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The role of prolactin in central nervous system inflammation

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Abstract: Prolactin has been shown to favor both the activation and suppression of the microglia and astrocytes, as well as the release of inflammatory and anti-inflammatory cytokines. Prolactin has also been associated with neuronal damage in diseases such as multiple sclerosis, epilepsy, and in experimental models of these diseases. However, studies show that prolactin has neuroprotective effects in conditions of neuronal damage and inflammation and may be used as neuroprotector factor. In this review, we first discuss general information about prolactin, then we summarize recent findings of prolactin function in inflammatory and anti-inflammatory processes and factors involved in the possible dual role of prolactin are described. Finally, we review the function of prolactin specifically in the central nervous system and how it promotes a neuroprotective effect, or that of neuronal damage, particularly in experimental autoimmune encephalomyelitis and during excitotoxicity. The overall studies indicated that prolactin may be a promising molecule for the treatment of some neurological diseases.

Keywords: cytokines; excitotoxicity; experimental autoimmune encephalomyelitis; neurodegenerative; neuroprotective; prolactin receptor.

Introduction

Over 300 functions have been described for prolactin (PRL) (Bole-Feysot et al. 1998), which are classified in six main categories: 1) water and electrolyte equilibrium, 2) growth and development, 3) endocrinology and metabolism, 4) brain and behavior, 5) reproduction and maintaining pregnancy, and 6) immunoregulation and protection (Bole-Feysot et al. 1998; Costanza et al. 2015).

Initially, functions such as activation of cells from the innate and adaptive immune system, increased release of inflammatory cytokines and reactive oxygen species (ROS), have all been described. Later, PRL was described to possess anti-inflammatory functions such as preventing cytokine secretion and inhibiting the mitogenic response of immune system cells (Ben-Jonathan et al. 2008; Costanza et al. 2015; Dogusan et al. 2001). Despite findings regarding PRL functions in the immune system, studies carried out in PRL-deficient mice ($\text{Prl}^{-/-}$), have shown that these mice have a similar count of pro-B, pre-B and mature B cells in bone marrow compared with control mice ($\text{Prl}^{+/+}$). Similarly, $\text{Prl}^{-/-}$ mice have the same frequency in subpopulations of thymocytes, lymphocytes, and mature myeloid cells compared to control mice ($\text{Prl}^{+/+}$) (Horseman et al. 1997). Similar results have been reported for PRL receptor-deficient mice ($\text{Prlr}^{-/-}$) (Bouchard et al. 1999; Dorshkind and Horseman 2000).

These results suggest that PRL is not an essential hormone for the functions of the immune system (Bouchard et al. 1999; Horseman et al. 1997). However, as we will see, PRL functions should not be interpreted in a reductionist manner, but rather as closely tied to other factors that regulate PRL functions. Under certain conditions, PRL mediates inflammatory functions and in others, anti-inflammatory functions. These are the two sides of the coin for PRL. In this review, we first described some general aspects of PRL and its receptor, further reviewed the inflammatory and anti-inflammatory functions of PRL, the factors that regulate these functions. Finally, we discuss recent findings of the implications of this dual function in some disease models of the central nervous system (CNS), such as autoimmune experimental encephalomyelitis (EAE) and excitotoxicity models, where attention has been drawn to possible therapeutic applications with PRL.

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Prolactin and prolactin receptor

PRL is produced principally by lactotrophs in the pituitary gland (Bernard et al. 2019). In humans, PRL is also synthesized in an *extrapituitary* manner by other tissues in the body such as the decidua, the brain, adipose tissue, skin follicles, endothelial cells, and immune cells (Marano and Ben-Jonathan 2014). *Extrapituitary* synthesis of PRL is directed by an alternative promoter known as the distal promoter (Ben-Jonathan et al. 2008). Both pituitary and extrapituitary PRL are formed by 199 amino acids and have a molecular mass of 23 kDa. However, PRL may suffer posttranslational changes such as phosphorylation, glycosylation, and proteolytic cleavage, giving rise to variants of different masses (14-, 16- and 22-kDa) (Freeman et al. 2000). In human serum, variants of a larger molecular mass have also been described, including big PRL (45–50 kDa) and macroprolactin (>100 kDa), and are formed by polymerization, or aggregation with immunoglobulins (Fahie-Wilson and Smith 2013; Fahie-Wilson et al. 2005).

PRL is the only pituitary hormone for which its secretion is not stimulated by the hypothalamus-releasing hormones, and it is the only one, among the major hormones, whose secretion is mainly under tonic inhibitory control of dopamine (Freeman et al. 2000; Hinson et al. 2010). Dopamine exerts an inhibitory effect on PRL secretion through D₂ dopamine receptors (D2Rs). Mice deficient in this receptor develop *hyperprolactinemia* (Kelly et al. 1997). Estrogen, thyrotropin-releasing hormone, and vasoactive intestinal peptide can stimulate PRL secretion (Bernard et al. 2019).

PRL functions are mediated through interaction of the prolactin receptor (PRLR), and belongs to the class-1 cytokine receptors superfamily which also includes receptors for interleukin-2 (IL-2), IL-6, granulocyte and monocyte colony-stimulating factor, erythropoietin, leptin, etc. (Bole-Feysot et al. 1998; Costanza et al. 2015). PRLR can function as a receptor for three hormones: PRL, placental lactogen, and growth hormone. The promiscuity of PRL has made it difficult to interpret the effects of PRL *in vivo* (Bernard et al. 2019; Brooks 2012). The binding of any of these hormones to PRLR may activate any of the following three principal signaling pathways: JAK2/STAT5 (Janus kinase 2/signal transducer and activator of transcription 5), MAPK/ERK1-2 (mitogen-activated protein kinase/extracellular signal-regulated kinase1-2) and PI3K/AKT (phosphoinositide 3-kinase/protein kinase B) pathways (Abramicheva and Smirnova 2019). However, activation of these pathways depends on the formation of homodimers or heterodimers of the PRLR and the different isoforms of this receptor (Abramicheva and Smirnova 2019).

Through alternative splicing, several isoforms of PRLR are produced from the same gene located on chromosome 5 in humans, and on chromosome 15 in mice (Abramicheva and Smirnova 2019). The most studied isoforms are the large (PRLR-L), intermediate (PRLR-I), and short (PRLR-S) isoforms, named accordingly for the size of the intracellular region they are found in. The PRLR isoforms have similar extracellular domains but differ in the size of the intracellular region and in the intracellular messengers they recruit. A soluble receptor for PRL has also been described in humans, but its functions have been not completely elucidated (Hu et al. 2001; Trott et al. 2004).

The binding of a PRL molecule to a homodimer of the PRLR-L occurs through two receptor-binding sites present in PRL, these domains have different structural and electrostatic characteristics, which control the binding of PRL in two sequential steps (Kossiakoff 2004). Each PRL domain binds to an extracellular domain of a PRLR molecule, causing the JAK2 kinases to phosphorylate each other. These kinases are constitutively associated with the proline-rich Box 1 motif of PRLR (Pezet et al. 1997; Rui et al. 1994). Once activated, JAK2 kinases can phosphorylate tyrosine residues in the cytoplasmic region of the PRLR-L. The phosphorylation of these residues creates binding sites for proteins with the Src-homologous domain (SH2) (Brooks 2012). The transcriptional factor STAT binds to these phosphorylated tyrosines through their SH2 domain and is activated by phosphorylation by the JAK2 kinases (Ben-Jonathan et al. 2008).

STAT1, STAT3, STAT5A and STAT5B participate in signaling through PRLR-L and once phosphorylated they can interact with each other to form homodimers or heterodimers that translocate to the nucleus (Abramicheva and Smirnova 2019; Ben-Jonathan et al. 2008). These STAT dimers specifically regulate gene expression by binding to gamma interferon-activated sequence (GAS) motifs (Litterst et al. 2005). The induction of certain genes depends on the STAT dimers that are activated. For example, in a mammary epithelial cell line (KIM-2), STAT3 dimerization induces apoptosis, whereas STAT5 dimerization protects from STAT3-induced apoptosis and increases the expression of Wap, a marker of differentiation in mammary epithelium (Clarkson et al. 2006; Liu et al. 1997).

Studies in STAT3-deficient mice show that STAT3 exerts an antiapoptotic and proliferative effect on T-lymphocytes (Akaishi et al. 1998). In macrophages, the activation of STAT3 by PRL mediates anti-inflammatory effects and secretion of IL-10 (Sodhi and Tripathi 2008; Williams et al. 2007). On the other hand, PRL stimulates the synthesis of IFN- γ , TNF- α , IL-12p40 and IL-1 β in macrophages through the activation of

the JAK2-STAT1 pathway, in addition, treatment with AG490, a JAK2 inhibitor, decreases the synthesis of these cytokines (Tripathi and Sodhi 2008). In some pathologies such as pancreatic ductal adenocarcinoma, PRL promotes fibrosis and collagen deposition by signaling through STAT3 and not STAT5 (Tandon et al. 2019).

In the case of STAT5, mice deficient in this factor, do not develop B cells or T cells (Hoelbl et al. 2006). In T cells, PRL induces the expression of T-box transcription factor TBX21 (T-bet) through STAT5, but not through STAT1 (Tomio et al. 2008). PRL induces the expression of iNOS in peripheral blood mononuclear cells through the activation of STAT5 and the expression of interferon regulatory factor 1 (IRF-1) and iNOS in granulocytes through STAT1 (Dogusan et al. 2001). In cultures of lung fibroblasts treated with PRL and pro-inflammatory cytokines (IL-1 β , IFN- γ , TNF- α) phosphorylation of STAT1 or STAT5 was mainly observed, the authors point out that STAT1 can activate IRF-1 (transcription factor that coordinates the expression of inflammatory genes, including cytokines, adhesion molecules and iNOS) while STAT-5 can inhibit the transcription of IRF-1 (Corbacho et al. 2003).

In the CNS, the activation of STAT5, by PRL, has been related to the development of chronic mild stress-induced depression and apoptosis of neurons in the CA3 region of the hippocampus in a murine model (Tian et al. 2019). Furthermore, in a mouse model of severe spinal muscular atrophy, which is caused by recessive mutations in the survival motor neuron protein, PRL stimulates the functional expression of this protein by activating STAT5 thereby increasing motor function and survival (Farooq et al. 2011).

The signaling of PRL through the JAK2-STAT pathway can be inhibited by several proteins. For example, cytokine signal suppressor proteins 1 and 3 (SOCS1-3) regulate PRL signaling and its receptor, contain an Src-homologous domain (SH2), and the C-terminal SOCS box. The SH2 domain enables these proteins to interact with phosphorylated tyrosine residues and inhibits the JAK2 signaling pathway (Abramicheva and Smirnova 2019; Bouilly et al. 2012). In addition, inducible cytokine SH2 containing protein (CIS) binds to PRLR thus modifying its structure to prevent STAT5 binding (Endo et al. 2003). The PIAS family of proteins includes four members in humans (PIAS1-4) (Abramicheva and Smirnova 2019). These proteins act at the nuclear level where they bind to STAT dimers and prevent binding to the target sequences of this transcriptional factor (Liao et al. 2000). PIAS1 and PIAS3 interact with STAT1 and STAT3, respectively (Chung et al. 1997; Liu et al. 1998). PIAS3 also binds to STAT5 at its DNA binding site to prevent DNA interaction with GAS motifs (Abramicheva and Smirnova 2019; Halim et al. 2020). PIAS can mediate the conjugation of

small ubiquitin-related modifier (SUMO) to transcriptional factors and thus regulate their activity, this process is called sumoylation (Shuai 2006). However, the role of this process in the regulation of STAT proteins is not clear (Abramicheva and Smirnova 2019; Shuai 2006).

Binding of PRL with PRLR-I activates JAK2 to promote cell survival, but not proliferation, in the Ba/F3 murine myeloma cell line (Kline et al. 1999). The PRLR-I lacks the intracellular motifs necessary for the union of STAT5, due to an upward displacement of the reading frame, so the lack of the mitogenic stimulus may be due to the lack of the C-terminal domain that prevents the activation of Fyn and STAT5 (Kline et al. 1999). Because PRLR-S is unable to bind to proteins with SH2 domains, such as STAT5, PRLR-S may exert an inhibitory function, because after dimerizing with long isoforms, it prevents signaling through the JAK2/STAT5 pathway in Ba/F3 cells and human kidney embryonic cell (Devi et al. 2011). Furthermore, PRLR-S has been reported to inhibit the activation of transcription factors such as Sp-1 and Forkhead box O transcription factor (FOXO), and may inhibit the MAPK pathway through dual specific phosphatase that dephosphorylates cascade proteins (DUPD1) (Devi et al. 2009; Halperin et al. 2008).

The soluble isoform of PRLR binds to PRL to increase its circulation time, but it also competes with membrane receptors to bind to PRL, which affects its availability to activate signaling pathways (Abramicheva and Smirnova 2019; Ben-Jonathan et al. 2008). Numerous soluble isoforms of cytokine receptors have been reported and have been proposed to play an important role in the regulation of inflammation (Lokau and Garbers 2020). Figure 1A shows some signaling pathways activated or inhibited by the different PRLR isoforms. The expression of the different isoforms of PRL and its receptor contribute to explain the more than 300 functions described for this hormone. The identification of changes in the proportion of PRL and their receptor isoforms variants, both in a normal and pathological state, could provide essential information for the design of new therapeutic strategies.

Prolactin effects in the immune system

PRL's role in the immune system has been documented since the 1970s. The first studies suggested an inflammatory role for PRL, but later other studies reported anti-inflammatory and repair functions for PRL (Costanza and Pedotti 2016; Costanza et al. 2015; Dorshkind and

Figure 1: Factors that regulate the function of prolactin.

(A) The image represents some of the signaling pathways activated or inhibited by different PRLR isoforms (the functions involved in these pathways are described in the text). (B) Schematic diagram of the dual function of PRL when it interacts with cytokines and PAMPs. PRL, when acting together with IFN- γ and LPS, favors inflammatory functions in macrophages and monocytes (top). Illustration of PRL's anti-inflammatory effect by acting alongside IL-4 and *M. bovis* proteins in these cells (bottom). (C) The schematic diagram contrasts the effect of PRL at low and high concentrations on transcription factor T-bet expression. (D) Short periods of exposure to PRL favors T-bet expression in T cells (green) and secretion of inflammatory cytokines in astrocytes (purple) (top). However, prolonged exposure times favor T-bet and TNF- α inhibition (bottom). PRL: prolactin, PRLR: prolactin receptor, PRLR-L: long isoform of the PRLR, PRLR-I: intermediate isoform of the PRLR, PRLR-S: short isoform of the PRLR, T-bet: T-box transcription factor TBX21, NO: nitric oxide, LPS: lipopolysaccharide, PAMPs: pathogen-associated molecular patterns.

Horseman 2000). PRLR is expressed by a large variety of cells in the immune system including monocytes, neutrophils, macrophages, lymphocytes, natural killer cells (NK), and microglia (Borba et al. 2018). PRL can mediate effects in all of these cells. Herein, we will describe inflammatory functions and later anti-inflammatory functions of PRL in the peripheral immune system.

Inflammatory effects of prolactin

Effects for PRL have been reported for all cells in the immune system. For example, PRL is capable of stimulating cytokine secretion such as with interferon- γ (IFN- γ), IL-12, and IL-10, in total blood cells with lipopolysaccharide (LPS) or phytohaemagglutinin administration (Matalaka 2003). In macrophages, PRL stimulates chemokine secretion such as macrophage inflammatory protein 1 α (MIP-1 α), monocyte chemoattractant protein 1 (MCP-1), interferon-gamma protein 1 (IP-10), and the CCL5 chemokine (Sodhi and Tripathi 2008). Furthermore, PRL increases ROS and macrophage cytotoxic activity in a tumor environment (Majumder et al. 2002; Malaguarnera et al. 2004). In granulocytes, PRL induces expression of inducible nitric oxide synthase (iNOS) and IRF1 (Dogusan et al. 2001). Also, PRL increases proliferation and secretion of IFN- γ in NK cells (Matera et al. 1999; Sun et al. 2004). In a Chagas disease rat model, PRL increased the number of activated NK cells, decreased apoptosis of splenocytes and increased the percentage of T CD4 $^{+}$ cells that produce IFN- γ (Del Vecchio Filipin et al. 2019).

PRL acts as a comitogen by favoring cellular survival in T cells (Bauernhofer et al. 2003; Sabharwal et al. 1992) and by increasing tumor necrosis factor- α (TNF α), IFN- γ , and IL-2 production in CD8 $^{+}$ T cells treated with phorbol myristate acetate (Dimitrov et al. 2004). Moreover, PRL decreases the suppressor function of T regulatory cells (Legorreta-Haquet et al. 2012; Wu et al. 2014). In B cells, PRL reduces apoptosis in T1 B cells during negative selection by inducing antiapoptotic gene INF- γ type II receptor (INF- γ R2) expression and by decreasing proapoptotic Trp63 gene expression. PRL also modifies B cell editing by abating the anergy activation threshold in these cells through and increment in intracellular calcium (Saha et al. 2009). In activated B cells, PRL increases antibody secretion (Lahat et al. 1993) and stimulates proliferation in B cell hybridomas (Richards et al. 1998).

Anti-inflammatory effects of prolactin

PRL decreases the immune response to LPS by preventing expression of the Toll 4 type receptor induced by LPS, and phosphorylation of Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which results in reduced secretion of TNF- α , IL-1 β , and IL-6 in Placental cotyledons (Olmos-Ortiz et al. 2019). *In vitro* studies using human fetal membranes, LPS stimulates the secretion of TNF- α and IL- β in the choriodecidual and amniotic regions,

however, co-treatment with PRL decreases the expression of TNF- α in the choriodecidual region and IL- β in both regions (Flores-Espinosa et al. 2017). Fetal membrane explants cultures express IL-1 β , TNF- α , and matrix metalloproteinase 9 at 24 h, but when they are cultured in the presence of PRL the expression of IL-1 β and matrix metalloproteinase 9 is reduced, the expression of TNF- α was not significantly reduced (Zaga-Clavellina et al. 2014).

In summary, most *in vitro* and *in vivo* studies indicate a proinflammatory and immunostimulatory function for PRL, however, it has been identified that under certain conditions PRL may mediate anti-inflammatory effects. These dual effects are highly influenced by the type of target cell and the molecular composition of the milieu.

Factors that regulate prolactin function

As has been described, PRL mediates inflammatory and anti-inflammatory actions. Consequently, we will describe some factors that participate in the regulation of PRL effects such as cytokines and pathogen-associated molecular patterns (PAMPs) in the environment, PRLR isoforms, PRL concentration, and length of exposure.

Regulation of prolactin effects by the inflammatory environment milieu

Molecules in the environment, such as cytokines and PAMPs, participate in the regulation of PRL effects by inducing or inhibiting its secretion and acting synergistically or antagonistically with PRL, or by inducing expression of different PRLR isoforms (Borba et al. 2018; Martínez-Neri et al. 2015). We will first mention studies that demonstrate how cytokines and PAMPs favor an inflammatory role for PRL, and later, how in other cases they may favor an anti-inflammatory response (see Figure 1B).

PRL secretion is partly regulated by cytokines in the environment. For example, IL-1, IL-2, and IL-6 stimulate PRL secretion while endothelin 3, transforming growth factor-beta (TGF- β) and IFN- γ inhibit its secretion (Borba et al. 2018; Domae et al. 1992; Farrow and Gutierrez-Hartmann 1999). TNF- α can stimulate release of PRL (Friedrichsen et al. 2006), but prolonged exposure of this cytokine can inhibit this action (Harel et al. 1995).

Cytokines not only participate in the regulation of PRL secretion but may also act synergistically with this hormone in the inflammatory response. For example, macrophage

cultures treated with both PRL and INF- γ display increased nitric oxide (NO) release and cytotoxic capacity against tumor cells compared to macrophages treated only with either PRL or with INF- γ (Majumder et al. 2002). Furthermore, IL-12 and PRL can act synergistically to induce differentiation of CD4⁺ T cells towards a Th1 phenotype, since PRL induces the release of IL-12 by macrophages as a positive feedback mechanism (Majumder et al. 2002). In the case of NK cells, PRL interacts jointly with IL-2 and IL-12 to increase INF- γ production (Matera et al. 2000) and with IL-15 to increase proliferation, perforin expression and cytotoxic activity of these cells (Sun et al. 2004).

In some cases, cytokines can also act antagonistically to PRL. For example, PRL has been reported to participate in macrophage activation (Chen and Johnson 1993). Yet, when macrophages are grown with PRL and Th2-type cytokines, such as IL4, PRL-induced activation is suppressed, and the cytotoxic capacity against tumor cells, and the production of NO and singlet oxygen (O₂⁻) in macrophages, is decreased (Majumder et al. 2002).

PAMPs can also induce or inhibit PRL expression in different cells or act synergistically with this hormone in the inflammatory response. This occurs in the same way that LPS induces PRL and PRLR expression in monocytes, as a positive feedback mechanism to increase inflammatory cytokine secretion. This was demonstrated by treating monocytes with LPS and anti-PRLR antibodies (MAB1167), which resulted in decreased secretion of IL-1 β , IL-6, and TNF- α , and increased secretion of IL-10 (López-Rincón et al. 2013a). Furthermore, monocytes from the THP-1 cell line treated with LPS or porins from *Salmonella enterica* serovar Typhimurium, and PRL, show a greater secretion of IL-8 compared to cells treated with only one of these stimuli (D'Isanto et al. 2004).

As previously mentioned, cytokines and some pathogen molecules that act together with PRL, favor an inflammatory response. However, cytokines can also act together with PRL to mediate anti-inflammatory and repair functions. PRL, by itself, is not essential for cartilage survival under normal conditions since Prlr^{-/-} mice do not present alterations in this tissue. Yet, in an inflammatory context, PRL has an antiapoptotic effect (Adán et al. 2013; Clément-Lacroix et al. 1999). In rheumatoid arthritis, cytokines such as TNF- α , IL-1 β , and IFN- γ stimulate chondrocyte apoptosis and extracellular matrix degradation (McInnes and Schett 2007). Although, when chondrocytes are treated *in vitro* with a mixture of proapoptotic cytokines (TNF- α , IL-1 β , and IFN- γ) together with PRL, the latter protects chondrocytes from apoptosis by inducing BCL-2 transcription through the JAK2/STAT3 pathway (Adán et al. 2013).

Similar results were reported in an adjuvant-induced model of inflammatory arthritis in rats, where PRL administration prevented cartilage degradation. PRL's protective effect was independent of NO production, but dependent on the JAK2/STAT3 pathway since this pathway inhibits the expression of the nuclear factor activator receptor κ B (RANKL), a key regulator of osteoclastogenesis in rheumatoid arthritis. This indicates that the antiapoptotic and repair effects of PRL are observed in an inflammatory environment (Adán et al. 2013; Ledesma-Colunga et al. 2017).

Another case where PRL has an anti-inflammatory function is with the control of ROS in rat lung fibroblasts. In these cells, PRL by itself, does not affect iNOS expression nor NO production. However, when PRL acts with inflammatory cytokines such as TNF- α , IL-1 β , and IFN- γ , it induces PRLR expression in fibroblasts, inhibits iNOS expression and blocks NO production. PRL's inhibitory mechanism of iNOS expression is related to an increase in STAT-5b phosphorylation and suppression of IRF-1 expression (Corbacho et al. 2003).

PAMPs and microbial antigens can also mediate anti-inflammatory actions of PRL. For example, in THP1 cells, PRL alone was found to activate the JAK2/STAT3-5 signaling pathway and did not affect cytokine secretion. However, when acting together with *Mycobacterium bovis* proteins, an increase in phosphorylation of STAT3-5, and a decrease in phosphorylation of protein kinase B2 (AKT2), ERK1-2, and p38 kinase were observed. A consequent decrease in IL-1 β , TNF- α , and IL-12 secretion, but an increase in IL-10 secretion, was also observed. This effect may be the result of a decrease in the proinflammatory response against mycobacterial antigens, through the activation of the MAPK kinase pathway by the short PRL receptor isoform (PRLR-S) (Martínez-Neri et al. 2015).

Following the above, PRL induces the production of TNF- α , IL-1 β , and NO in epithelial cells of the bovine mammary gland. But when these cells are stimulated with PRL and *Staphylococcus aureus*, there is a decrease in the production of IL-1 β , NO, and β -defensin. This suggests that bovine PRL plays an inhibitory role in the immune response against *S. aureus* (Gutiérrez-Barroso et al. 2008). Finally, cytokines and PAMPs may induce a change in the expression of PRLR isoforms. The effect of the expression of different PRLR isoforms will be discussed below.

Differential expression and activity of the prolactin receptor isoforms

In humans, PRLR-L is expressed mainly in the placenta, adrenal gland, pituitary gland, and hippocampus, while

PRLR-I is expressed in the placenta, adrenal gland, small intestine, and kidney (Kline et al. 1999). PRLR-S expression has been reported in organs such as the prostate, liver, and uterus (Abramicheva and Smirnova 2019). Different PRLR isoform expression depends the tissue type and on the molecular composition of the milieu, such as cytokines and hormones. Change in the proportion between isoforms of PRLRs is a mechanism that regulates cellular function in processes such as pregnancy, lactation, tissue development, inflammatory response and in pathological conditions, such as autoimmunity and tumor formation (Abramicheva and Smirnova 2019; Shemanko 2016). For example, in samples of healthy uterine tissue the expression of PRLR-S was observed and in cervical cancer tissue both PRLR-S and PRLR-L are expressed (Ascencio-Cedillo et al. 2015).

In the study carried out by López-Rincón et al. (2013b), the expression of PRLR isoforms was evaluated in cattle infected with *M. bovis*. It was detected the expression of the PRLR isoforms of 130, 120, 100, 75, 65, 50 and 40 kDa in thymus and spleen, being the most abundant isoform the one of 50 kDa. The 75 and 100 kDa isoforms were found in the liver and the 40, 50 and 100 kDa isoforms in the lungs. In the cells of the bronchoalveolar lavage, the 40 kDa isoform was found in half of the infected animals, while the 50 kDa isoform was expressed in all of them. No PRLR isoforms were found in healthy animals.

Expression of different PRLR isoforms has also been reported in cells of the immune system after stimulation with cytokines or PAMPs, for example, LPS stimulation of THP1 monocytes produced an increase in total PRLR mRNA expression, particularly PRLR-L (100 kDa) and PRLR-I (50 kDa). This PRLR-I expression increased in a time-dependent manner between 8 and 72 h after the stimulus. In the culture of untreated monocytes, obtained from healthy participants, the basal expression of the 50, 60 and 23 kDa PRLR isoforms was observed, but not the PRLR-L. After stimulation with LPS for 8 h there was an increase in the expression of PRLR-L and PRLR-I. At 48 h of stimulation an increase in the expression of the isoforms of 100, 90, 65 and 50 kDa was observed (López-Rincón et al. 2013a). Therefore, the different isoforms of the PRL receptors could contribute to maintaining homeostasis during an inflammatory response. Accordingly, Pereira Suarez et al. (2015) have suggested that the interaction of 60 kDa PRL with PRLR-L could activate JAK/STAT1 and release pro-inflammatory cytokines, while the interaction of pituitary PRL with the intermediate isoforms (40, 50 and 65 kDa) of the PRLR could activate the PI3K/AKT pathway and release IL-10.

THP-1 monocytes express basal PRLR-I, but not PRLR-L. *In vitro* treatment with culture filtrate proteins-

M. bovis increases in a time-dependent manner (1–72 h) the expression of PRLR-I and significantly less the expression of PRLR-L (López-Rincón et al. 2015). Martínez-Neri et al. (2015) reported that *M. bovis* induces the expression of PRLR-S in THP1 cells. Furthermore, in lung fibroblasts pretreated with proinflammatory cytokines (IL-1 β , TNF- α e IFN γ) the expression of PRLR-L was induced, but not the expression of PRLR-S (Corbacho et al. 2003). Therefore, the different signaling pathways activated by the PRLR isoforms could mediate the various inflammatory effects related to PRL.

The concentration of prolactin and control of inflammation

One factor that possibly influences the function of PRL is its concentration; however, the main experimental data that support this observation were obtained *in vitro*. It has previously been reported that conditions of hypoprolactinemia and hyperprolactinemia usually produce anti-inflammatory and immunosuppressive effects, respectively. By contrast, increased PRL in physiological ranges favors inflammation and activation of the immune system (Brand et al. 2004; Matera 1997). Low concentrations of PLR (15–30 ng/ml) favor the secretion of cytokines such as IL-12 and IFN- γ in whole blood cells, but high concentrations (100–300 ng/ml) do not affect the secretion of these cytokines (Matalka 2003). Concentrations of PRL in a physiological range (12–25 ng/ml) have been reported to increase the proliferation of NK cells and the response to mitogenic agents by NK cells, B cells, and T cells. However, concentrations 5–10 times higher than physiological concentrations inhibit the mitogenic response (Matera et al. 1992).

In CD4⁺ T cells, low concentrations of PRL (10–30 ng/ml) activate the JAK2/STAT5-mediated signaling pathway. In turn, phosphorylated STAT5 binds to the regulatory region of T-bet, which is a transcriptional factor that directs the Th1 type inflammatory response and the release of IFN- γ , IL-12 and NO (Rincón et al. 1998; Tomio et al. 2008). Conversely, high doses of PRL (100 ng/ml) activate the signaling pathway through SOCS1 and 3, leading to suppression of T-bet activation (see Figure 1C). Activation of SOCS1 and 3 is dose-dependent on PRL concentration (Tomio et al. 2008). PRL function is mediated by its concentration by changing signaling in the PRL/JAK2/STAT5 and PRL/SOCS1-3 pathways and therefore T-bet activity.

In macrophages, concentrations of 100 ng/ml PRL were the most effective in inducing the expression of IL-1 β , IL-12p40, and IFN- γ . Although concentrations of 20 ng/ml

and 500 ng/ml also induced an increase in the expression of these cytokines, the effect was less. Concentrations of 1000 ng/ml PRL only induced a significant increase in IL-12p40, but the increase was less than that observed with 100 ng/ml PRL. Furthermore, in this study the effect of PRL on the expression of chemokines MIP-1a, RANTES, MCP-1 and IP-10 was determined. The production of these chemokines was dependent on the concentration of PRL, and the highest expression was observed with a dose of 100 ng/ml (Sodhi and Tripathi 2008). A significant increase in IL-10 production was observed only with the treatment of 1000 ng/ml PRL (Sodhi and Tripathi 2008). Concentrations of 1000 ng/ml PRL, but not 100 ng/ml, favor activation of the JAK2/STAT3 pathway with the consequential release of IL-10 (Sodhi and Tripathi 2008). In turn, IL-10 stimulates the expression of PRLR-S and this isoform reduces the inflammatory response of cells to PRL (Abramicheva and Smirnova 2019; Paul et al. 2010). Therefore, IL-10 secretion serves as a negative feedback mechanism to regulate the response to PRL.

Under certain conditions, PRL's anti-inflammatory and immunosuppressive effects can cause negative responses in the body. For example, in a murine sepsis model, hyperprolactinemia dramatically increases mortality from 47 to 81% in 48 h, due to PRL's immunosuppressive effect which decreases the proliferation of immune cells, blocks IL-2 production and increases splenocyte apoptosis and IFN- γ release (Oberbeck et al. 2003).

Time of exposure regulates the effect of prolactin

Another important factor that regulates PRL's functions is the time of exposure. As previously described, low PRL concentrations that act during short periods activate T-bet expression. However, long periods (> 10 h) of stimulation inhibit the activation of this transcription factor (Tomio et al. 2008). *In vitro* studies with astrocytes show that stimulation with recombinant PRL for 6 h favors TNF- α and IL-1 α expression, but not that of TGF- α . On the other hand, with longer periods of stimulation (18 h) an increase in the expression of TGF- α and IL-1 α is observed, yet TNF- α expression is decreased (DeVito et al. 1995) (see Figure 1D). At transcriptional level, time course experiments *in vivo* demonstrated that PRL modified the expression of different genes in the rat hippocampus, including changes in those relate to microglia activation (Cabrera-Reyes et al. 2019).

In conclusion, the function of PRL depends on several factors among which the influence of inflammatory factors such as cytokines and PAMPs, and the effect these have on

the expression of PRLR isoforms (see Table 1). Knowing how these factors interact will allow us to have a more holistic view of the function of this hormone and predict the effect of its modulation as a therapeutic strategy.

Control of inflammation by prolactin in the central nervous system

Traditionally, the CNS was seen as an immune-privileged site; although the concept of immunological privilege has been questioned during the last years, it is clear that the innate and adaptive immune response is lower than that of peripheral organs, given the anatomical, cellular and molecular peculiarities of the CNS (Schiller et al. 2020). Numerous cellular and humoral immune interactions maintain a two-way communication between the CNS and the immune system, both in physiological and pathological conditions (Norris and Kipnis 2018). Such is the case of T reg cells, which are capable of suppressing astrogliosis in an ischemic stroke model, producing amphiregulin (AREG) an epidermal growth factor (EGF)-like molecule (Ito et al. 2019). Another example is the case of B-1a cells that promote the proliferation of oligodendrocyte precursors and myelination during the development of the CNS (Tanabe and Yamashita 2018). *In vitro* studies, a potential interaction between primed cells and astrocytes has been shown through the interaction of CD40 and CD40L, leading astrocytes to the production of inflammatory cytokines (Kim et al. 2011).

In addition to the interaction between the CNS and the immune system, the endocrine system may mediate functions on these, we previously described the effect of the hormone PRL on the immune system and will now review the effect of this hormone on the immune response in the CNS.

Prolactin in the central nervous system

The PRL present in the CNS comes mainly from the circulation. It can enter the cerebrospinal fluid through a PRLR-mediated transport system in the choroid plexus (Walsh et al. 1987). However, it was observed that iodine-125 labeled PRL administered intravenously is transported mainly to the brain and not to the cerebrospinal fluid in mice lacking the PRL receptor. This identified transport mechanism in PRL receptor deficient mice was saturated with a high dose of unlabelled ovine PRL, indicating the

involvement of an unidentified transporter (Brown et al. 2016). Another way that PRL can enter the brain is through regions that lack a blood-brain barrier, for example, the median eminence of the hypothalamus and circumventricular structures (Peruzzo et al. 2000). In addition, in the middle eminence of the ventromedial arcuate nucleus are fenestrated capillaries with pores 50–80 nm in diameter through which hormones of up to 40 kDa can diffuse (Schaeffer et al. 2013). From these pathways, PRL could have access to the brain so that exploratory neurons can detect changes in the amount of circulating PRL (Bridges and Grattan 2019).

Additionally, the synthesis of PRL messenger RNA by RT-PCR has been reported in the hypothalamus, cerebellum, amygdala, cortex and hippocampus (Emanuele et al. 1992). Induced PRL synthesis was observed in the hypothalamus during lactation, pregnancy, or under stress (Torner et al. 2002; Torner et al. 2004). In addition, the expression of PRL has been described in neurons and glial cells under conditions of damage (Mödersheim et al. 2007; Vergara-Castañeda et al. 2016). Regarding PRLR, its expression in astrocytes, microglia, oligodendrocytes and neurons has been described (Anagnostou et al. 2018; Cabrera-Reyes et al. 2019; De Vito et al. 1995). This PRLR expression has been observed in the hypothalamus, choroid plexus, medial amygdala, and terminal septum (Brown et al. 2010). PRL and its putative expression in many brain areas has been compiled by Cabrera Reyes et al. (2017).

Inflammatory effects

In the brain, PRL acts synergistically with other factors, such as cytokines, increases the release of inflammatory mediators, and the activation of glial cells (Anagnostou et al. 2018). As previously mentioned, PRL stimulates TNF- α and IL-1 α expression in astrocytes (DeVito et al. 1995). In rats with a hippocampal injury performed by needle puncture, PRL increases TNF- α and glial fibrillary acidic protein (GFAP) expression, a marker of microglial activation (De Vito et al. 1995).

In the nervous system, PRL enhances inflammatory responses when it acts synergistically with proinflammatory cytokines. In a study performed in a murine model with *Acanthamoeba castellanii* infection, pretreatment of the microglia with recombinant PRL in combination with recombinant IFN- γ synergistically triggered the release of IL-1 α , IL-1 β , IL-6 and TNF- α (Benedetto and Auriault 2003). Similarly in another study, rats pretreated with recombinant tumor necrosis factor and PRL

Table 1: Inflammatory and anti-inflammatory effects of PRL.

Cell or model	Prolactin concentration	Exposure time to PRL	Effect	Reference
Inflammatory functions				
Total blood cells	PRL (15–30 ng) + LPS or phytohaemagglutinin	48 h	↑ IFN- γ , IL-12	(Matalka 2003)
Macrophages	PRL (50–100 ng/ml)	12–24 h	↑ IL-1 β , IL-12p40, IFN- γ , MIP-1a, RANTES, MCP-1, IP-10, CCL5, ROS and macrophage cytotoxic activity	(Majumder et al. 2002; Malaguarnera et al. 2004; Sodhi and Tripathi 2008)
Monocyte THP-1 cells	PRL (1000 ng/ml)	12–24 h	↑ IL-12p40	(Sodhi and Tripathi 2008)
	PRL (50 ng/ml) + INF- γ	18 h	↑ NO and cytotoxic capacity against tumor cells	(Majumder et al. 2002)
	PRL (200 mg), murine model PRL (10–200 ng/ml) + LPS or porins from <i>S. enterica</i>	Days 0 and + 1 30 min	↑ Phagocytosis, NO and H ₂ O ₂ ↑ IL-8	(Chen and Johnson 1993) (D'Isanto et al. 2004)
Granulocytes	PRL (10 ng/ml)	30 min	↑ iNOs and IRF1	(Dogusan et al. 2001)
NK cells	PRL (12–200 ng/ml)	6–72 h	↑ IFN- γ , proliferation and activation	(Matera et al. 1992; Matera et al. 1999; Sun et al. 2004)
T cells	PRL (100 ng/ml) + IL-12, IL-15 or IL-2	12 h	↑ Cytotoxicity and IFN- γ and perforin	(Matera et al. 2000; Sun et al. 2004)
	PRL (200 ng/ml)	72 h	↑ Comitogen and survival	(Bauernhofer et al. 2003)
CD4 ⁺ T cells	PRL (10–30 ng/ml) PRL (40 μ g), rat model	10 min–4 h For 60 consecutive days	↑ T-bet ↑ CD4 ⁺ INF- γ + T cell	(Tomio et al. 2008) (Del Vecchio Filipin et al. 2019)
CD8 ⁺ T cells	PRL (20 ng/ml) + Phorbol myristate acetate	6 h	↑ TNF α , IFN- γ , and IL-2	(Dimitrov et al. 2004)
T regulatory cells	PRL 50 ng/ml	Five days	↓ suppressor function	(Legorreta-Haquet et al. 2012)
T1 B cells	PRL(0.1 mg)	Every day for four weeks	↓ Apoptosis and modifies B cell editing	(Saha et al. 2009)
Activated B cells	PRL (0.2–100 ng/ml) + IL-2	Nine days	↑ Antibody secretion	(Lahat et al. 1993)
Astrocytes	PRL (1 nM)	6 h 18 h	↑ TNF- α and IL-1 α ↑ TGF- α and IL-1 α	(DeVito et al. 1995)
Anti-inflammatory functions				
Placental cotyledons	PRL(100–500 ng/ml) + LPS	48 h	↓ TNF- α , IL-1 β , and IL-6	(Olmos-Ortiz et al. 2019)
Human fetal membranes	PRL (250–4000) + LPS	24 h	↓ TNF- α and IL- β	(Flores-Espinosa et al. 2017)
Fetal membrane explants	PRL (500 ng/ml)	48 h	↓ IL-1 β and matrix metal- loproteinase 9	(Zaga-Clavellina et al. 2014)
Epithelial cells of the bovine mammary gland	PRL (5 ng/ml) + <i>S. aureus</i>	24 h	↓ IL-1 β , NO, and β -defensin	(Gutiérrez-Barroso et al. 2008)
Chondrocytes	PRL (2.3 μ g/ml) + TNF- α , IL-1 β , and IFN- γ	6–24 h	Protects chondrocytes from apoptosis	(Adán et al. 2013)
Rat lung fibroblasts	PRL (10 nM) + TNF- α , IL-1 β , and IFN- γ	24 h	↓ iNOS and NO	(Corbacho et al. 2003)
Monocyte THP1 cells	PRL (20 ng/ml) + <i>M. bovis</i> proteins	48 h	↓ IL1- β , TNF- α , and IL-12 ↑ IL-10	(Martínez-Neri et al. 2015)
NK cells	PRL (100 ng/ml)	Four days	↓ mitogenic response	(Matera et al. 1992)
CD4 ⁺ T cells	PRL (100 ng/ml)	10 min–4 h	↓ T-bet activation	(Tomio et al. 2008)
Macrophages	PRL (1000 ng/ml)	12–24 h	↑ IL-10	(Sodhi and Tripathi 2008)
Murine sepsis model	Hyperprolactinemia (4 mg/kg)	Days 0 and +1	↓ Proliferation of immune cells and IL-2 production ↑ Splenocyte apoptosis and IFN- γ release	(Oberbeck et al. 2003)
Adjuvant-induced model of inflammatory arthritis	Serum PRL (80 ng/ml)	21	↓ Cartilage degradation	(Adán et al. 2013)

responded better to *Toxoplasma gondii* infection. Pre-treatment induced intracellular death of *T. gondii* and release of IL-1 β , IL-3, and IL-6 by microglia (Benedetto et al. 2001).

PRL not only favors the production of cytokines in the brain, it also activates iNOS, and increases the production of NO in paraventricular and supraoptic hypothalamic nuclei in male rats administered PRL (Vega et al. 2010). Additionally, when PRL acts in synergy with IFN- γ , the release of NO in C6 glial cells is further increased. These findings highlight the neuroimmunoregulatory function of PRL (Raso et al. 1999).

Antiinflammatory effects

Other studies have shown that PRL decreases microglial activation. For example, in a retinal degeneration model by phototoxicity, rats were exposed to constant bright light. PRL was found to decrease retinal gliosis evaluated by measuring GFAP expression (Arnold et al. 2014). Moreover, treatment with PRL for 24 h in ovariectomized rats decreased microglial activation in the hippocampus, determined by a decrease in immunodetection of CD11b/c. Additionally, PRL produces morphological changes in glial cells and favors the expression of the genes involved in the maintenance of microglial functions, such as Ador2a, Car3, Chat, Drd2, Egr2, Hif3a, Notch, Penk, Tac1 and Ttr (Cabrera-Reyes et al. 2019).

PRL can decrease oxidative stress under certain conditions. For example, PRL reduces ROS production in retinal levels in retinal epithelial cells treated with hydrogen peroxide (H₂O₂). Furthermore, it protected against oxidative damage by promoting cell survival and reducing apoptosis in these cells. PRL's protective effect was mediated by an increase in the production of the antioxidant glutathione. PRL did not increase cell survival or glutathione concentration alone. These effects were observed only under conditions of oxidative stress by exposure to H₂O₂ (Meléndez García et al. 2016).

In cultured rat hippocampal neurons, PRL protects against glutamate-induced apoptosis by preserving mitochondrial function and by increasing the production and activity of superoxide dismutases 1 and 2, enzymes that participate in an antioxidant-type defense. Furthermore, PRL decreased lipid peroxidation in hippocampal neurons exposed to glutamate (Glu) excitotoxicity (Rivero-Segura et al. 2019). Regarding the effect on cytokines, PRL has been reported to increase the secretion of IL-10 and IL-4 in the hippocampus of ovariectomized rats. By immunodetection it was determined that the expression of IL-4 and

IL-10 was mainly located in neurons of the hilum of the dentate gyrus (Reyes-Mendoza and Morales 2020).

Effect of prolactin in diseases of the central nervous system

PRL concentration in the CNS is increased in diseases such as multiple sclerosis, ischemia, and epilepsy as well as in their experimental models. Early studies showed that PRL mediated inflammatory processes, but recent findings indicate that it also has anti-inflammatory and protective functions (Costanza and Pedotti 2016). Next, we will describe the dual role of PRL in some disease models of the CNS.

Effect of prolactin in experimental autoimmune encephalomyelitis

Experimental autoimmune encephalomyelitis (EAE) is the most-studied animal model of multiple sclerosis (MS). EAE may be induced in mice with different genetic backgrounds, such as SJL/J, C57BL/6 and NOD. When EAE is actively induced, animals are immunized subcutaneously with a myelin-associated antigen. When it is passively induced, EAE is performed by activation or adoptive transfer of myelin-specific T cells (Procaccini et al. 2015).

EAE is characterized by loss of integrity of the blood-brain barrier and infiltration of immune cells (Aubé et al. 2014). At disease onset, monocyte-derived macrophages produce IL-1 β that induces the secretion of granulocyte-monocyte colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) by endothelial cells (Wesselingh et al. 2019). These factors promote the differentiation of the infiltrated monocytes into antigen-presenting cells (dendritic cells). Through the secretion of IL-12p70, IL-6 and TGF- β , differentiation of CD4⁺ T lymphocytes towards Th1, and Th17 is favored, which participate in the development of the disease (Ifergan et al. 2008). Once differentiated, Th1 and Th17 enter the central nervous system where they are reactivated by antigen-presenting cells (Brambilla 2019). Both Th1 cells and Th17 cells are capable of inducing EAE (Goverman 2009).

EAE has allowed the development of therapeutic strategies against multiple sclerosis such as Glatiramer acetate a drug that induces deviation from the pro-inflammatory to the anti-inflammatory pathways and natalizumab, a monoclonal antibody to α 4 β 1 integrin which is able to block lymphocyte adherence to blood vessels in EAE brains (Steinman et al. 2005).

Moreover, the microglia are activated before the onset of symptoms. Activated microglia present an upregulation of CD45, Major histocompatibility complex (MHC) class II, CD40, CD86, and dendritic cell marker CD11c. The increase in MHC class II, CD40, and CD86 levels suggest that microglia also act as an antigen-presenting cell in the EAE model (Ponomarev et al. 2005). Additionally, in EAE, astrocytes with an A1 activation phenotype have been observed (Liddelow et al. 2017), and are characterized by the secretion of proinflammatory cytokines (IL-6, IL-1 β , and TNF) which favor the activation of the microglia (Brambilla 2019). Therefore, there is an activation of various cell types of the immune system, such as monocytes, microglia, astrocytes, and lymphocytes in EAE. These are cells in which PRL mediates the activation, differentiation, and secretory functions of inflammatory mediators. Therein, we will describe the main findings of PRL's role in these cells and their relationship with EAE.

Serum PRL levels have been reported to increase during the induction phase of acute EAE. Also, prior treatment with bromocriptine (a drug that inhibits the release of PRL) reduces the severity and incidence of clinical signs of the disease (Riskind et al. 1991). However, bromocriptine can suppress the proliferation of T cells independently of PRL by blocking the production of IL-2 (Morikawa et al. 1994). However, bromocriptine does not block the production of extrapituitary PRL (Golander et al. 1979; Lehtovirta and Ranta 1981), making it difficult to assess the role of PRL in studies with this drug.

The role of PRL was evaluated in PRL-deficient mice (Prl^{-/-}) and PRL receptor-deficient (Prlr^{-/-}) mice that developed EAE. These mice were found to develop EAE with late-onset, but eventually, the severity of the disease was similar to that of mice that expressed PRL and its receptor. An interesting observation of this study was that the lymph node cells of Prl^{-/-} and Prlr^{-/-} mice, collected seven days after induction of EAE and stimulation with the 35–55 peptide of the oligodendrocyte myelin glycoprotein (MOG_{35–55}), showed less proliferation and secretion of cytokines IFN- γ , IL-17 A, IL6, and IL-10, compared to control mice (Prl^{+/+} and Prlr^{+/+}, respectively). However, when lymph node cells were collected 10 days after induction of EAE, at the onset of clinical symptoms of the disease and stimulation with the MOG_{35–55} peptide, cells from Prl^{-/-} and Prlr^{-/-} mice presented greater proliferation and secretion of cytokines compared to cells of control mice (Costanza et al. 2013).

In a late phase of EAE, a subset of the cytotoxic Th cells that express the transcription factor T-box eomesodermin (Eomes), called Th Eomes⁺ cells, which are the mainly involved in the inflammatory response (Raveney et al. 2015). This shift from naive CD4⁺ T cells to Th Eomes⁺ is

mediated by PRL and PRLR. Although the pituitary production of PRL and its concentration in the blood are decreased in mice in the late phase of EAE, the concentration in cerebrospinal fluid is increased. This is due to the extrapituitary production of PRL by MHC class II⁺ (Major Histocompatibility Complex Class II) myeloid cells and by B cells. Blocking the production of PRL through antibodies produces a decrease in Th Eomes⁺ cells and markedly improves the late-onset of EAE (Zhang et al. 2019).

Also, treatment with PRL in conjunction with IFN- β at the onset of EAE reduces the clinical symptoms of the disease and reduces the number of immune cells that infiltrate the spinal cord, probably due to the re-melting actions of PRL and the immunosuppressive effect of IFN- β . This protective effect was not observed when mice with EAE were treated only with PRL or only with IFN- β (Zhornitsky et al. 2015).

Effect of prolactin in events of excitotoxicity

PRL protects neurons against damage in excitotoxicity damage models since PRL stimulates processes such as neuroplasticity, neurogenesis, and remyelination (Costanza and Pedotti 2016). Furthermore, PRL has protective functions in astrocytes and microglia, where it regulates activation and secretion of inflammatory cytokines (Anagnostou et al. 2018; Costanza and Pedotti 2016).

Excitotoxicity is a pathophysiological phenomenon in the nervous system caused by continuously increased stimulation of glutamatergic receptors by excitatory amino acids, such as Glu and kainic acid (KA), resulting in an exacerbated intracellular calcium flow, generation of free radicals, and mitochondrial damage, which activates necrotic and apoptotic pathways (Abramov and Duchon 2008; Quillinan et al. 2016). Excitotoxicity is a common phenomenon seen in various neurodegenerative conditions such as epilepsy, hypoxia-ischemia, traumatic damage, and diseases such as Alzheimer's and Huntington's (Dong et al. 2009; Muddapu et al. 2020).

Astrogliosis and microglial activation are processes involved in neurodegeneration due to excitotoxicity. The activated cells secrete inflammatory cytokines, such as TNF- α , IL-1, IL-12, and IL-18, and ROS (Chen et al. 2005; Zhang and Zhu et al. 2011). Blocking receptors like Toll 4 type receptor or inhibiting the release of inflammatory cytokines has been shown to reduce neuronal death in models of neurodegeneration by excitotoxicity (Nikolic et al. 2018; Zhu et al. 2019).

Since PRL participates in the control of cytokines and inflammation, the role it plays under conditions of

excitotoxicity has been evaluated. The KA damage model mimics Glu excitotoxicity and is used as a model of temporal lobe epilepsy (Lévesque and Avoli 2013). In this model, treatment with PRL in rats administered with KA favored increased expression of PRLR in all the subfields of the hippocampus accompanied by neuroprotection (Morales et al. 2014; Ortiz-Pérez et al. 2019; Tejadilla et al. 2010). PRL favors neuronal survival through a mechanism with an effect on the astroglia, found to decrease astrogliosis in the CA1 region of the hippocampus, and that correlates with neuronal survival (Reyes-Mendoza and Morales 2016). In addition, treatment with PRL in ovariectomized rats administered with KA decreases microglial activation in the CA1 and CA4 regions, in addition to decreasing iNOS expression and increasing IL-4 expression (Reyes-Mendoza and Morales 2020).

Another mechanism where PRL favors neuronal survival under conditions of KA excitotoxicity is through inductions of increased expression of the vesicular glutamate transporter 1 (VGLUT1). This transporter is related to Glu transport in neurons and has been reported to participate in the plasticity and survival of hippocampal neurons (Mayor and Tymianski 2018). Treatment with PRL alone does not significantly change the number of hippocampal neurons or increase PRLR expression. These effects were only observed in an environment of damage and inflammation caused by KA (Ortiz-Pérez et al. 2019).

In vitro models of primary cultures of rat hippocampal neurons with excitotoxic Glu damage show protection against neuronal death by PRL, which maintains mitochondrial function by mechanisms related to intracellular calcium release and NF- κ B factor activation (Rivero-Segura et al. 2017). PRL does not, on its own, affect cell viability in neuron cultures, since the protective effect is only observed under conditions of Glu excitotoxicity mediated by the activation of PRLR (Vergara-Castañeda et al. 2016).

Effect of prolactin on ischemia

In ischemia, the concentration of PRL increases in the nervous system as a possible mechanism of protection and control of the glia (Vermani et al. 2020). A study compared the concentration of PRL in blood and cerebrospinal fluid in cases of hypoxia-ischemic death due to suffocation with cases of death from other causes. No differences in plasma PRL concentration between both groups was found. However, the concentration of PRL in cerebrospinal fluid was three times greater in hypoxia-ischemic death cases. This was due to the transport of PRL through epithelial cells of the choroid plexus in hypoxic conditions (Tani et al. 2018).

In a rat hypoxia-ischemia model due to brain injury, the injury triggered both PRL and PRLR expression in glial cells and neurons. However, PRL and PRLR expression in astrocytes lasted longer, compared to that observed in neurons (one day in neurons and more than seven days in astrocytes). PRLR expression was not observed in microglia, but PRL expression was observed three days after damage. Although, in this study, PRL did not promote neuronal survival, trophic and proliferative effects were observed in the glia (Mödersheim et al. 2007). In another rat ischemia model, treatment with PRL did attenuate neuronal damage by reducing increased levels of the neurotransmitter gamma-aminobutyric acid (GABA) and cerebral calcium (Vermani et al. 2020).

Under hypoxic conditions, the organism appears to seek increased PRL concentrations in the nervous system, either by promoting its transport from the blood or by inducing its extrapituitary expression. However, further studies are required to clarify the role of PRL in ischemia and to assess its effect on glial cells.

Therefore, therapeutic strategies that seek to stimulate or inhibit PRL function should be carefully evaluated given the inflammatory, anti-inflammatory, or restorative functions that it presents, since its inhibition could be beneficial or harmful in specific contexts.

Conclusions and future perspectives

PRL possibly exerts a dual activity on inflammation; it either inhibits or promotes this type of immune response. PRL's antagonistic effects respond to factors such as target cell type, PRL concentration, stimulus duration, PRLR isoform, and cytokines in the medium. PRL mediates different effects depending on the cytokines that it interacts with. In the nervous system, PRL exerts inflammatory and anti-inflammatory effects in various neurodegenerative diseases such as EAE, ischemia, and under conditions of excitotoxicity. However, more research is needed to elucidate the function of PRL in a context of inflammation which will allow identification of new therapeutic alternatives to treat neuronal damage in different neurological diseases. In this sense, in EAE, studies are required to evaluate the conditions in which PRL mediates favorable effects, looking for treatments that combine PRL with more immunomodulators or evaluating the effect of administering PRL in different stages of development of EAE, since, as discussed in the role of this hormone changes according to the molecules present in the medium or according to the development of the disease. In

the case of EAE treatment with PRL plus IFN- γ , it is necessary to determine if it favors remyelination and the mechanism involved. Models of excitotoxicity treated with PRL require determining which cells secrete anti-inflammatory cytokines and evaluating the effect on polarization of microglia, macrophages, or astrocytes. Another option would be to study PrL^{-/-} or PrLr^{-/-} mice administered with KA or Glu to clarify the role of PRL. Under hypoxic conditions, the organism appears to seek increased PRL concentrations in the nervous system, either by promoting its transport from the blood or by inducing its extrapituitary expression. However, further studies are required to clarify the role of PRL in ischemia and to assess its effect on glial cells.

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