Prolactin receptor in regulation of neuronal excitability and channels

Keywords: neuroendocrinology, prolactin receptor, neuronal channels, TRP channels, neuropeptides, Ca$^{2+}$-dependent K$^+$ channels

Prolactin (PRL) represents a multifunctional endocrine hormone that was originally discovered in the 1930s as a factor controlling milk production and secretion. The vast number (> 300) of physiological functions influenced by PRL include reproduction, electrolyte balance, metabolism, regulation of the endocrine and immune systems, and modulation of the nervous system.1,2 The PRL-induced regulation of the nervous system is manifested in such important and relevant processes as maternal behavior, energy balance and food intake, stress and trauma responses, anxiety, neurogenesis, migraine and pain. PRL controls these critical functions by regulating receptor potential thresholds, neuronal excitability and/or neurotransmission efficiency. PRL also influences neuronal functions via activation of certain neurons, resulting in Ca$^{2+}$ influx and/or electrical firing with subsequent release of neurotransmitters. Although PRL was identified almost a century ago, very little specific information is known about how PRL regulates neuronal functions. Nevertheless, important initial steps have recently been made including the identification of PRL-induced transient signaling pathways in neurons and the modulation of neuronal transient receptor potential (TRP) and Ca$^{2+}$-dependent K$^+$ channels by PRL. In this review, we summarize current knowledge and recent progress in understanding the regulation of neuronal excitability and channels by PRL.

**Introduction**

Prolactin (PRL) activates PRL receptor isoforms to exert regulation of specific neuronal circuitries, and to control numerous physiological and clinically-relevant functions including; maternal behavior, energy balance and food intake, stress and trauma responses, anxiety, neurogenesis, migraine and pain. PRL controls these critical functions by regulating receptor potential thresholds, neuronal excitability and/or neurotransmission efficiency. PRL also influences neuronal functions via activation of certain neurons, resulting in Ca$^{2+}$ influx and/or electrical firing with subsequent release of neurotransmitters. Although PRL was identified almost a century ago, very little specific information is known about how PRL regulates neuronal functions. Nevertheless, important initial steps have recently been made including the identification of PRL-induced transient signaling pathways in neurons and the modulation of neuronal transient receptor potential (TRP) and Ca$^{2+}$-dependent K$^+$ channels by PRL. In this review, we summarize current knowledge and recent progress in understanding the regulation of neuronal excitability and channels by PRL.

To understand mechanisms responsible for PRL-induced regulation of neuronal functions and the channels involved.

**Prolactin in the Nervous System**

To understand mechanisms responsible for the regulation of nervous system function by PRL on normal physiological processes and in pathological conditions, it is essential to outline PRL concentrations in the different regions of the nervous system during various conditions. Serum levels are important since PRL from the peripheral circulation can gain access to the CNS either via receptor-mediated mechanisms3 or through circumventricular structures that lack a blood–brain barrier.16 The major source of serum PRL is from specialized anterior pituitary cells called lactotrophs.2 Under normal conditions, concentrations of PRL in blood are closely regulated by estrogen and depend on sex, the reproductive cycle, pregnancy and lactation.2 In rodent and human males, blood PRL levels are 5–20 ng/ml. In female rodents, blood PRL levels peak at prooestrous phase (12–14 h) and reach up to 120–150 ng/ml.17 After prooestrous phase, PRL drops and plateaus for 25–27 h (estrous phase) to 60–80 ng/ml, and then declines even further to 30–60ng/ml during diestrous phase (55–57 h).17 In contrast, the blood PRL level in women is not strongly influenced by menstrual cycle and stays in a range of 10–60 ng/ml.2 The profile of PRL release during human pregnancy is entirely different from that in rodents.18,19 Nevertheless, in both rodents and humans, observed blood PRL levels are as high as 200–300 ng/ml during pregnancy.2 During lactation, PRL remains as high as that found during pregnancy, but then gradually declines.20,21 PRL levels in blood are also significantly up-regulated in several notable pathological conditions, the first being inflammation. The inflammatory conditions associated with increased PRL serum levels include a severe form of progressive systemic sclerosis,22,23 the active phase of systemic lupus erythematosus,24,25 rheumatoid arthritis,26 polymyalgia rheumatica27 and autoimmune thyroid diseases.28 In contrast to these chronic inflammatory conditions, acute inflammation does not cause elevated levels of PRL in blood.29 The second pathological condition associated with elevated levels of PRL is physiological and physical stress (i.e., trauma). Burn,30 surgical procedures,31,32 osteoarthritis,33 migraine34 and post-traumatic stress disorder (PTSD)35 are all...
associated with increased PRL serum levels. Although estrogen is important in the regulation of PRL, PRL could be considered as a dually regulated hormone, where both estrogen and a specific pathological condition, especially stress and inflammation, also control the levels of PRL in blood. This dual regulation is an important point and needs to be considered by investigators when studying the regulation of neuronal functions by PRL.

Circulating PRL in the blood from the anterior pituitary is not the only source of PRL in the nervous system, since extra-pituitary sources include PRL from neurons and non-neuronal cells located in both the peripheral (PNS) and central nervous system (CNS). Dual regulation of extra-pituitary PRL by estrogen and pathological condition is not well studied. Therefore, direct measurements on alterations in PRL concentrations in relevant parts of the nervous system triggered by pathological conditions are important to consider. PRL concentrations can be measured from total protein extracts obtained from particular regions of the CNS and these extracts contain both extracellular and intracellular proteins. For example, rat male lumbar (L4-L6) spinal cord contains 2ng/ml PRL and 6 ng/ml PRL 24h after hindpaw surgery. In contrast, female rats in estrous phase have 8ng/ml PRL, but this is elevated to 40ng/ml 24h after surgery. The exact source(s) responsible for these elevated PRL levels seen in the spinal cord after surgical trauma is unknown. In addition, it is possible that PRL concentrations in the vicinity of neuronal synapses located in important pain processing regions of the spinal cord will be even higher than the concentrations obtained from total proteins. Nevertheless, although exact physiological PRL concentrations acting on particular neurons cannot be established, evaluations of total PRL protein concentrations in defined regions of the nervous system do offer fundamental information when considering possible contributions of changed PRL concentration levels to neuronal activation in pathological conditions.

**Prolactin Receptor in the Nervous System**

PRL actions are specifically mediated via the PRL receptor (PRLR). PRLR belongs to the cytokine type I subfamily, which also includes receptors for growth hormone, leptin, leukemia inhibiting factor and erythropoietin. Along with an extracellular domain (ECD) that binds PRL, PRLR also has a single trans-membrane domain (TD) and intracellular domain (ICD) (Fig. 1). PRLR is encoded by a single gene, but has numerous tissue and cell-specific splice variants. A complete gene structure of PRLR is presented in detail in several excellent reviews, and the exon-intron structure can be found on the website Atlas of Genetics and Cytogenetics. Briefly, PRLR has three isoforms: long (PRLR-L), intermediate (PRLR-I in rodents = S1a in humans) and short (PRLR-S in rodents = S1b in humans). PRLR exists either as a homodimer or heterodimer between PRLR-L and PRLR-S. Isoforms have identical ECD and TD, but differ in ICD lengths and sequences (Fig. 1). In this respect, the most noticeable difference between long and short forms is that PRLR-L and PRLR-I (an uncommon form) have Box-1 and Box-2 domains, while PRLR-S also has Box-1 but lacks the Box-2 domain (Fig. 1). Consequently, cellular signaling pathways via PRLR-L and PRLR-S are substantially different. For example, PRLR-L, but not PRLR-S, activates both Janus kinase2/signal transducer and activator of transcription 5 (JAK2/STAT5) pathways.

PRL is the main mediator inducing phosphorylation of STAT5 (phospho-STAT5) in the nervous system, with dopamine D2 receptor (D2R) agonist-bromocriptine-treatment blocking a majority of basal phospho-STAT5 expression (Table 1). In addition to PRL, growth hormone and leptin have the capability of phosphorylating STAT5 in the hypothalamus. The phosphorylation of STAT5 likely controls long-term changes in the nervous system. The regulation of neuronal excitability and channels often requires the activation of a transient signaling pathway and the role of PRLR-L and -S in transient signaling in neurons will be discussed in detail below.

To understand the wide spectrum of PRL actions in the nervous system, it is essential to first identify PRL-sensitive neurons. PRLR-L and PRLR-S expression patterns in the CNS of female and male rodents have been characterized in detail, and are summarized in Table 1. The PRLR expression pattern in the CNS is notably similar in rats and mice. Importantly, PRL can modulate the function of sensory neurons, and this modulation...
can help account for sex differences in pain mechanisms.\textsuperscript{7,8} A PRLR antibody, which does not discriminate between the different forms of PRLR, labels 50–60% of neurons in female rat trigeminal ganglia (TG) and a majority of the satellite glial cells (SGC) surrounding neuronal cell bodies.\textsuperscript{40} However, use of in situ hybridization showed that PRLR-L is only present in 3–5% of male and female rat TG neurons thus suggesting that much of the PRLR immunoreactivity is due to PRLR-S. Evaluation of human dental pulp tissue that is innervated by the trigeminal nerve, showed that PRLR-L is mostly absent in afferent nerves, but is present in the myelin sheath of peripheral glia that surrounds nerves.\textsuperscript{40} In contrast, the use of isoform-specific human antibodies, showed that both PRLR-S\textsubscript{1a} and \textsubscript{1b} were present in both nerve axoplasm and glia.\textsuperscript{40} In the female rat dorsal

| The nervous system                                      | PRLR-L | PRLR-S | pSTAT5 |
|---------------------------------------------------------|--------|--------|--------|
| Anteroventral periventricular nucleus (AvPv)            | +++    | ++++   | +++    |
| Medial preoptic nucleus (MPN)                           | +++    | +++    | +      |
| Median preoptic nucleus (MEPO)                          | -      | +++    | -      |
| Anteroventral preoptic nucleus (ADP)                    | ++     | ?      | ?      |
| Parastrial nucleus (PS)                                 | +++    | ?      | ?      |
| Supraoptic nucleus (SO)                                 | ++     | +++    | ?      |
| Medial septal nucleus (MS)                              | ++     | ?      | ?      |
| Rostral preoptic periventricular nuclei (rPvpo)         | ++     | ?      | +      |
| Caudal preoptic periventricular nuclei (cPvpo)          | ++     | ?      | +      |
| Choroid plexus (CP)                                     | ++     | -      | ++     |
| Anterior bed nuclei stria terminalis (aBST)             | ++     | ++     | +      |
| Posterior bed nuclei stria terminalis (pBST)            | ++     | ++     | +      |
| Lateral septum (LS)                                     | ++     | +++    | +      |
| Anterior hypothalamic area (AHA)                        | ++     | ?      | -      |
| Paraventricular nucleus thalamus (PVT)                  | +      | +++    | -      |
| Paraventricular nucleus (PVN)                           | +      | +++    | -      |
| Arcuate nucleus (ARN)                                   | ++++   | +++    | +++    |
| Ventromedial nucleus, ventrolateral part (VMH)          | ++     | +++    | +      |
| Dorsomedial nucleus (DMN)                               | ++     | ?      | +      |
| Posterior hypothalamic nucleus (PH)                     | ++     | ?      | -      |
| The medial nucleus of the amygdale (MEA)                | ++     | ++     | +      |
| Dorsal supraoptic nucleus (SON)                         | +      | ?      | ?      |
| Lateral hypothalamus (LHA)                              | +      | ++     | ?      |
| The lateral preoptic area (LPO)                         | +      | ?      | ?      |
| Periaqueductal gray (PAG)                               | ++     | ?      | ?      |
| Interpeduncular nucleus (IPN)                           | ±      | ?      | ?      |
| Substantia nigra (SN)                                   | ±      | ?      | ?      |
| Pontine gray (PG)                                       | ±      | ?      | ?      |
| The dorsal horn of the spinal cord                       | ++++   | ?      | +++    |
| The ventral horn of the spinal cord                      | -      | -      | -      |
| Trigeminal ganglion                                      | +      | ++++   | ?      |
| Dorsal root ganglion                                     | ++     | ++++   | +++    |

| Regular font, the central nervous system (CNS) regions, excluding brain stem and spinal cord. Underlined, the brain stem and spinal cord regions. Bold, the peripheral nervous system (PNS), including sensory, sympathetic, parasympathetic and enteric nervous systems. | Celiac ganglion |
|---|---|---|

can help account for sex differences in pain mechanisms.\textsuperscript{7,8} A PRLR antibody, which does not discriminate between the different forms of PRLR, labels 50–60% of neurons in female rat trigeminal ganglia (TG) and a majority of the satellite glial cells (SGC) surrounding neuronal cell bodies.\textsuperscript{40} However, use of in situ hybridization showed that PRLR-L is only present in 3–5% of male and female rat TG neurons thus suggesting that much of the PRLR immunoreactivity is due to PRLR-S. Evaluation of human dental pulp tissue that is innervated by the trigeminal nerve, showed that PRLR-L is mostly absent in afferent nerves, but is present in the myelin sheath of peripheral glia that surrounds nerves.\textsuperscript{40} In contrast, the use of isoform-specific human antibodies, showed that both PRLR-S\textsubscript{1a} and \textsubscript{1b} were present in both nerve axoplasm and glia.\textsuperscript{40} In the female rat dorsal
root ganglia (DRG), PRLR staining as seen with the U5-clone 5 monoclonal antibody is also observed in many neurons and satellite glial cells (Fig. 2A) like seen in the female rat TG. In addition, many of the DRG sensory neurons with prominent PRLR-expression are small in size and almost all co-express TRPV1 (Fig. 2A), and both of these features are common in nociceptors. Interestingly, basal levels of activated phospho-STAT5 are present in ≈10–15% rat female DRG neurons labeled with phospho-STAT5 antibody (Fig. 2B), along with a subset of spinal cord neurons located primarily within the superficial laminae of the dorsal horn, an important region responsible for the modulation of nociceptive stimuli (Fig. 2C). In contrast, the satellite glial cells typically do not show significant phospho-STAT5 labeling under basal conditions. The phospho-STAT5 positive DRG neurons are also predominantly small in size. It is not yet clear if this basal expression of phospho-STAT5 in DRG and dorsal horn neurons is regulated by PRL as it is within other regions of the CNS. In this respect, it comes as no surprise that a majority of studies on regulation of neuronal functions by PRL have been done on neurons from ARN, AVPV, DRG and TG.

Activation of Neurons by PRL

Activation of neurons can evoke action potentials and/or Ca^{2+} influx in neurons, culminating in the release of neurotransmitters or neuropeptides. An excellent example of this is the PRL-stimulated release of oxytocin or dopamine from hypothalamic neurons that control feedback mechanisms. In addition, PRL is capable of exerting action potential firing in different types of neurons. Thus, tuberoinfundibular dopamine (TIDA) neurons located in ARN provide a dopaminergic inhibitory tone on lactotrophs and fire action potentials upon application of 20nM (≈450ng/ml) PRL. TIDA neurons, identified as tyrosine hydroxylase-positive, display hallmark oscillation, when recorded in male rat hypothalamic slices using the whole-cell patch clamp technique. However, loose patch recording from mouse TIDA neurons, identified as dopamine transporter-expressing cells, found oscillation only in 20% neurons. Interestingly, these TIDA neurons displayed irregular spontaneous action potentials, and PRL-induced firing rates were not modified during lactation. PRL (20nM) is also capable of generating electrical firing in < 5% of female rat hypothalamic AVPV, but not gonadotropin-releasing hormone (GnRH)-positive neurons. When rat male TIDA neurons are voltage clamped at resting membrane potentials (≈-65mV), exposure to high concentrations of PRL (40–500 nM, ≈1–12 µg/ml) generates small inward currents (I_{PRL}; 15–25 pA). This depolarizing I_{PRL} could change the membrane potential above threshold levels and produce action potentials, which are observed in TIDA neurons upon PRL application. The precise nature of the channels mediating I_{PRL} in TIDA neurons is not known. However, it has a certain pharmacological profile. Thus, I_{PRL} was abolished by 2-aminoethyl diphenylborinate (2-APB), but not by SKF96365, a TRPC6/TRPC7 channel antagonist. I_{PRL} was also larger in the presence of low extracellular Ca^{2+}. These results imply that I_{PRL} could be mediated by a TRP-like channel, probably one of the TRPC1-TRPC4 channels. To identify intracellular cascades involved in generation of I_{PRL}, JAK and phosphoinositide 3 (PI3)-kinase pathways were blocked with AG490 and wortmannin.
Neither JAK nor PI3-kinase pathways are engaged in generation of \( \text{IPRL} \). Activation of TIDA neurons by PRL is similar in effect to that of leptin on ARN neurons, which is also mediated by TRPC-like channels. The difference is that leptin's action is via a JAK pathway. PRL is also able to produce inward current (at resting membrane potentials) in non-neuronal, glioma cells. This \( \text{IPRL} \) appeared several minutes after PRL application, independent from intracellular Ca\(^{2+}\), but triggered by an unknown second messenger. In summary, even though the precise nature of PRL-activated channels in neuronal as well as non-neuronal cells is still unknown, there is evidence to suggest these channels may belong to TRP-like channel family. The signaling pathway involved in this activation of the TIDA neurons by PRL has also not been clearly identified. Furthermore, the activation of these neurons at resting membrane potentials required a substantial amount of PRL, which is typical of concentrations observed during pathological hyperprolactinaemia resulting from pituitary adenomas. Even so, PRL concentrations (20–40 nM) observed during pregnancy and lactation can produce action potential firing in TIDA neurons via an unknown mechanism. Interestingly, non-neuronal cells can be activated by PRL concentrations as low as 1 nM.

Activation of neurons can lead to action potential firing, inward currents at resting membrane potential as well as intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) accumulation. Even though PRL-evoked [Ca\(^{2+}\)]\(_i\), rise has been studied in non-neuronal cells, similar studies in neurons are mostly lacking. In non-neuronal cells, PRL-evoked [Ca\(^{2+}\)]\(_i\), rise is mediated by intracellular stores and by an unknown plasma membrane channel activated by PRL (see above). In neurons, it is presumed that PRL can produce Ca\(^{2+}\) influx via both TRP-like channels and L-type voltage-gated Ca\(^{2+}\) channels (Fig. 3). The role of intracellular stores in PRL-evoked [Ca\(^{2+}\)]\(_i\) rise in neurons is unknown. Moreover, PRL-induced [Ca\(^{2+}\)]\(_i\) rise depends on neuronal type. Thus, some data indicate that GABAergic, but not dopaminergic TIDA neurons, react to PRL with a rapid increase in [Ca\(^{2+}\)]\(_i\).

**Regulation of TRP Channels by PRL in Sensory Neurons**

Sensory neurons play a key role in nociception/pain. Certain TRP channels, especially TRPV1, TRPA1 and TRPM8 are highly expressed in sensory neurons and contribute to nociception/hypersensitivity/pain. Strong evidence links these channels to mediating sensory neuronal activities by modulating the neuronal threshold of activation and efficiency of neurotransmission between sensory and spinal cord neurons. A variety of pain conditions such as those associated with surgical procedures, migraine, burn, rheumatoid arthritis, osteoarthritis trigger an increase in serum PRL levels in men, and even more so in women. Recent evidence also suggests that inflammation, tissue injury and trauma lead to a sex-dependent up-regulation in extra-pituitary PRL in the vicinity of peripheral and central terminals of sensory neurons. Interestingly, TRPV1 activities are enhanced by concentrations of PRL as low as 10–25 ng/ml in DRG neurons from estrous female mice. In contrast, TRPV1 in DRG neurons from male mice requires > 0.8–1 ug/ml PRL for up-regulation of activities. Similar contrasting results were observed in the regulation of TRPV1 activities by PRL in TG neurons from female ovariectomized (OVX) vs. OVX with estrogen replacement (OVX-E2) rats. These results showed that PRL (100 ng/ml; ~4 nM) up-regulates the activity of TRPV1 in neurons from OVX-E2 rats, but not in rats with OVX alone, thus demonstrating influence of estrogen. Moreover, TRPV1 is not even sensitized by 1 ug/ml PRL in sensory neurons from OVX rats. PRL is also capable of increasing TRPA1 and TRPM8 activities in a sex-dependent manner in a subset of DRG neurons from mice. In summary, PRL concentrations increase to 25–100 ng/ml within both peripheral tissues and the lumbar spinal cord of females after inflammation and trauma, and this concentration is effective in enhancing TRPV1, TRPA1 and TRPM8 activities in sensory neurons of female, but not male rodents. Together, these findings suggest possible important contributions of PRL to the regulation of nociceptors after inflammatory and trauma in a sex-dependent manner.

**PRL and Rapid Signaling Pathways in Neurons**

Activation of PRLR initiates multiple second-messenger cascades, including JAK/STAT5, MAPK and PI3-kinase signaling pathways in non-neuronal cells. The role of these cascades in transcription control and endpoints such as cell differentiation, proliferation and survival of non-neuronal cells are relatively well studied. There is a general agreement that a majority of these long-lasting PRL effects in non-neuronal as well as neuronal cells are mediated via PRLR-L and a recruitment of the JAK/STAT5 pathway or at times the MAPK pathway. Multiple studies performed in the nervous system suggest that besides long-lasting effects, neuronal responses to PRL can also be as fast as 1–5 min. These rapid responses are essential for the control of neuronal excitability as well as modulation of neuronal circuitries by PRL. In this section, we will present current views on the mechanisms underlying PRL-induced rapid signaling pathway in neurons.

PRLR expression in a heterologous system along with reconstitution of PRLR in PRLR KO sensory neurons showed that the transient/rapid effects of PRL on TRPV1 activities are mediated by PRLR-S. PRLR-L does not have any direct involvement in the transient modulation of TRPV1 by PRL. However, PRLR-L can suppress PRLR-S function, and this suppression can indirectly inhibit TRPV1 activity. The C-terminal portion of PRLR-L (290–540 aa rat clone), especially the 290–430aa domain containing Box2 (Fig. 1), contributes to inhibition of PRLR-S function. PRLR-S has a short and unique amino acid C-terminal sequence (30 aa) that does not contain any noticeable motifs for activation of kinases. This could suggest that perhaps both PRLR-L and PRLR-S have the potential for rapid signaling. However, this capability is suppressed for PRLR-L homodimers. Altogether, the rapid PRL effects in neurons could be mediated mainly by the PRLR-S homodimer (Fig. 3), which
is extensively expressed in the nervous system (see Table 1). In sensory neurons, expressions of PRLR-L and PRLR-S seldom overlap. In the CNS, PRLR-L, and PRLR-S expression overlaps are common. In such circumstances, it is not clear which cellular mechanisms are responsible for separating the effects of PRLR-L and PRLR-S when expressed together in the same neurons.

Studies on signaling mechanisms responsible for the PRL (0.01–1ug/ml) regulation of hypothalamic neuroendocrine dopaminergic (NEDA) neurons demonstrated that PRL rapidly induces synthesis of catecholamine via protein kinase A (PKA) and C (PKC), but not Ca²⁺-dependent calmodulin kinase-II (CaMKII) pathways. PRL application also led to a PKC-dependent activation of ERK1/2 within the MAPK pathway (Fig. 3). Interestingly, PRL-activated transcription does not involve PKA, PKC, CaMKII, or ERK1/2. The role of PRL-triggered rapid signaling pathways in neurons was further studied in TRPV1 sensitization. Inhibition of several kinase pathways demonstrated that the transient actions of PRL on TRPV1 activities in sensory neurons are directed by PI3-kinase or PKC (Fig. 3). PKA and Src-kinase inhibitors however failed to affect TRPV1 activity enhancement by PRL. Detailed investigation showed that the main PKC isoform involved in mediating PRL effects on TRPV1 in sensory neurons is PKCe. However, it is not clear whether the PKC pathway is downstream of the PI3-kinase pathway (Fig. 3). Rapid induction of PI3-kinase by PRL in neurons of the CNS has been reported, and includes the suppression of BK-type Ca²⁺-dependent K⁺-currents in TIDA neurons by way of the PI3-kinase pathway (Fig. 3). In summary, it appears that PRL activates two principally distinct signaling pathways in neurons. The first involves PRLR-L and the phosphorylation of STAT5, leading to long-lasting effects based on transcription activation (Fig. 3). The second pathway recruits PRLR-S, leading to rapid activation of PI3-kinase, ERK1/2 and/or PKC, with subsequent modulation of neuronal channels, excitability and neurotransmission (Fig. 3). The schematic presented in Figure 3 is a simplified pathway, but underscores the primary signaling events following activation of neurons by endogenous or exogenous PRL. PI3-kinase, PKC and ERK1/2 can regulate a variety of ligand- and voltage-gated channels in neurons. Thus, PKC activation results in production of diacylglycerol (DAG), a potent activator of many TRPC-like channels. Finally, it must be taken into account that PRL regulation of certain pathways and kinase recruitments could be cell-dependent. Thus, kinases can influence each other’s activities, and in certain neurons, PI3-kinase or PKC could be directed by PRLR-L.

**Regulation of Neuronal Excitability by PRL**

To fulfill its function in the nervous system, PRL has to regulate spontaneous or evoked neuronal activities (i.e., firing rates). There are several well characterized neuronal circuitries in which PRL exhibits such regulations. The magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei express oxytocin and demonstrate spontaneous firing. The magnocellular neurons are highly responsive to PRL, which up-regulates or inhibits oxytocin secretion depending on physiological conditions. Both PRLR-L and PRLR-S have been identified in the SON and PVN (Table 1). Spontaneous firing rates in oxytocin neurons, which are identified by responsiveness to cholecystokinin (CCK), are suppressed by PRL (lуг/ml) in non-pregnant and non-lactating diestrous rats. PRL (100 ng/ml) is also able to elicit hyperpolarizing currents in the magnocellular neurons of female rats at membrane potentials more negative than ≈ 60 mV, leading to the inhibition of firing rates. Interestingly, PRL shows both excitatory and inhibitory effects on spontaneous activities of SON oxytocin neurons of male rats. The mechanisms underlying the actions of PRL on spontaneous firing activities in oxytocin neurons of female and male rats are not known as yet.

In TIDA neurons, PRL (>40 nM) generates large inward currents at positive holding potentials that can influence action potential shape. Indeed, PRL broadens action potential width by ≈20% in TIDA neurons. Broadening of action potentials is a hallmark indicator for modulation of voltage-gated Ca²⁺- and/or BK-type Ca²⁺-dependent K⁺-channels. Even small changes in action potential width could lead to a several-fold rise of synaptic neurotransmitter release.
mechanisms underlying an increase in action potential width and induction of inward currents by PRL at positive potentials revealed that PRL stimulates Ca$^{2+}$ influx into TIDA neurons via L-type voltage-gated Ca$^{2+}$ channel (VGCC). PRL-activated inward current at +40 mV was blocked by paxilline, a BK-type Ca$^{2+}$-dependent K$^+$ channel blocker, but not affected by the SK-type Ca$^{2+}$-dependent K$^+$ channel blocker, UCL1684. Altogether, PRL modulates the shape of action potentials and can increase neurotransmitter release at pre-synaptic sites of TIDA neurons by inhibiting BK-type Ca$^{2+}$-dependent K$^+$ channels coupled to Ca$^{2+}$ influx via L-type VGCC. This suggests that rapid effects of PRL in TIDA neurons may be mediated via PRLR-S, and may also involve PKC.

There is a substantial difference in the effects of PRL on BK and other K$^+$ channels in non-neuronal cells compared with those in neurons. First, it appears that in non-neuronal cells PRL exerts transient effects through PRLR-L via the JAK2 pathway. Second, in Chinese hamster ovary (CHO) and glioma cells, PRL (4 nM) increases activity of BK-type Ca$^{2+}$-dependent K$^+$ channels. PRL has also shown to activate other K$^+$ currents and alter open probability in a prostate cancer cell line via a Fyn pathway. This difference in effect of PRL in neuronal as compared with non-neuronal cells suggests involvement either specialized adaptor proteins for PRLR or different cellular sub-domain organization (i.e., lipid rafts for example).

The regulation of evoked neuronal activities (i.e., firing rates) by PRL is the least studied property of this multifunctional hormone. Since PRLR is expressed on sensory neurons, they could be physiologically relevant and a suitable model to study regulation of evoked neuronal activities by PRL. Action potential firing in sensory neurons can be evoked by environmental cues acting on peripheral terminals, or by endogenous agents released in dorsal horn of spinal cord (or brain stem) and activating central terminals of sensory neurons. Under pathophysiological conditions, PRL can assess peripheral and central sensory neuronal terminals via an autocrine, paracrine and/or endocrine mechanisms. Action potential firing can be modeled by injecting current into neurons recorded in whole-cell configuration under current clamp mode (Fig. 4B). Acutely cultured DRG neurons from estrous rat females showed a low firing rate upon current injection (Fig. 4A and B). However, following pre-treatment (15 min) with PRL (4 nM; 100ng/ml), DRG firing rates were substantially increased (Fig. 4A and C). Firing rates are controlled by a wide variety of voltage-gated channels. Thus, the post-translational or transcriptional inhibition of K$^+$-channels, such as BK-type, SK-type and/or M-type by PRL can increase the firing rate of neurons. Since contribution of voltage-gated Na$^+$ channels (VGSC) to firing rates are well documented, enhancement of VGSC activities or threshold of activation by PRL could likely lead to an increase in neuronal excitability.

**Conclusion**

Although PRL controls a vast number of physiologically critical functions in the nervous system, we are still far from understanding the molecular mechanisms underlying these processes. Nonetheless, research on PRL mediated regulation of neuronal functions, excitability, neurotransmission and involved channels has recently made notable progress. We now know that PRL can generate small currents in neurons via putative TRP-like Ca$^{2+}$ channels and influx Ca$^{2+}$ into neurons via L-type VGCC. We also know that rapid PRL responses in neurons are probably mediated by the short PRLR isoform via PI3-kinase and PKC.
pathways. Importantly, PRL is able to rapidly modulate evoked firing rates and neurotransmission by suppressing certain K⁺ channels in neurons. This is a principal difference for neuronal PRL-induced pathways compared with non-neuronal pathways where PRL activates K⁺ channels that will lead to overall inhibition. Although we are now just beginning to understand the mechanisms responsible for PRL actions in the nervous system, there are numerous critically important unaddressed aspects regarding the regulation of neuronal functions by PRL. In this respect, several understudied issues include: (1) Mechanisms contributing to extra-pituitary PRL production in a variety of neurological disorders. (2) The role of PRL in orchestrating the regulation of neuronal excitability in physiological vs. pathophysiological conditions. (3) Differences in the regulation of PRL and PRLR expressions in neurons of males and females as compared with pregnant or lactating females. (4) Defining the specific cellular mechanisms involved in the PRL regulation of the different PRL-responsive neuronal subtypes. (5) Identifying mechanisms involved in regulation of a variety of ligand and voltage-gated channels by PRL in neurons. As our understanding of PRL actions continues to evolve, future research will hopefully focus on some of these important issues in attempts to even better understand the mechanisms of action of this exciting and intriguing hormone on a wide variety of physiological and pathophysiological conditions affecting the nervous system.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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