PROLACTIN: The New Biology of an Old Hormone

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Abstract Prolactin (PRL) is a paradoxical hormone. Historically known as the pituitary hormone of lactation, it has had attributed to it more than 300 separate actions, which can be correlated to the quasi-ubiquitous distribution of its receptor. Meanwhile, PRL-related knockout models have mainly highlighted its irreplaceable role in functions of lactation and reproduction, which suggests that most of its other reported target tissues are presumably modulated by, rather than strictly dependent on, PRL. The multiplicity of PRL actions in animals is in direct opposition to the paucity of arguments that suggest its involvement in human pathophysiology other than effects on reproduction. Although many experimental data argue for a role of PRL in the progression of some tumors, such as breast and prostate cancers, drugs lowering circulating PRL levels are ineffective. This observation opens new avenues for research into the understanding of whether local production of PRL is involved in tumor growth and, if so, how extrapituitary PRL synthesis is regulated. Finally, the physiological relevance of PRL variants, such as the antiangiogenic 16K-like PRL fragments, needs to be elucidated. This review is aimed at critically discussing how these recent findings have renewed the manner in which PRL should be considered as a multifunctional hormone.

PROLACTIN

Structure and Regulation

Prolactin (PRL) is a polypeptide hormone discovered more than 70 years ago (1) to be a pituitary factor that stimulates mammary gland development and lactation in rabbits, a function from which its name “pro-lactin” originated (2). The gene encoding PRL is unique and is found in all vertebrates. In humans, it is located on chromosome 6 (3). It was initially described as containing five exons and four introns, for an overall length of 10 kb (4); 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
(5), some of which considerably enlarge the field of action of the hormone (see below).

PRL is mainly secreted by lactotrophic cells of the anterior pituitary. It is widely accepted that pituitary PRL secretion is positively and negatively regulated, but it is mainly controlled by inhibitory factors originating from the hypothalamus, the most important of which is dopamine, acting through the D2 subclass of dopamine receptors present in lactotrophs. It is interesting to note that mice in which the PRL receptor (PRLR) gene has been invalidated (6) are hyperprolactinemic, reflecting feedback of PRL on its own secretion (7). This negative regulation may be direct on lactotrophs or indirect via an action on neuroendocrine dopaminergic neurons that have been shown to express PRL receptors. The current view of the regulation of PRL synthesis integrates an extremely wide spectrum of molecules (hormones, neurotransmitters, neuropeptides, etc.), the description of which has been recently reviewed (8).

The PRL gene is regulated at the transcriptional level by two distinct promoters. The proximal promoter, also referred to as the pituitary promoter, covers \(~5\) kb upstream of the transcription site, in which the 250 bp just before the Cap (capping of polymerase on RNA) site (in exon 1b) are necessary and sufficient for transcription (9). The second promoter, referred to as the extrapituitary promoter, includes \(~3\) kb upstream of exon 1a (itself located \(~5.8\) kb upstream from the initiation site) and was initially described as directing PRL expression in lymphoid and decidual cells (10, 11). Depending on promoter usage, PRL mRNAs differ in length by 134 bp, but they encode identical mature protein. The dichotomy of promoter usage (pituitary versus extrapituitary) needs to be revisited in view of recent data, which may considerably enlarge our understanding of the mechanism of action of PRL, especially when acting locally (autocrine/paracrine effect).

**PRL Is a Cytokine**

The amino acid sequence of PRL is similar to that of two other polypeptide hormones, growth hormone (GH) and placental lactogen (PL). Because these homologous proteins share genomic, structural, immunological, and biological features, they have been grouped within a protein family called the PRL/GH/PL family (12, 13). More recently, PRL/PL/GH hormones have been linked to a still more extended family of proteins, referred to as hematopoietic cytokines (14). Considering PRL as a cytokine is based on both molecular and functional evidence. The first, although indirect, argument is that its receptor is a member of the cytokine receptor superfamily. Second, PRL is predicted to adopt the up-up-down-down four \(\alpha\)-helix bundle fold (15) characteristic of hematopoietic cytokines (14). Third, similarly to well-recognized cytokines, PRL was shown to act on cells of the immune system, although considerable controversy exists concerning the true immunomodulatory role of PRL (see below).
PROLACTIN RECEPTOR

Structure and Distribution

Nearly three decades ago, PRLR was identified as a specific, high-affinity, saturable membrane-bound protein (16). In addition to the short PRLR isoform whose cDNA was originally cloned (17), other isoforms referred to as long or intermediate, based on their overall length, have been identified. Within a given species, the extracellular (ligand-binding) domain is identical, with only the cytoplasmic tail differing. Extensive descriptions of these various PRLR isoforms have been published (18).

The gene encoding the human PRLR is unique and is located on chromosome 5; it contains at least 10 exons, for an overall length >100 kb (19). It is interesting that the genomic organization (coding sequences of exons) closely parallels the functional/folding domains of the mature proteins (20). The 5′ UTR of the PRLR gene contains several promoters whose tissue-specific usage in various species has been proposed (20–22). PRLR is virtually expressed in all organs and/or tissues, and the level can be very low in certain cells, such as those of the immune system; depending on cell type, levels vary from ~200 to ~30,000 receptors per cell. The expression of the various isoforms has been shown to vary as a function of the stage of the estrous cycle, pregnancy, and lactation. However, because of the extremely broad distribution of PRLR, it is currently difficult to propose a general overview of its regulation of expression (18).

The receptors for GH (23), PRL (17), and a few other cytokines were the pioneering members of the superfamily of cytokine receptors (24), which currently includes more than 30 members (25). These receptors share typical features, such as two disulfide bonds and a duplicated Trp-Ser sequence named the WS motif within the receptor extracellular domain (18).

Activation of PRLR

The PRLR binds to at least three types of ligands: PRL, PL, and primate GHs (13). This complicates our understanding of the biological effects induced by PRL in vivo because ligands other than PRL activate the prolactin receptor, especially in humans, where the contribution of GH to PRLR-mediated effects occurs. This multiplicity of PRLR ligands may be one of the molecular reasons why the phenotypes observed in PRL knockout (KO) mice are less severe than in PRLR KO mice, because PLs (synthesized during gestation) can still exert their effects via PRLR in PRL-deficient animals (see below). In this context, it is also noteworthy that the receptor for PLs was recently proposed to be a PRLR-GHR heterodimer, although the biological relevance of this observation remains to be demonstrated (26).

Binding of these ligands to PRLR is the first step of receptor activation. Thus far, and in contrast to most cytokine receptors (27), no accessory membrane protein has been shown to be required for effective PRLR signaling. Several studies...
argue that PRLR is activated by dimerization (13), which is mediated by a single molecule of ligand (28). This involves two regions (so-called binding sites 1 and 2), each interacting with one molecule of PRLR (13). Based on this mechanism of activation, PRLR antagonists have been designed by introducing sterically hindering residues within the second binding site of its various ligands, which consequently maintain the ability to bind to the receptor through their site 1 but are no longer able to induce its dimerization (29, 30).

Signal Transduction

Once bound to one of its ligands, PRLR triggers intracellular signaling cascades; there is currently no evidence that the type of ligand (PRL, GH, or PL) affects the nature of the signal transmitted into the cell. Like all cytokine receptors, PRLR lacks intrinsic enzymatic activity and transduces its message inside the cell via a wide number of associated kinases, which in turn activate downstream effectors. The main and best-known cascades involve the Jak/Stat pathway, the Ras-Raf-MAPK pathway, and the Src tyrosine kinases, but other transducing proteins are also involved that have been extensively detailed elsewhere (18, 31). Recently, we generated a list of the genes that are activated by PRL in rat Nb2 lymphoma cells, which shed new light on the genomic targets of this hormone (32).

Site-directed mutational studies have identified within the PRLR cytoplasmic domain some features specifically linked to certain transducing properties, such as specific tyrosine residues that can be phosphorylated and participate in recruiting Stats, insulin receptor substrates (IRS), and adaptor proteins to the receptor complex (18). Depending on the presence or absence of these features, the various PRLR isoforms are thus expected to exhibit different signaling properties. For example, the short PRLR is not tyrosine-phosphorylated, which prevents this isoform from interacting directly with SH2-containing proteins, such as Stat factors. However, such interactions may occur via indirect mechanisms mediated by adaptor proteins (33), or by other proteins included in the receptor complex, such as Jak2, whose phosphotyrosines can recruit Stat5 (34). These alternative mechanisms of protein recruitment by PRLR probably require classical structure-function interactions to be revisited in part. It is interesting to note that heterodimerization of different PRLR isoforms produces inactive complexes (35, 36), which might also be of importance in the physiological context because PRL target cells usually express more than a single PRLR isoform.

Protein tyrosine phosphatases are believed to be part of the signaling down-regulation network, although their mechanism of action is still poorly understood (33, 37). Recently, the SOCS (suppressor of cytokine signaling) gene family was identified as targets of the Jak/Stat pathway and was shown to encode proteins down-regulating this pathway at the level of activation. Although the involvement of individual SOCS proteins in PRLR signaling has been studied mainly using cell transfection approaches (38), the mechanisms by which endogenous SOCS regulate PRLR signaling in more physiological contexts are still to be elucidated.
In contrast to SOCS-2 KO mice whose gigantism presumably identifies this SOCS as a down-regulator of GH function (40), linking any phenotype observed in mice lacking a particular SOCS gene to PRL function has proven to be difficult. Obviously, regulation of SOCS expression is a means by which cross talk between cytokine receptors (including PRLR) could occur, which complicates the interpretation of data provided by KO models.

Finally, another emerging field in PRLR signaling is the occurrence of cross talk with members of other receptor families, such as tyrosine kinases (41, 42) or nuclear receptors (43). Interactions of activated Stats with the latter obviously represents a possible molecular mechanism underlying the integrated regulation of multiple hormone-dependent functions known to involve PRL and, for example, sex steroids.

A NEW LOOK AT THE MULTIPLE FUNCTIONS AND MECHANISMS OF ACTIONS OF PRL

PRL was originally isolated based on its ability to stimulate mammary development and lactation in rabbits, and soon thereafter to stimulate the production of crop milk in pigeons. PRL was also shown to promote the formation and action of the corpus luteum (44). As emphasized by the phenotypes of PRL-related KO models, milk production and reproductive properties are functions that cannot be taken over by other hormones or cytokines. However, the biological role of PRL can no longer be restricted to these actions. We recently listed up to 300 separate functions or molecules activated by PRLR, which we organized into categories related to water and electrolyte balance, growth and development, endocrinology and metabolism, brain and behavior, reproduction, and immunoregulation and protection (18). This extremely broad spectrum of activities should probably be regarded as a panel of functions modulated by, rather than unique to, PRL (or its receptor).

In the next part of this chapter, our goal is not to again make an exhaustive list of these biological functions but rather to suggest why PRL should be considered differently. For this purpose, we summarize some recent experimental findings that may open new avenues of PRL research.

Does PRL Act in the Nucleus?

Although internalization of receptor-bound PRL has been clearly demonstrated, whether hormone-receptor complexes are translocated to the nucleus after internalization has long been debated. Obviously, one of the potential areas of concern is the choice of antibodies used for immunocytochemical studies, which may cross-react with other cellular/nuclear proteins and, hence, lead to misinterpretation of experimental observations. Our group failed to detect PRL or its receptor within the nucleus (45). Others reported that significant nuclear translocation of PRL required the presence of costimulatory factors, such as epidermal growth factor (EGF) or
 interleukins (46), although the molecular mechanisms underlying this observation are unknown. To delineate how nuclear retrotransport of PRL could occur (PRL lacks any consensus nuclear localization sequence), a yeast two-hybrid screen was performed that identified cyclophilin B (which possesses a nuclear localization sequence) as a candidate for interaction with PRL (46). This interaction is thought to occur within the extracellular space, without affecting PRL affinity for its receptor or activation of signaling pathways (46). Exogenous cyclophilin B was shown to enhance not only anti-PRL immunoreactivity in nuclei of target cells, but also cell proliferation, establishing this protein as a chaperone-facilitating nuclear translocation and mitogenic activity of PRL. However, how these molecules/pathways interact to synergistically increase cell responses remains open to investigation, although interactions between PRL–cyclophilin B complex and transcription factors (e.g., Stats) have been cited (46, 47).

Relevance of Extrapituitary PRL

Although the large majority of circulating PRL is of pituitary origin, in the past few years, interest has been raised in locally produced, extrapituitary PRL (48). Recent reports have identified human umbilical vein endothelial cells (49), prostate (50, 51), and myeloid leukemic cells (52) as new PRL sources.

The control of extrapituitary PRL secretion is still poorly understood, both at the transcriptional and at the stimulatory levels. Conventional wisdom has linked the proximal PRL promoter to pituitary gene expression (involving Pit-1 as major activating transcription factor and dopamine as major negative regulator) and the decidual/lymphocyte promoter to extrapituitary gene expression (independent of Pit-1 and dopamine). It is interesting to note that PRL cDNA obtained from various human breast cancer cell lines or biopsies showed that both types of PRL mRNA (differing in their 5’ UTR) are present, reflecting a duality of promoter usage (53). Still more surprising, a recent study has shown that in the SK–BR-3 human mammary tumor cell line, the pituitary and not the decidual/lymphocyte promoter is active, despite the absence of Pit-1 in mammary cells, and a potential stimulatory role has also been proposed for EGF (I. Manfroid, J.A. Martial & M. Muller, personal communication). This pioneering study should shed new light on possible mechanisms of extrapituitary PRL regulation.

Demonstrating the occurrence of an autocrine-paracrine mechanism in PRL target cells is a tricky issue that requires using neutralizing antibodies, hormone antagonists, specific inhibitors, or PRL antisense oligonucleotides to highlight the effects resulting from inhibition of the endogenous hormone. It has been shown that endogenously produced hormones are active at much lower concentrations than those administered exogenously (54, 55). Therefore, although the contribution of local PRL production to circulating PRL levels is presumably low, it may be sufficient to exert significant activity on its local environment. In human breast cancer cells, PRL synthesis has been detected using various approaches (56, 57), and autocrine secretion has been shown to constitutively activate cell proliferation.
via activation of Jak2, which in turn activates both ErbB-2 (one of the multiple EGF receptors) and downstream pathways (58). Similarly, endometrial PRL has been proposed to be an active player in the morphological and functional changes undergone by the decidua from implantation to delivery (59, 60). More than showing a local PRL secretion, these studies demonstrate the functionality of the autocrine/paracrine loop.

**Specific Functions of PRL Isoforms**

Posttranslational modifications are not required for the hormone to be fully active (61). In fact, posttranslational modifications are more often detrimental than beneficial to PRL bioactivity (5). For example, glycosylation lowers biological activity, phosphorylation generates PRL antagonists in some species, and proteolytic cleavage of PRL into 16K PRL abolishes PRLR binding. In this section, we discuss recent findings related to two of these variants, with the aim of integrating their functional specificity within the spectrum of PRL functions.

**(PSEUDO)PHOSPHORYLATED PRL**

To mimic natural PRL phosphorylation (covalent linkage of a phosphate group to serine and/or threonine residues), a recombinant pseudophosphorylated (PP)-PRL has been engineered by substituting an aspartate for serine 179 in the human sequence (62). As anticipated from former reports (63), PP–human (h)PRL was first reported to act as a PRLR antagonist in vitro (62). Accordingly, this analog was shown to reduce tumor incidence of prostate cancer cells injected into Nude mice (64), and to alter maternal behavior in nulliparous female (65) and development of pup tissues (66) in rats. In contrast, PP-hPRL was also reported to promote lobuloalveolar differentiation and casein expression during rat pregnancy (67) and to be even more potent than wild-type hPRL on bone tissue (68), which indicates that this analog also displays agonistic properties in some circumstances. In our hands, using various in vitro bioassays, this PP analog has been shown to be an agonist with no antagonistic properties (69). Hence, using PP-PRL raises more questions than it solves about the true physiological role of phosphorylated PRL, one of which is whether this analog is a true molecular mimic of phosphorylated PRL in vivo. Obviously, further studies using PP-hPRL will need careful interpretation.

**16K PRL**

16K PRL was discovered more than 20 years ago as the N-terminal 16-kDa fragment resulting from the proteolysis of rat PRL by acidified mammary extracts (70). Since then, this PRL fragment has received considerable interest from the scientific community. The protease responsible for the cleavage of rat PRL into 16K PRL was identified as cathepsin D, whose implication in tumor progression is relevant (71). 16K PRL was shown to have lost PRLR binding ability but otherwise to have acquired the ability to specifically bind another membrane receptor (72) through which it exerts antiangiogenic activity (73). Although this receptor is still not identified, some of its downstream signaling targets have been elucidated (74–77).
However, many questions related to the biology of 16K PRL remain unanswered. First, although the majority of investigations have used rat 16K PRL, results are much less clear for other species, especially humans, in which PRL was recently reported to be resistant to cathepsin D (78). This contrasts with our findings indicating that hPRL yields partial, but reproducible, proteolysis leading to N-terminal 16K-like PRL fragments when incubated with this protease. Second, because it may be generated both centrally (79) and at the periphery, such as in pulmonary fibroblasts (77) and endothelial cells (49), the site(s) of 16K PRL generation remain(s) to be clearly identified. Hence, whether all sites of extrapituitary PRL synthesis can generate 16K PRL from endogenous 23K PRL or, alternatively, whether circulating PRL is internalized before the proteolyzed form is exported (or both) remains open to investigation. Also, the subcellular compartment(s) in which appropriate proteolysis conditions are found remain(s) to be identified, although one can not discard the possibility that the cleavage takes place in the extracellular milieu. These few examples underscore the necessity of posing the appropriate questions to demonstrate the physiological relevance of PRL fragments in vivo. In humans, although various recombinant forms of 16K hPRL were shown to be antiangiogenic (76), they do not provide any insight into the biological relevance of 16K hPRL in vivo. Also, the question is raised whether the effects of PRL on tumors in vivo should be viewed from a new angle, considering a balance between the mitogenic and angiogenic (pro-tumor) activities of full-length PRL versus the antiangiogenic (anti-tumor) activity of 16K-like PRL (76, 80).

Lessons from Animal Models

One of the most important recent advances in the study of mammalian genes has been the development of techniques to obtain defined mutations in mice. Often the deletion of a gene that has accepted functions from biochemical and cell biological experiments results in unexpected phenotypes. Frequently this takes the form of no or mild phenotypes and evokes the possible existence of redundantly functioning genes. Efforts to define the functions of PRL and its receptor from the phenotypes of null mutants illustrate these complexities. In this section, we describe the phenotypes resulting from null mutation of PRL or PRLR genes, which are presented as expected, unexpected, and controversial phenotypes.

EXPECTED PHENOTYPES

Reproductive phenotypes  A large body of literature attests that lactogenic hormones play a role in reproductive function. Accordingly, PRL−/− female mice are completely infertile. After several matings with males of established fertility, no litters were produced. 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 (81).
PRLR−/− females also showed an absence of pseudopregnancy and an arrest of egg development immediately after fertilization, with only a few reaching the stage of blastocysts. The outcome is a complete sterility. Divergent effects of PRL on the rate of implantation and development of mouse embryos have been reported (18). Uterine preparation for embryo implantation is dependent on continued estrogen and progesterone secretion by the corpus luteum, which in rodents is supported by a functional pituitary during the first half of pregnancy (82). PRL has been shown to stimulate progesterone synthesis by dispersed ovarian cells from mice in midpregnancy (83), demonstrating that lactogenic hormones can directly stimulate ovarian progesterone secretion. Thus, whereas PRLR−/− females cannot implant blastocysts, the defect of the preimplantation egg development can be completely rescued by exogenous progesterone. However, although implantation occurs, full-term pregnancy is not achieved (7).

Both PRL and PRLR genes are expressed in the uterus (84), which suggests that a paracrine/autocrine effect might be involved. Our observations indicate that preventing PRL action by disruption of the PRLR gene alters the maternal decidual transformation in response to the implanting blastocyst, demonstrating an essential role of PRL in reproduction. PRLR expression has also been reported in human endometrial tissue. PRL is known to be expressed in the decidualized human endometrium and secreted into amniotic fluid. By using in situ hybridization histochemistry techniques, PRL specific hybridization signals were distributed over the decidual cells in early and term pregnancy. Knowing that PRLR is expressed in the uterus (85), it would be interesting to determine whether production of PRL or the expression of PRLR is altered in pathological conditions associated with female sterility.

Furthermore, the expression pattern of such progesterone-dependent genes as amphiregulin, COX-1, and Hoxa-10 was similar in wild-type and steroid-supplemented PRLR−/− mice (85). These results suggest that the correction of reproductive deficits by progesterone in PRLR−/− mice is accomplished by proper expression of progesterone-dependent genes that are essential in early pregnancy. Thus, the rescue of pregnancy failure by progesterone and the cause of pregnancy loss at a later stage in PRLR−/− mice cannot be ascribed to an aberrant spatial expression of genes that normally contribute to the establishment of pregnancy. At early stages of pregnancy, uterine PRLR expression is restricted to a subpopulation of undecidualized cells adjacent to the uterine crypt and in the antimesometrial stroma. Although the function of PRLR in these cells is unknown, we cannot exclude their contribution to normal decidual function.

**Mammary gland development** PRLR+/− mice showed impaired mammary development and alveolar differentiation during pregnancy, which corresponded to reduced phosphorylation levels of Stat5; they also showed impaired expression of milk protein genes. Development of the glands in these mice was arrested at midpregnancy. Although PRL activated Stat5 only in the epithelium, GH and EGF activated Stat5 preferentially in the stroma. Epithelial PRLRs are required for
mammary development and milk protein gene expression during pregnancy. Although GH is not required for alveolar development, we were able to demonstrate its lactogenic function in cultured PRLR-null mammary epithelium. However, ductal development in GHR-null mice was impaired, supporting the notion that GH signals through the stromal compartment. Our findings demonstrate that GH, PRL, and EGF activate Stat5 in separate compartments, which in turn reflects their specific role in ductal and alveolar development and differentiation (86). These results demonstrate that two functional alleles of PRLR are required for efficient lactation and that this phenotype in heterozygotes is primarily due to a deficit in the degree of mammary gland development.

The mammary gland undergoes development in utero, at puberty, and during pregnancy. The essential hormonal factors regulating the later two phases in mice have been established as estrogen, glucocorticoids, and growth hormone during puberty, and estrogen, progesterone, and placental lactogen and/or prolactin during pregnancy (87, 88). These hormones produce some development with each estrous cycle and massive development at pregnancy, which following estrus or weaning never fully regresses, resulting in ever-increasing alveolar and ductal development with each episode (89). Our observations suggest that the epithelial cell proliferation during pregnancy and the postpartum period depends on a threshold of PRLR expression that is not achieved with just one functional allele, given that the level of PRLR is closely controlled in mammary gland (90). Heterozygous mice on C57BL/6 pure background never lactate, even after multiple pregnancies. In contrast, on 129Sv background, mammary gland proliferation is insufficient to insure lactation at the first pregnancy, but further estrous cycles or a single pregnancy lead to the development of a mammary gland capable of producing milk. This demonstrates either that continuous hormonal stimulus can overcome the block, or that compensatory mechanisms are established.

Initial histological investigation of virgin glands of mature wild-type, PRL−/−, or PRLR−/− animals indicated no dramatic differences with ductal tissue present, confirming that PRL stimulus is not essential for this stage of development (6, 81). Because PRLR−/− females are sterile, the effect of the receptor mutation on mammary development during pregnancy has been analyzed by transplanting PRLR−/− mammary epithelium into PRLR+/+ mammary fat pads cleared of endogenous epithelial cells before puberty (91). These results demonstrate that epithelial PRLR is required not for alveolar bud formation but for lobuloalveolar development.

Behavior A number of experimental behavioral studies have clearly established PRLR as a regulator of maternal behavior. A deficiency in pup-induced maternal behavior was observed in PRLR−/− and PRLR+/− nulliparous females. Moreover, primiparous PRLR+/− females exhibited a profound deficit in maternal care when challenged with foster pups (92). By contrast, the PRL gene mutation did not prevent female mice from manifesting spontaneous maternal behaviors (81). Otherwise, eating, locomotor activity, sexual behavior, configural
learning, and olfactory function exploration were normal in PRLR mutant mice (92).

UNEXPECTED PHENOTYPES

**Bone**  Although no particular growth phenotype was observed in PRL−/− animals, examination of the calvariae of PRLR−/− embryos indicates lower ossification compared with controls. In PRLR−/− adults, histomorphometric analysis showed decreased bone formation rate and reduced bone mineral density. We identified PRLR mRNA encoding the long form in osteoblasts, but not in osteoclast-like cells, which suggests that a direct effect of PRL on osteoblasts could be required for normal bone formation and maintenance of bone mass (93).

**Male fertility**  PRL was reported to regulate testosterone production by Leydig cells via modulation of the effects of luteinizing hormone and of the level of its receptor. PRL has been also proposed to be involved in sperm capacitation and to enhance in vitro fertilization rates (94, 95), although others failed to confirm these findings (96). PRL can also influence the function of the accessory reproductive glands (97, 98). We thus expected some abnormalities in PRL-related KO models. In contrast, PRLR−/− (N. Binart, C. Pineau, H. Kercret, A.M. Touzalin, P. Imbert-Bollaré, et al., manuscript in preparation) and PRL−/− (99) males are fully fertile, indicating that PRL is not a key player in the control of male fertility in mice. Histological analysis of testes and accessory glands PRLR−/− did not show any abnormality. Moreover, no alterations were detected in plasma testosterone, and the response to exogenous administration of gonadotropins was also completely normal. Although it was previously shown that PRL can repair the reproductive defect in male pituitary dwarf mice, our data are totally consistent with observation, indicating that PRL deficiency alone is not sufficient to cause male infertility and alterations in basal plasma testosterone concentrations (99).

**Development of lacrimal and Harderian glands**  Analysis of the PRLR KO model suggests that PRL plays a weak role in establishing the sexual dimorphism of male lacrimal glands. In females, hyperprolactinemia causes a hyperfemale morphology, which suggests a role for PRL in dry-eyes syndrome. PRL is required for porphyrin secretion by the Harderian gland but plays no essential role in the secretory immune function of the lacrimal gland (100).

**Metabolic status**  PRL and PLs are known to play a role in carbohydrate metabolism through effects on pancreatic insulin production and peripheral insulin sensitivity; however, the roles of lactogens in lipid metabolism are poorly understood (101). Progressive reduction in body weight associated with a reduction in total abdominal fat mass and in leptin concentrations was observed in PRLR−/− females but not in males (102). This reduction in abdominal fat reflects in part the absence of lactogen action in the adipocyte because the presence of PRLR mRNA was demonstrated in white adipose tissue. No apparent decrease of weight has been described
in PRL−/− mice, which suggests a role for lactogens in adipose tissue growth and metabolism in pregnancy when PLs are present at a very high level.

CONTROVERSIAL PHENOTYPE: IMMUNITY Among the very broad spectrum of actions attributed to PRL (18), some (103) remain conflictual; one example is its actual role in the immune system. Indeed, an amazing body of literature has described PRL as involved in proliferation, differentiation, and apoptosis in various immune cells, which argues for the immunomodulatory role of PRL (104). However, no clear immune phenotype could be detected in PRL-related KO models. In PRLR−/−, thymic or splenic cellularity, composition of the lymphocyte subset present in primary or secondary lymphoid organs, and immune system development and function appeared unaltered (105). In PRL−/−, myelopoiesis and primary lymphopoiesis were not affected (81). These data argue that PRL does not play any critical role in primary lymphocyte development and homeostasis, although an immunomodulatory role is not ruled out.

HUMAN PATHOPHYSIOLOGY

To date, and contrary to all other pituitary hormones, no disease has been linked to any genetic abnormality of PRL or its receptor. This would suggest either that mutations have no detectable effect in vivo, thereby preventing their phenotypic detection, or that such mutations might be lethal and thus never detected. PRL/PRLR KO mice are viable, so it is clear that PRL stimulus is not essential for survival. However, the major reproductive defects in females could explain the lack of genetic transmission.

In the current state of the art, the only characterized pathology related to PRL is hyperprolactinemia, which is frequent and is responsible for almost 25% of menstrual cycle disorders in women. It can result from pituitary adenoma or particular physiological circumstances (pregnancy, stress), or it can be secondary to drug intake. Lactotroph adenomas, which arise from monoclonal expansion of a single cell that has presumably undergone somatic mutation (106), can be treated efficiently by surgery or by dopaminergic agonists. At the other extremity, hyperprolactinemia is not a well-described syndrome, except in the few cases resulting from genetic defects of Pit-1 (which alters production of several pituitary hormones). In view of hyperprolactinemia leading to absence of milk secretion in postpartum PRL resistance in related family members, a genetic hypothesis has recently been suggested (107).

Besides these pathologies linked to abnormal levels of circulating PRL, it can be argued that PRL should be included in the list of factors favoring the proliferation of certain tumors. In vitro studies clearly show that PRL stimulates proliferation of many breast cancer cell lines, irrespective of their estrogen/progesterone receptor status (56, 108). Moreover, in contrast to steroid receptors, PRLR expression has been observed in (almost) all human breast tumors analyzed (109–111). In some instances, a higher level of expression could be detected in tumor versus normal
breast tissue (111), which could indicate a greater sensitivity of mammary tumors to PRL. In vivo studies using genetically modified mice have shown that elevated PRL levels (PRL transgenic mice) accelerate the rate of spontaneous mammary tumor appearance (112), and that absence of PRL (PRL KO mice) retards the appearance of genetically induced mammary tumors (113). Finally, the recent analysis of the American nurse cohort has clearly shown that breast cancer risk in postmenopausal woman is correlated to high-normal serum PRL levels (114). Despite these convincing reports, the involvement of PRL in the progression of breast cancer has been debated for decades and is still partly disregarded. One of the reasons is that no real benefit was reported when patients suffering from breast cancer were treated with dopamine agonists, although disease stabilization was observed in some cases (115, 116). The failure of dopamine agonist may be linked to the fact that it is local, rather than systemic, PRL that is involved in breast cancer cell proliferation via an autocrine-paracrine mechanism (56, 57, 110). Similar arguments also begin to accumulate for the involvement of PRL in prostate tumor growth (50, 51, 117), the strongest of which resides in the recent observation that transgenic mice overexpressing PRL in the prostate develop dramatic hyperplasia of this organ (118).

As discussed above, there is currently no known inhibitor of local synthesis of PRL. Our group thus turned to the strategy of developing PRL antagonists (29), which may be an alternative to the use of dopamine for the inhibition of PRLR-mediated effects (108). Obviously, in vivo studies will be necessary to confirm whether antagonists complement currently available anti-PRL molecules in clinical use.

Finally, a role of PRL in the pathogenesis of autoimmune diseases has also been suggested (systemic lupus or rheumatoid arthritis). However, experimental more than clinical data suggest such an interaction because most of the therapeutic trials using dopamine agonists in lupic patients have been disappointing (119). Again, because various cells of the immune system express PRL (120), the eventual role of local PRL needs to be addressed. Although the absence of immune phenotype in PRL-related KO models has led to questions about the immunomodulatory role of this hormone, it has recently been suggested that the role of PRL (and GH) in the immune system may be limited to immune responses associated with stress (121). Accordingly, clinical use of PRL in the treatment of immunosuppressed patients suffering from such diseases as AIDS and cancer has been proposed (122).

CONCLUSIONS

Although the main biological functions of PRL are related to its historically described actions on breast and reproductive tract, it can no longer be considered as acting exclusively on these targets. The broad distribution of PRLR and the increasing list of tissues identified as PRL sources are probably correlated to the
unusually large number of functions reported for this hormone, some, but obviously not all of which were confirmed by the phenotypes observed in KO models. Until a few years ago, there was a strong discrepancy between the biological versatility of PRL and the paucity of clinical arguments that suggested a role for PRL in human diseases. While awaiting the eventual identification of pathologies resulting from genetic defects of PRL or PRLR, one immediate goal in this field will be to understand how the amazing number of puzzling reports describing targets, mechanisms of actions, or functions of PRL can be linked and integrated into an overall physiological relevance of this old hormone (Figure 1). In particular, elucidating the mechanisms of extrapituitary PRL regulation and its real in vivo contribution (especially in tumors), the functional specificity of the numerous PRL variants and PRLR isoforms, and the in vivo impact of signaling cross talks constitute major challenges for the future.

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Figure 1  Schematic representation of the prolactin (PRL)/PRL receptor (PRLR) system, which can be divided into three main levels (color coded). The first (blue) involves all features concerning PRLR ligands (origin, regulation, nature, posttranslational modification, etc.). The second (green) involves the events occurring once PRLR ligands meet their target cells (receptor activation, intracellular signaling, cross talk, gene activation, etc.). The third (pink) involves the phenotypic consequences of these molecular/cellular events, i.e., the biological actions of PRLR ligands (including pathophysiological aspects). Some of the major current questions regarding these three levels are indicated on the right. Although complex, this figure is not meant to be exhaustive.
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