients from 2 to 80 years old. Prolactinomas are more common in women, with a peak incidence during the childbearing years.

The *postpartum* period is characterized hormonally by elevated levels of PRL and low levels of gonadotropins and sex steroids. During breast feeding, this state of *postpartum* amenorrhea can persist for an extended period, even though PRL levels decrease slowly. Although the action of PRL on multiple target sites has frequently been suggested as the cause of this ovarian quiescence, a

Table 1 Causes of pathological hyperprolactinaemia.

Pituitary disorders	Pituitary tumours: micro- or macroprolactinoma, hypothalamic stalk interruption	Disruption of dopamine delivery from the hypothalamus and/or secretion of prolactin by the adenoma
Medications	Empty Sella syndrome Infiltrative diseases (tuberculosis, sarcoidosis) Anti-psychotics (phenothiazines, haloperidol, butyrophenones, risperidone, monoamine oxidase inhibitors, fluoxetine, sulphiride) Anti-emetics (metoclopramide, domperidone) Anti-hypertensives (methyldopa, calcium channel blockers, reserpine) Tricyclic antidepressants Selective serotonin re-uptake inhibitors Opiates Estrogens Verapamil Protease inhibitors	Damage to/regression of the pituitary Infiltration of pituitary Inhibition of dopamine release Stimulation of hypothalamic opioid receptors Positive action on lactotrophs Unknown
Primary hypothyroidism		Increased hypothalamic thyrotrophin-releasing hormone and decreased metabolism
Reduced prolactin elimination	Renal failure Hepatic insufficiency	

suckling-induced alteration in hypothalamic gonadotropin releasing hormone (GnRH) production has also been suggested (Zinaman *et al.* 1995). Indeed, some authors demonstrated that hyperprolactinemia produces hypogonadism primarily by interfering with pulsatile GnRH release in males (Bouchard *et al.* 1985).

In young women, hyperprolactinemia is one of the most common endocrine disorders of the hypothalamic–pituitary axis. It can be defined as the presence of an abnormally high level of PRL in the blood (normal levels are typically 10–28 µg/L in women and 5–10 µg/L in men). The prevalence of hyperprolactinemia varies in different patient populations, from 0.4% in an unselected normal population to up to 17% of women with reproductive disorders (Crosignani 1999). The increase in PRL levels observed in pathological hyperprolactinemia results in effects equivalent to those observed during the *postpartum* period, namely inhibition of the release of GnRH from the hypothalamus and subsequent inhibition of LH and follicle stimulating hormone (FSH), suppressed gonadal function. The consequence is that an elevated serum PRL from any cause results in anovulation with amenorrhea in women, but the degree of hypogonadism is frequently proportionate to the size of increase in PRL levels. In cases of marked hyperprolactinemia, amenorrhea and galactorrhea are frequently observed, while mild hyperprolactinemia may be associated with a short luteal phase and anovulatory infertility.

Multiple sampling has again revealed that LH (GnRH) pulse secretion is slow and of irregular amplitude. However, suppression of PRL by dopamine agonists rapidly restores pulsatile LH release, and ovulation usually ensues within few weeks. The irregular, slow-frequency GnRH pulses again appear to reflect increased hypothalamic opioid activity (Marshall *et al.* 2001).

Hyperprolactinemia in men may result in decreased libido, impotence, decreased sperm production,

infertility (Colao *et al.* 2004). When hyperprolactinemia is caused by a prolactinoma, additional pressure symptoms may include headaches, visual field defects and abnormal pituitary function. The objectives of hyperprolactinemia therapy are to improve the symptoms associated with high PRL levels and to reduce the size of a pituitary tumor. Pharmacotherapy involves the use of dopamine agonists, which are able to decrease tumor size and reduce PRL secretion (Molitch 2003). The treatment of most patients is readily achieved using dopamine D2 receptor agonists, which can normalize serum PRL levels and shrink prolactinomas suitably to prevent any need of surgery.

Conclusion

The generation of a number of targeted mutagenesis models is increasingly being used to study reproductive function and also to understand fundamental processes of development. Until a few years ago, there was a strong discrepancy between the biological versatility of PRL and the paucity of clinical arguments to suggest a role for PRL in any human diseases. While awaiting the eventual identification of pathologies, resulting from genetic defects of PRL or PRLR, one immediate goal will be to understand how the amazing number of puzzling reports describing targets, mechanisms of actions or functions of PRL can be linked together and integrated into an overall physiological relevance of this pleiotropic hormone.

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