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The Regulation of Pituitary Prolactin Secretion: Hypothalamic, Intrapituitary and Intracellular Factors and Signaling Mechanisms

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1. Introduction

A consensus view developed over the last decades holds that the basal secretion of PRL from the anterior lobe (AL) is spontaneous (i.e., occurs without stimulation by the hypothalamus) (Neill 1994; Freeman 2000) since PRL is secreted from lactotropes with a high secretory rate for prolonged periods after disconnecting the hypothalamic influence (when it transplanted to a site distant from the hypothalamus under the kidney capsule or when cultured in vitro). Consequently, PRL secretion appears to be severely restrained by the hypothalamus in vivo as the main source prolactin inhibiting factor (PIF). Results of the extensive research of the last decades have clearly demonstrated that the withdrawal of DA tone is not sufficient to account for the surge of PRL secretion observed in response to the suckling (physiological) stimulus during lactation. Similarly, DAerg tone would not completely recover the chronic elevation of PRL during pregnancy, lactation or in other pathophysiological stages. The research has subsequently included the search for putative prolactin-releasing factors (PRF) controlling PRL peaks occurring after mating or triggered by ovarian steroids. These can be termed by central (i.e hypothalamic) or peripheral (within the pituitary gland) sites of actions. However, it may be better to classify them by the levels of control mechanisms to regulate PRL secretion: *(i)* action on hypothalamic DAerg neurons; *(ii)* binding to specific receptors on lactotrophs in the pituitary gland; *(iii)* paracrine/autocrine compounds.

i.   The hypothalamic regulating factors acting on *hypothalamic DAergic neurons* may have direct activation or inhibition of their activity, or alter DA, resulting in a consequent changes in PRL secretion (Freeman, 2000; Ben-Jonathan, 2008). The final common pathways of the central stimulatory and inhibitory control are the neuroendocrine neurons producing DA available to be delivered into the hypophyseal portal vessels or

to be released to pituitary neurointermediate (NIL) lobe in order to reach ultimately the anterior lobe (AL).

- Prolactin itself, bombesin, gastrin-releasing peptide (GRP), neuromedins B and C, neurotensin, neuropeptide Y, acetylcholine (Ach), PACAP, angiotensin II, calcitonin, atrial natriuretic peptide (ANP) family members demonstrated to stimulate tyrosine-hydroxilase (TH) activity, increase DA release and a consequent depression in PRL secretion.
- Others induce opposite effect, such as opioids, norepinephrine, somatostatin, CCK, serotonin, and GABA act as inhibitors on TH activity of TIDA cells and associated with PRL release.
- Several compounds (e.g. TRH, oxytocin, VIP, neurotensin, neuropeptide Y, histamine, acetylcholine, somatostatin, CCK) may have different central or peripheral effect or exert dual phase actions on PRL release.

ii. The other group of regulators of PRL secretion act *directly on lactotrophs*. These hypothalamic releasing/inhibiting factors bind to specific receptors on pituitary lactotrophs, cause alterations in expression or release of PRL resulting in changes of secretion pattern. PIF and PRF from the neuroendocrine neurons can be released either at the level of median eminence (ME) into the long portal veins or at the level of NIL, which is indirectly connected to the anterior lobe of the pituitary gland by the short portal vessels.

iii. Additional to the hypothalamic PRF and PIF factors, lactotrophs are also influenced by compounds that released from the surrounding cells and act as *a paracrine regulation* or potentially from the *lactotrophs themselves via autocrine regulation*. These putative paracrine or autocrine factors or combined interactions are potentially also responsible for regulation of PRL release, but the robust and dynamic regulation of PRL secretion more likely controlled and strongly relies on hypothalamic factors (*vide supra*).

On the other hand, there is an ever-growing list of peptides with the potential to act as intrapituitary agent to control PRL secretion, and even more of those which may have dual features: enhance or suppress PRL release in the presence/ absence of other factors. That is a challenge for researchers to demonstrate the presence and to prove the precise mode of actions, since data obtained from *in vitro, in vivo* or *ex vivo* experiments may have discrepancies in results. The effect of a putative autocrine-paracrine control is also hard to demonstrate, since the active substance may have an extremely low local level. Detection however, on a single cell level of PRL release; or by presence of those factors using immunocytochemistry and in situ hybridization, or a combination of these techniques has been found successful. The list of proved factors that act as a local regulator on pituitary lactotroph cells considered as a cumulative contributions of "*Pros and Cons*" over the last two-three decades (Freeman 2000; Wenger 1999; Takaya 2000; Brogilo, 2008; Ondo, 1989; Schettini, 1990; Ben-Jonathan, 2008; Toth 2001; Rettori, 2011):

Suppress/ inhibit PRL secretion:

- dopamine (DA),
- somatostatin (SST), cortistatin (CST),
- gamma-aminobutyric acid (GABA),

- calcitonin (in vitro)
- prolactin (PRL),
- TGF-β isoforms,
- endothelin-like peptides, ET1,
- acetylcholine (ACh),
- glucocorticoids,
- cannabinoids,
- adenosine or analogues (in vitro).

Stimulate/enhance PRL secretion:

- VIP,
- TRH (locally in anterior pituitary),
- oxytocin (OT),
- dopamine (DA, in certain low concentrations only)
- cytokines/ IL6, IL1,
- EGF,
- vasopressin (VP),
- angiotensin (ANG) II,
- galanin (GAL),
- Substance P,
- bombesin/ gastrin-releasing peptide (GRP),
- neurotensin (NT),
- serotonin (5-HT),
- GnRH,
- α-MSH,
- estradiol (E2),
- ghrelin and growth hormone secretagogue (GHS),
- adenosine (icv),
- salsolinol,
- ethanol (alcohol).

1.1. Anatomy of hypothalamic structures in control of PRL secretion

The established hypothalamic hypophysiotropic inhibitor of PRL secretion is DA produced by the arcuate-periventricular nucleus and travels from the median eminence (ME) to the anterior lobe (AL) via the long portal vessels. The well known hypophysiotrophic DAergic neurons in the hypothalamus consist of at least two different areas (named as 'A12' and 'A14') by anatomical location. Despite the extensive supportive evidences for this simplistic view, it has recently proven to be incomplete. It has been demonstrated, that the early studies failed to account for an important source of DA reaching the AL through the short portal vessels from the neurointermediate lobe (NIL). It was clarified that these DAergic neurons can be divided into three distinct systems based on functionality due to the anatomical distribution of neurons and the terminals within the pituitary and sensitivity of internal control mechanisms (summarized in Table 1.).

a. Tuberoinfundibular DAergic (TIDA) neurons located mainly in the middle and posterior portion of the arcuate nucleus ('A12'), project to the ME, and the functions are well accepted as a physiological regulator of hypophysial PRL secretion.

b. The periventricular-hypophysial dopaminergic (PHDA, 'A14') neurons' branched axons terminate in the intermediate lobe (IL), (but not in the neural lobe) (Ben-Jonathan 1980; Peters 1981; Ben-Jonathan, 1982; Goudreau, 1995). Taken together that hypothalamic neurons release DA into portal blood flow (rather than into synaptic clefts) and also the fact that no DA autoreceptors found in TIDA neurons, both assist to abolish the effect of the negative feedback by secreted DA on tyrosine-hydroxilase (TH) activity in DA neurons themselves and consequently maintain the high DA output. These cells are not affected by stimuli of nonselective DA agonists, which cells otherwise increase or decrease the DA output and provide consequent change in PRL secretion as a response by selective D1 or D2 agonist's actions, respectively (Ben-Jonathan, 2001).

c. Experimental evidences have been presented to prove that DA derived from the tuberohypophysial DAergic (THDA, 'A12') system also may serve as an important regulator of PRL secretion. Neurons of this THDA system are located in the most rostral part of the arcuate and periventricular nucleus and axons terminate in the NIL of the pituitary gland rather than in the ME of the hypothalamus (Holzbauer, 1978; Holzbauer, 1985; Ben-Jonathan, 1982, Ben-Jonathan, 1985; Neill 1994; Freeman 2000). Due to the anatomical situation that axon terminals branch in NIL, the amount of DA released by THDA neurons would not be present in pituitary stalk blood. These cells carry different functionality and interestingly no differences in activities between males and females were recognized compared these characteristics to neurons of the TIDA system (Higuchi 1992; Freeman 2000).

From the regulatory aspects, DA concentrations reaching the AL, measured in portal blood only may have been underestimated. Indeed, removal (Ben-Jonathan, 1982; Peters, 1981; Ben-Jonathan, 1985) or denervation (Vecsernyes, 1997) of the NIL elevates basal PRL secretion in cycling or lactating females, but also in males. Hypothalamic regions of the main DAergic control (i.e. TIDA neurons) in arcuate nucleus, keep the balance of stimulation and inhibition and maintain the regularly low basal PRL secretion, due to combined action of putative control mechanisms (*vide infra*). On the other hand, elevated PRL levels during pregnancy and lactation mediate actions also in other hypothalamic regions such as the paraventricular nucleus (PVN), rostral preoptic area (rPOA) as demonstrated by Sapsford et al. (2012). The observation that electrochemically detectable DA in the AL is reduced after surgical removal of the NIL was found to be consistent with this finding (Mulchahey, 1985). Thus, DA of hypothalamic origin which is delivered to the AL by way of the long and the short portal vessels (from TIDA or THDA, respectively) together, seems quantitatively sufficient to account for inhibition of PRL release (Nagy, 1992; Freeman, 1993; Nagy, 1990).

The aim of this summary is to review the evidences accumulated to date and outline several new aspects of the regulation and the tonic inhibitory role of DA on PRL secretion in various physiological stages.

1.2. Physiology of responsiveness: The balance

The feedback interaction in which the released PRL controls its own secretion called "short loop feedback", only partially has been characterized. Due to the primary influence as inhibition on PRL release by hypothalamic structures, the mechanism of this feedback is considered fairly complex in process to change the activity of TIDA or PHDA/THDA neurons and trigger effect of immediate reactions, or in course of the physiological regulation, such as estrus cycle or by chronic increase of PRL levels, such as pregnancy or lactation. Elevation of serum PRL levels consequently increases hypothalamic DA synthesis and DA release into hypophysial-portal blood to complete the negative feed-back loop (Gudelsky, 1980). Since hypothalamus is within the brain, PRL that released to circulation should be transferred through the blood-brain barrier (BBB) in order to reach and manifest a feedback to TIDA cells, potentially by an uptake of the choroid plexus (Mangurian, 1992).

For the action of PRL on TIDA neurons several potential mechanisms have been proposed: such as control of TH expression by activation of PRL-receptor in DA neurons (Gonzalez, 1988) or activation of early genes, nerve growth factors (NGF-1), etc (Sagrillo, 1998). Since PRL receptors are found in all the three described subpopulations i.e. TIDA, THDA and PHDA neurons in hypothalamus, the short PRL feedback supported by the relevance of potential activation in all three systems (DeMaria, 1999). Alternatively, PRL can be synthesized *de novo* locally within the hypothalamus, by the stimuli of estrogens may have other functions and questionable the direct action controlling of TIDA neurons (DeVito, 1992; DeVito, 1993; Freeman 2000; Ben-Jonathan 2001). Interestingly, subpopulations of oxytocin neurons in the hypothalamus were also found to be differentially sensitive to PRL, and may have effect controlling PRL secretion, as PRF (Kennett, 2012).

According to the most recent report of Lyons et al. (2012) in which they focused on rapid electrophysiological changes induced by PRL within the hypothalamic TIDA neurons utilizing a whole-cell current- and voltage-clamp recordings. The presence of relatively high doses of PRL in arcuate nucleus resulted in a change to tonic inhibition, manipulating mainly those rapid mechanisms of depolarization on the spontaneously oscillating DA secreting cells. Experiments on slices of hypothalamus, tested to ion substitution and pharmacological manipulations proved that the transparent switch to tonic discharge and consequent APs are composed of low- and high-voltage components: activation of a transient receptor potential-like current and the change of a calcium ion-dependent BK-type K^+-current as a slow component, then broadened APs increase terminal Ca^{2+} influx, and change the vesicular DA release at the terminals in the median eminence. The PRL-induced depolarization is reversible and dose dependent; it involves direct, postsynaptic actions because it persists when AP discharge is blocked by TTX. PRL levels (higher than the physiological range of circadian rhythm) that required for normal reproduction (may be out of the normal range) have been effective in these electrophysiological actions. Accordingly this feedback „option" may primarily play a role during pathophysiological elevations of PRL. (Lyons, 2012)

Perhaps the most intriguing aspect of this neuroendocrine reflex mechanism is the dramatic change in responsiveness of lactotrophs due to a brief application of the suckling stimulus

(Nagy, 1990; Hill, 1991; Nagy, 1991). Previously we have provided experimental evidences that these responsiveness changes (induced by 10 minutes suckling in vivo) can be detected in primary cultures prepared from dissected ALs (Nagy, 1990; Hill, 1991; Nagy, 1991; Murányi, 1997; Murányi, 1998; Horváth, 1999). Pituitary cells from non-suckled rats (separated for 4 hours) exposed to various concentrations of DA exhibit only a dose-dependent inhibitory effect and TRH or Angiotensin II (AII) cannot release PRL. In striking contrast, lactotrophs derived from suckled rats are less responsive to the inhibitory actions of DA as well as more responsive to the stimulatory effects of TRH and AII. The suckling-induced changes in responsiveness related to decrease in protein phosphatase-2A (PP2A) activity which possibly plays a role in the uncoupling of D2 receptors on lactotrophs from the tonic inhibitory influence of DA (Murányi, 1998). Parallel with these, 10 minutes suckling stimulus applied immediately before sacrifice rendered PRL cells to respond with an increase of PRL release to picomolar concentrations (10^{-10} M-10^{-12} M) of DA. Thus, a brief suckling stimulus primes pituitary lactotrophs to respond with an increase of PRL secretion to low dose of DA (Nagy, 1990).

Another distinct advantage of this experimental model (non-suckled and suckled rats) is that it also shows a striking difference in the effectiveness of DA removal-induced increase of PRL release. It is well known, that dissociation of DA from its receptor induces PRL release. AL cells have been dispersed from non-suckled and suckled mothers then subjected to treatment of the inhibitory dose of DA for 2 hours. In the next step DA has been washed out before initiating the plaque assay by infusing the PRL antibody. Withdrawal of DA has clearly induced release of PRL compared to the medium pretreated cells obtained from non-suckled mothers. In contrast, the DA removal signal has been completely missing on cells from suckled animals (Nagy, 1991; Horváth, 1999; Murányi, 1998). Being able to distinguish between dissociation- and stimulation-induced elevation of PRL release is a critical part of the interpretation of these mechanisms.

Salsolinol (SAL), isolated from the NIL is present in the hypothalamic neuroendocrine dopaminergic system, appears to be a selective and potent stimulator of PRL secretion rat *in vivo* and with a moderate effect *in vitro* (Toth, 2001). It seems that SAL does not act through the dopamine D2 receptors, but utilize receptor independent mechanisms to stimulate PRL High affinity binding sites for this putative PRF have been detected in median eminence where TIDA projects and the NIL that are known terminal fields of THDA/PHDA DAergic systems (Toth; 2002; Homicsko, 2003). Reserpine pretreatment (blocking VMAT) prevented the effect of SAL on PRL release. Within the similar experimental model, suckling stimulus increased SAL content of NIL, but there was no change in SAL binding in the anterior lobe. Moreover, structural analogue of SAL (1-methyl-3,4-dihydroisoquinoline) can block salsolinol-induced release of PRL, but does not affect PRL release in response to TRH, TH inhibitors or D2 receptor antagonist domperidone (Homicsko, 2003). Taken together these results suggested that D2 receptor independent mechanisms can play a pivotal role in regulation of PRL secretion in reflection to suckling induced physiological stimuli (Toth, 2002; Bodnar, 2003).

1.3. Tonic inhibition of prolactin secretion

The PRL production and release to circulation is under a predominant inhibitory control of DA. Without the sustained regulation by hypothalamic DA dispersed pituitary cells increase the basal secretion and similarly, disconnected pituitary gland increase the released PRL (Neill, 1982; Leong, 1983; Vecsernyes, 1997; Murányi, 1998). Since most of experimental reports discuss the role of DA in females, it should be noted here that there are marked sexual differences in activity of TIDA (not relevant with PHDA and THDA) neurons and responsiveness to physiological and pharmacological stimuli, even though similar density nerve terminals found in both sexes (See also Table 1.). Higher basal activity of these neurons is seen in females that suppressed by removal of ovaries (OVX) and restored by treatment with E$_2$. The opposite, i.e. control of lower basal activity of TIDA neurons in males effected by presence of testosterone and may be due also to tonic inhibition by endogenous opioids (Ben-Jonathan 2001, Pan 1996; Freeman 2000).

Name Location	Termination	Function	Characteristics / Differences
TIDA dorsomedial part of the arcuate nucleus (A12)	External zone of the median eminence (EM) around long portal vessels	- Main physiological regulator of PRL secretion. - Daily rhythm by SCN . PRL surges in estrus cycle - Response to suckling stimulus. - Sensitive to feedback to PRL levels.	- Discharge in a highly robust and synchronized oscillations. - Significant difference in activity b/w male and females. - Activity increased by OVX, decreases by orchidectomy.
THDA rostral arcuate nucleus (A12)	Intermediate and the neural lobe (NIL)	- Response to suckling stimulus. - Activated by dehydration. - Release of DA to AL through the short portal vessels.	-No sex differences in activities, - function is independent of gonadal steroids
PHDA periventricular nucleus (A14)	Intermediate lobe (IL)	- tonic inhibition α-MSH and on basal PRL - Release of DA to AL through the short portal vessels.	-No sex differences in activities, - function is independent from gonadal steroids

Table 1. Dopaminergic neurons in control of PRL secretion

All of our recent data clearly indicate that wherever this signal originates and whatever is its nature, suckling-induced desensitization/sensitization of pituitary tissue to PRL-release inhibiting stimuli is manifested at the cellular level of lactotrophs as a proportional increase of subpopulation of those cells less sensitive to inhibition by high doses and more sensitive to stimulatory effect by low doses of DA. The mechanism(s) leading to these suckling-induced changes in DA responsiveness of lactotroph cells is (are) currently only in parts clarified. Efforts have been made to determine the mechanism(s) of this change in responsiveness have indicated that it is mediated through the D2 dopamine receptors (Horváth, 1999; Murányi, 1997). There are certain theories in hormonal- and receptor-level or intracellular signaling cascades-related, which may serve as potential explanation for the complexity of responsiveness to external stimuli and internal controls of pituitary cells during physiological and stress reactions.

TIDA cells form a network that discharges rhythmically in a robust 0.05 Hz synchronized oscillations. Thyrotropin-releasing hormone (TRH), which stimulates PRL release at the level of pituitary, cause transition from phasic pattern to tonic firing at the level of TIDA-cell. The results of these electrophysiology experiments are in concert with earlier findings on dispersed pituitary cell, suggesting a useful model for PRL regulation. TIDA network switches from oscillations to sustained discharge converting DA at high concentrations to a functional agonist as the net DA output decreases. (Lyons, 2010; Nagy, 1991)

Utilizing the analogy that has been reported by Conductier (2011) on hypothalamic neuronal populations, may serve as another potential explanation of this dose-dependent biphasic regulatory process of DA. The theory is based on the observation that certain biological actions believed to be associated with DA are not due to activation of DA receptors, but likely mediated via other receptors (such as α2-noradrenergic) and a subsequent action to open G-protein activated inward rectifier K^+ (GIRK) channels, which leads to hyperpolarization of cells (*vide infra*). It is also possible that DA modulates other hypothalamic inputs in a complex and biphasic manner: at low concentrations DA activates D2-like receptors, promoting presynaptic activity and upregulation, but high concentration of DA activates the D2-like receptors resulting in inhibition, which consequently blocks the presynaptic activity. (Conductier, 2011)

It has been recently discovered that sustained presence of the ligands of G-protein-coupled receptors (GPCRs) can promote specific intracellular signaling adaptation mechanisms parallel with the internalization process of the receptors. The receptor desensitization or "tolerance" is based on these mechanisms. Desensitization has been described in the AL of pituitary gland as well, where dopamine D2 receptors are permanently activated on lactotrophs. Ceasing of the dopaminerg inhibition is essential for the maintenance of the high secretory rate of PRL in lactotrophs during lactation.

2. Receptor mechanisms regulating lactotroph cells

Among those cellular and variety of receptor mediated mechanisms that regulating the pituitary hormone secreting cells only the key elements in this chapter that may have direct

impact on PRL release, will be highlighted. DA released from the specific DAerg nerve terminals and bind to its appropriate receptors located at the post-synaptic membrane is the main down-stream process. However, the potential effects of presynaptic receptor mechanisms in regulation can not be completely ruled out. To achieve biological response in cellular level, it is necessary to activate the G-protein-coupled receptors (GPCRs), which described with five distinct but closely related subunits that carry different and versatile subcellular messenger functions. A new concept of GPCR receptor theory describes the options of modified ligand selectivity and alternative intracellular responses on the same receptor type, increasing the down-stream effector mechanisms.

2.1. Dopamine receptors in mammotrop cells

The two major groups of receptors are the D1 and D2 classes: D1 class (D1 and D5 subtypes) has a stimulatory effect on intracellular signaling pathways, while D2 class (D2, D3 and D4 subtypes) has mainly inhibitory influence on cAMP. The predominant D2 type receptor in pituitary lactotroph cells exists in two alternatively spliced isoforms, termed D2-short (D2S) and D2-long (D2L) receptors, which differ from each other in the insertion of 29 amino acids of the third cytoplasmic loop. To date, there are no specific ligands to discriminate the D2S from D2L actions, only KO animal models in use to identify isoform specific effects. (Beaulieu, 2011; Vallone, 2000; Ben-Jonathan, 2001; Radl, 2011). The receptor isoforms exhibit fairly similar pharmacological properties, activation in rat lactotrophs mediates DA suppression of the PRL gene (McChesney, 1991). These two isoforms of D2 dopamine receptor are both present on lactotroph cells. Because each form is selectively coupled to different G proteins, they serve different functions: (i) inhibition of adenylyl cyclase, (ii) activation of voltage-gated calcium channels, and (iii) inhibition of potassium channels in a similar manner but only the D2S coupled to the phospholipase signaling pathway. (The G_i/G_o family of proteins is not involved in this pathway, since insensitive to PTX) (Senogles, 2000).

D2L is the main isoform present in the anterior pituitary both in rat and in human (Guivarc'h, 1995) and instead of D2S, the D2L isoform is expressed in an elevated manner level during estradiol-induced PRL secretion and cell proliferation in mammotrop cell. However, in cells contained only D2L form, (lactotroph-derived PR1 cells, with no D2S) there was only diminished response to the same stimuli demonstrated. It was concluded that D2S-receptor regulates the Gi3 inhibitory action on Gs; also D2S is more efficient for inhibiting adenylyl cyclase than D2L, thus could serve as mechanism controlling the PRL release and proliferation of mammotrop cells (Sengupta, 2012). The role of D2 receptor is essential to maintain the physiological PRL levels. In receptor knock-out (KO) mice pituitary hyperplasia and persistent hyperprolactinemia is seen. Other data suggest some additional function associated with DA, describing it not just a major inhibitor of PRL secretion and cell proliferation, but also induces apoptosis of mammary cells (Kelly 1997).

Experimental data support the hypothesis that D2S and D2L receptors are functionally distinct in terms of coupling to MAPK pathways, since DA-induced apoptosis in neurons in

lactotrophs, via p38 MAPK. Similarly, cabergolin-induces apoptosis of lactotrophs in the presence of E_2. The effect of DA on apoptosis is reverted by a p38 MAPK inhibitor in primary cultures and in PR1-D2S (D2S predominant, transfected pituitary tumor) cells, indicating that p38 MAPK is involved in the apoptosis of lactotrophs induced by D2 receptor activation. Alterations in the proportion of D2L and D2S isoform expression could be involved in the clinical resistance of D2R agonist therapies (i.e. cabergoline). In addition, estrogens sensitize anterior pituitary cells to different proapoptotic behavior. The phosphorylation of p38 MAPK induced by DA seems to be a necessary but not a sufficient event to induce apoptosis of lactotrophs, and moreover the hormonal milieu could affect the action of D2R agonists in patients with prolactinomas (Radl, 2011).

2.2. GIRK channel in lactotroph cells

The theory that a G-protein activated inward rectifier K^+ (GIRK) channel can be considered as a physiological cellular level effector of DA action in pituitary lactotroph cell has been proposed by Gregerson et al (2001). The experiments focused on specific hormonal changes on days of estrus cycle in rat, associated with DA sensitivity and effects of dispersed cell in vitro. In proestrus and not on other days of the reproductive cycle in rats, the functional expression of this DA-activated channel could be observed in lactotrophs isolated from female rats. (Gregerson, 1989, Gregerson, 2001)

This experimental design demonstrated that estradiol (E_2) up-regulates the GIRK channel subunits and controls the functional activation of the D_2R-GIRK pathway on mammotrop cells. The functional D_2R-GIRK pathway is the pertussis toxin (PTX)-sensitive heterotrimeric G-protein complex. The ability of DA to activate the GIRK channel of lactotroph demonstrated on isolated cells prepared from animals on different days of estrus cycle seems to be regulated by the hormonal status and property of the cells obtained only of the day of proestrus. Rising levels of circulating E_2 during the transition from diestrus result in a functional switch in DA signaling to include GIRK channel activation. On the morning of proestrus DA activates membrane hyperpolarization. This negative membrane potential "primes" the lactotroph population by removing inactivation of voltage-gated Ca^{2+} channels (VGCC). The drop of hypothalamic DA levels in portal vessels, i.e. on the afternoon of proestrus, the primed lactotrophs depolarize, initiating increased Ca^{2+} influx through VGCC and a consequent PRL release. (Gregerson, 1989; Gregerson, 2001)

E_2 does not have a direct effect on D2 receptor, but may influence the expression of G protein $\beta\gamma$ subunit isoforms, which are known to bind and potentially activate the GIRK channel. A receptor antagonist that competes with E_2 for binding at both $ER\alpha$ and $Er\beta$ blocks the induction of GTPγS-activated GIRK current. It was concluded, that there are 3 „essential components" work in synergy regulating the mammotrop cells during the hormonal changes of estrus cycle:

- D2 receptor, which couples to $G\alpha_i$ and thereby inhibits adenylate cyclase (AC), decrease in density on the afternoon of proestrus or with exposure to high concentrations of E_2. (Enjalbert 1983; Pasqualini 1984)

- D$_2$R-GIRK pathway: a pertussis toxin-sensitive heterotrimeric G protein complex, where the dissociated $\beta\gamma$ complex directly binds to GIRK proteins to open the K$^+$ channel. (Krapivinsky 1996; Yamada 1998)
- GIRK channel itself: E$_2$ significantly increase the percentage of lactotrophs with detectable levels of GIRK transcripts in the rat AP gland, measured by single-cell RT-PCR. (Christensen 2011

2.3. DA transporter (DAT)

This member of the Na+/Cl−-dependent transporter superfamily displays the characteristic twelve transmembrane domains and actively transports DA. It there is a bi-directional mechanism behind, since DAT can not only remove DA from the synaptic cleft but under certain conditions can also pump DAT out of cells which is generally referred to as the "reverse transport" (Barnes 2008). The functionality of dopamine transporter of TIDA neurons that is effective in regulating PRL secretion, but the transporter effective in THDA and PHDA neurons as well. Within the dynamics of DA secretion, the termination of DA action is primarily achieved by its reuptake utilized a dopamine transporter (to inwards) located on the terminals of dopaminergic neurons. Dopamine is translocated from the cytoplasm into the vesicles by the vesicular monoamine transporter (VMAT). DA is stored in synaptic vesicles at extremely high at a 100- to 1000-fold higher concentration than neuropeptides, which is near its limit of solubility. These vesicles intend to store and protect DA from degradation but also prevent leakage and control precise release from the synaptic vesicles (Ben-Jonathan, 2001).

DAT-knock-out mice have increased dopaminergic tone and anterior pituitary hypoplasia, and decreased number of lactotroph cells with down-regulation of the PRL gene (Bosse, 1997). „Clinically" the signs summarized as increased DA, consequent reduction (70-80%) in pituitary PRL content, the lack of milk production (since no PRL) and the inability to lactate (since no suckling-induced PRL release). Interestingly, the DAT-KO mice has the basal PRL level unchanged, which is probably due to activated compensatory mechanisms either in DA terminals, or at the lactotroph level. (Ben-Jonathan, 2001; Ben-Jonathan, 2008)

DAT is a common target of several drugs used in both the therapeutic field of psychiatry (psychostimulants, antidepressants), and subjects of drug abuse (like cocaine or amphetamine). Manipulations with the function of DAT and its potential influence on DA levels might have regulatory potential, but more likely causing side-effect of drugs or in certain cases the drug abuse.

2.4. Non DA related receptor mechanisms

2.4.1. Endothelin receptors

The alternative path in regulation of PRL secretion is the actions of endothelins (ETs) in pituitary cells: all members of the mammalian endothelin family of peptides exert significant effects on PRL release *in vitro* that mediated by ET$_A$ receptors (ET-AR). ET-AR is encoded by

an intron-containing gene. Selective ET$_A$ receptor antagonist can block the effects of the ETs in a competitive manner (Samson, 1992). Functional ET$_A$ receptors are expressed in all five secretory pituitary cell types included lactotrophs. The ET receptors are connected to both stimulatory and inhibitory (Gs, Gi/o, Gz) G-protein pathways (Tomic, 2002; Andric, 2005).

Generally in lactotrophs and also in somatotrophs, ETs activate the Ca^{2+}-mobilization pathway and transiently can stimulate hormone release. There is a post transient inhibitory effect of PRL release observed for several hours, underlining the importance of desensitization period. Endothelin, similar to D2 receptors coupled to Gi/o, in a picomolar concentration range inhibits adenylyl cyclase (AC) activity in a dose-dependent manner and consequently the PRL release from cultured anterior pituitary cells (Kanyicska, 1992; Samson, 1990). However, this inhibition of basal cAMP production does not abolish spontaneous firing of PRL cells, and only partially inhibits basal PRL release (Stojilkovic 2009).

2.4.2. Adenosine receptors

The presence of adenosine has been identified in the anterior pituitary gland. The experimental results and the direct effect of adenosine are controversial and depend on site of administration or the experimental conditions (Ondo, 1980; Schettini, 1990; We, 1998).

As it was revealed earlier, adenosine inhibits the basal adenylate cyclase activity in a dose-dependent manner and decreased PRL secretion. Adenosine and analogues affects the basal as well as stimulated secretion of PRL in pituitary cells, *in vitro* cell culture. It has a biphasic pattern of effect: low concentrations inhibit both AC activity and that affect the PRL release from primary pituitary cells; high concentrations may restore the action. The mechanisms involve the action on the cAMP coupled adenosine receptors at the level of pituitary gland (Schettini, 1990) or the short-loop negative feedback of released PRL.

The adenosine receptor subtypes (A$_1$, A$_{2a}$, A$_{2b}$, and A$_2$) are involved in mechanisms of action and in regulation of cell proliferation: A$_1$ receptors have been shown to activate G$_i$ inhibitory G-proteins that decrease intracellular calcium concentrations which decrease the activity of NOS and lower the NO. NO activatins guanylate cyclase that synthesizes cGMP from GTP, and increase the level, which leads to inhibition of PRL release. Since the A$_1$ and A$_2$ receptor blockers did not alter PRL release adenosine plays no role in basal PRL release *in vitro* (We, 1998). There subtypes of purinergic G protein-coupled adenosine receptors (AR), the adenosine nucleotide-controlled receptors (P2YR) and ion-conducting receptor-channels (P2XR) have been characterized in the pituitary by Koshimizu et al. (2000). Lactotrophs cells express three subtypes of P2XR channels, and homomeric and/or heteromeric receptors may utilize also the extremely complex but effective mechanisms to activate the cell- and receptor-specific Ca^{2+} signaling patterns (Koshimizu, 2000; Zemkova 2010).

2.4.3. Cannabinoid receptors

Preclinical studies suggest a predominantly inhibitory effect of cannabinoids on PRL secretion and some other pituitary hormones like GH, THS. The cannabinoid receptors (CB-

1R) are found in hypothalamic regions (Murphey, 1998; Wenger 1999) and in pituitary (Yasuo, 2010), co-localized with DA receptors in hypothalamic DA projections and modulate the DA transmission that regulates the PRL secretion (Rodríguez De Fonseca, 2001). Treatment with specific endogenous ligand of cannabinoid receptor anandamide inhibited the postnatal development of hypothalamo-pituitary axis in offspring (Wenger, 1999). Anandamide blocks human breast cancer cell proliferation through CB1-like receptor-mediated inhibition of endogenous PRL action by suppressing the levels of the long form of the PRL receptor (Petrocellis, 1998). In human, however, Δ-9-THC did not change plasma PRL levels across a wide range of relevant doses presented in a study by Ranganathan (2009), however frequent users may have lower baseline plasma PRL levels relative to healthy controls. Accordingly, clinical evidence suggesting effects by dose dependent and the development of tolerance and a long-term adaptation to several cannabinoid effects in association with chronic exposure (Block, 1991).

2.4.4. Ghrelin and GHS receptors

Expression of functional growth hormone secretagogue receptors (GHS-R) and the co-expression of the two GHS-R isoforms (Ia and Ib) was found in GH-, GH-PRL- and PRL-secreting adenomas and in pituitary cells. (Lanfranco, 2010). The PRL mRNA is co-localised with GHS-R mRNA. Triple in-situ hybridization showed co-localization of GHS-R mRNA with messengers of GH and PRL, conjointly or separately, in individual cells of somatotroph, somatomammotroph, and lactotroph adenomas (Barlier, 1999).

The administration ghrelin, serves as an endogenous ligand for the GHS-receptor (and primarily expressed in stomach and hypothalamus), as a direct action on somatomammotroph cells significantly stimulates PRL secretion in in human (Takaya, 2000; Lanfranco, 2010), but may exert inhibitory effects in prepubertal rats (Tena-Sempere, 2004). On the other hand, synthetic cortistatin-derived ghrelin receptor ligand (CST-8) does not modify the stimulatory endocrine responses to acylated ghrelin or hexarelin in humans and seems devoid of any modulatory action on either spontaneous or ghrelin-stimulated lactotrophs (Prodam, 2008).

3. Intracellular regulation of PRL secretion

Over the last two-three decades number papers discussing the signal transduction mechanisms have been presented describing the direct or the indirect regulatory effects of dopaminergic inhibition (or stimulation in certain circumstances) of PRL secretion. DA connected through its D2 receptors to G proteins and subunits to control the cAMP levels, but also activates various ion channels, triggering potassium current; influences voltage-activated calcium and potassium currents that induce changes in membrane depolarization, or cause hyperpolarization (Enjalbert, 1983; Freeman 2000). Most of these plasma membrane channels have been characterized in PRL secreting cells, but the mechanism underlying their extracellular Ca^{2+}-dependent action potentials and pacemaking activity is still not fully understood. The cAMP signaling pathway is probably in control of pacemaking, voltage-

gated Ca^{2+} influx and also the PRL release are PKA-independent mechanisms (Gonzalez-Iglesias, 2006). The highlights of key intracellular mechanisms regulating PRL secretion in lactotroph cells discussed below.

3.1. G-protein signaling pathways

The classical dopaminergic inhibition of PRL release from the anterior pituitary mediated through both the adenylate cyclase (AC) and Ca^{2+} mobilization/phosphoinositide pathways. D2 receptors are functionally associated with pertussis toxin (PTX)-sensitive G proteins (i.e. affected by activation of PTX-sensitive blockade). Activation of these receptors via $G_{i-3}\alpha$ causes inhibition of basal AC activity and consequent inhibition of cAMP production. Concomitantly D2 receptors trigger the voltage-sensitive potassium channels (via $G_{o}\alpha$) and inhibit the voltage-sensitive calcium channels. The $G\beta\gamma$-subunits can enhance the activity of type II AC, influencing the several other intracellular pathways. These complex intracellular messenger mechanisms alter the cAMP levels and a consequent PKA activity. PKA then in the cascade phosphorylates cytoplasmic and nuclear proteins and this also regulates ion channel function and may cause a desensitization of G protein coupled receptors. Accordingly, both Ca^{2+} and cAMP play important roles also in controlling the fusion of secretory vesicles with the plasma membrane to trigger the release hormones in these endocrine cells. (Lledo 1992; Freeman 2000)

That is in concert with the earlier observations, demonstrated the signaling through G protein-dependent pathways resulting in decreased cAMP levels. The $G_{i/o}$ signaling pathway is involved in DA-related inhibition, since resulted in depressed cAMP production. On the other hand, inhibition of basal cAMP production in pituitary lactotrophs does not completely abolish the spontaneous firing of APs and only partially inhibits basal PRL release (Gonzalez-Iglesias 2006). Similar to D2 DA receptor coupled subunits, the endothelin (ET_A) receptors are also connected to both stimulatory and inhibitory (Gs, Gi/o, Gz) G-protein pathways. (Tomic, 2002; Andric 2005).

The original theory discussed the activated heterotrimeric guanine nucleotide-binding (G) proteins dissociated $G\alpha$ and $G\beta\gamma$ subunits resulting in activate or inhibit various downstream effector molecules, impact the consequence of receptor activation in the same fashion (i.e. G-protein coupling, receptor desensitization, internalization, or trafficking). Utilizing frontiers in scientific approach supported by recent experimental evidences (Nb: not specified all cell types of pituitary gland to date) evolved a new concept for receptor theory of multiple active receptor states. That concept has critical implications since leaves room for alternative mechanisms of signaling; for the optimal receptor conformation of G protein activation that differs between G protein pools and that synthetic ones; or for cases when ligands can selectively promote different coupling conformations of the receptors. It is possible that only subsets of potential G protein partners being activated or induction of G protein coupling happens without triggering desensitization. On the other hand, receptor antagonists can cause receptor desensitization or initiate G protein-*independent* signals

without producing detectable activation of heterotrimeric G proteins. As concluded, receptor dimerization and interactions with scaffolding or signaling proteins can specifically modify ligand selectivity and determine the intracellular response from alternatives. Accordingly even within a single cell, multiple copies of the same receptor, may have coupled to different signal transduction cascade (especially if the receptor is susceptible to G protein switching induced by heterologous desensitization or capable of signaling through β-arrestins), so they can activate different down-stream effectors in response to the same ligand. (Maudsley, 2005)

The conclusion of recent reports on activation of G-protein coupled receptors (GPCR) by Stojilkovic et al (2009) outlined the complexity of actions of ET_A and D2 receptors in inhibition of basal PRL release. Pertussis toxin could only partially reverse the action of DA agonist induced inhibition of PRL release indicating the place for the pathway that independent from the $G_{i/o}$ pertussis toxin blocking effect. This PTX insensitive step in agonist-induced inhibition of PRL release is not affected by inhibition of PI3-kinase and GSK-3, but reversed at least partially by down-regulation of the $G_z\alpha$ expression. Moreover, the parallel activation of sensitive and insensitive pathways is affecting the PRL release through actions blocking electrical activity and also by desensitizing calcium-secretion coupling. (Stojilkovic 2009)

3.2. G-protein independent pathways

Update on mechanisms of intracellular events related with D2-dopamine receptor may help to understand several conflicting results that exist in the regulation of pituitary PRL secretion. In 2004, a new, D2-receptor coupled signaling was described in the mouse striatum that involves the ser/thr protein kinase Akt (protein kinase B) and was used to study efficiency of psychotropic drugs and lithium. This alternative vs. "canonical" (Gi-cAMP-PKA) signaling pathway is regulated mainly by β-arrestin2, a ubiquitous molecule of receptorial desensitization. The arrestin type 1 has been associated with the dark-adaptation of the retinal cones. The GPCR-β-arrestin2-Akt-PP2 complex plays a dual role: It can terminate the G-protein-mediated signaling of the receptor and initiates a G-protein independent signaling through different downstream phosphorilation cascades (e.g. Glycogene Synthase Kinase-3, GSK-3) (Beaulieu, 2005; Beaulieu, 2008; Csercsik 2008).

The expression of D2 receptor was found to be associated with filamin-A (FLNA). Recently Pervelli et al (2012) demonstrated reduced FLNA and D2R expression in DA-resistant tumors in human samples in vitro experimental conditions. According to clinical results, a subset of patients is resistant to DA probably D2 receptor alterations. The results indicate that (Peverelli, 2012): *(i)* FNLA is crucial for D2 receptor expression, *(ii)* depression of FNLA expression resulted in loss of inhibitory effect of DA due to decreased D2 receptor expression in 60%, in those prolactinomas that characterized as originally DA-sensitive; *(iii)* in DA-resistant prolactinomas forced FLNA expression restored D2 receptor expression and consequently the responsiveness to DA

3.3. Voltage-gated calcium channels

Dopamine exerts diverse effects on lactotroph, however the two most rapid events are: *(i)* the membrane hyperpolarization leading to inactivation of voltage-gated calcium channels, and resulting in a reduced intracellular free calcium ($[Ca^{2+}]_i$) and a consequent inhibition of PRL release from secretory granules; and *(ii)* suppression of AC activity and inositol phosphate metabolism resulting in diminished of PRL expression of the cells (Ben-Jonathan 2001). All other events associated to DA actions are more redundant on the time-scale, assuming that controls exerted via calcium channels play a critical role in immediate actions of PRL regulations.

The speed that noticed in electrophysiology experiments in the action of DA inhibiting PRL release also underline the involvement of ion channels and harmony in interplay between sodium current, T- and L-type calcium channels and calcium-dependent potassium channels. All of these membrane and ion-concentration related effects contribute to the generation of spontaneous, voltage dependent action potentials (APs) at frequencies between 0.2 and 0.5 Hz and a resting membrane potential -40 to -60 mV. However it should be noted, that a certain population of cells are considered as „quiescent" cells with a stable membrane potential, characterized with lack of changes in electrical activity, compared to „active" cells with higher basal PRL release. As it was reported earlier calcium is required to release PRL from the secretory granules. Action of DA induces a rapid change in cytosolic $[Ca^{2+}]_i$ concentration, particularly in those spontaneously active lactotrophs, as proposed via D2 receptor coupled with voltage-gated calcium channels and consequent phosphorylation/dephosphorylation or via indirect action on GIRK and resulting in a marked decline in intracellular calcium. (Corrette, 1995; Ho, 1996; Curtis, 1985; Nastainczyk, 1987; Ben-Jonathan, 2001; Gregerson, 2001)

As the membrane potential (Vm) of cultured anterior pituitary cells oscillates from a baseline and when it reaches the threshold level, pituitary cells fire action potentials (APs). The types of "active " secretory pituitary cells differ with respect to the pattern of electrical activity and AP-driven calcium signaling and secretion *in vivo* and *in vitro.* Cultured lactotrophs and somatotrophs frequently exhibit larger V_m oscillations, on top of which the depolarizing plateau and bursts of APs are generated, with spikes that do not usually reach the reverse potential. The differences in the patterns of spontaneous firing of APs among secretory pituitary cells are reflected in their respective pattern of calcium signaling. In elegant experiments of Stojilkovic (2009), measuring simultaneously the V_m and intracellular calcium concentration ($[Ca^{2+}]_i$) the lactotrophs cell have been characterized. Generally in lactotroph-somatotroph cells the slow resting V_m oscillations superimposed with bursts of APs, with an average duration of seconds shown high amplitude calcium transients, which reflects the increases in intracellular calcium concentration ($[Ca^{2+}]_i$ (Stojilkovic 2009).

The *in vitro* basal PRL secretion from lacto-somatotroph pituitary cells is high and is dependent on the extracellular calcium concentration. Basal hormone secretion is dependent also on the duration of the AP wave form, since that is the volume to drive calcium through voltage-gated calcium (Ca_v) channels. These non-stimulated cells secrete in a „constitutive

and regulated manner", since handled by Ca^{2+} influx and resulted in Ca^{2+}-dependent exocytosis. The voltage-gated calcium influx however, triggers secretion in lactotrophs, resulting in an organized superimposition of APs, so called „plateau-bursting" action potentials that generate high amplitude calcium signals. Since the firing of spontaneous APs depends on the presence of calcium in extracellular medium the transient removal of calcium leads to cease spiking and accompanied by abolition of basal hormone release. These results indicate that a specialty of these „active" lactotroph cells (i.e. the extended duration of the AP wave due to the high amplitude $[Ca^{2+}]_i$ signals) probably is the main reason for their high level of basal PRL secretion profile. (Van Goor 2001)

That is in accordance of earlier results which has been part of experimental protocols also in our laboratory that animals bearing ectopic pituitary grafts (e.g. under kidney capsules) *in vivo*, release high levels of PRL for a prolonged period. It is also plausible that a well-balanced control mechanism is necessary to regulate the PRL release in a precise manner during the different physiological stages, such as needed during the estrus cycle, peaks of diurnal rhythm during pregnancy, suckling induced PRL release, etc. DA serves as the hypothalamic controlling agent, regulating the spontaneous release by the cascades of $G_{i/o}$-coupled receptors for inhibition, controlling ion-channels for immediate action, but in addition to support need for release stimulation via G_s-coupled receptors mobilizing Ca^{2+} is also utilized.

3.4. Cyclic nucleotides in signaling

As discussed above the hypothalamo-hypophyseal system, or more generally the neuroendocrine and also immune systems are prominently regulated by G-protein coupled receptors that utilize the cAMP signal transduction cascade. Increases in cAMP lead to functional activation of PKA by binding to the regulatory subunits and liberating the catalytic subunits, phosphorylates cytoplasmic and nuclear proteins, and desensitize the G protein coupled receptors. Decrease in cAMP suppresses PKA by maintaining it in an inactive conformation and moreover in the presence of secretagogues, that potentially mediates other cellular responses of lactotrophs in relation to DA activation. Involvement of different membrane enzymes in the context of varied levels of activation of G-proteins as well as Ca^{2+}- and protein kinase C-dependent processes are equally important to generate the characteristic cAMP signal and cut-off at certain level by the cAMP-degrading phosphodiesterases. (Antony, 2000; Diamond 1999; Gonzalez-Iglesias, 2006)

The level of Ca^{2+} concentration in cytosol and the cyclic nucleotide signaling pathways are connected in regulation of physiological processes through messenger generation and also influencing additional other signaling pathways and *vice versa*, there is a distinct molecular machinery responsible to modulate the calcium concentration ($[Ca^{2+}]_i$) (Bruce 2003).

- *From one side*, the intracellular calcium concentration influences cAMP levels and balance, may also activates or inhibits some isoforms of adenylyl cyclase (AC), moreover the calcium concentration ($[Ca^{2+}]_i$), and selectively stimulates some phosphodiesterase (PDE) subtypes. Besides the cAMP, calcium also affects cGMP levels, NO synthase, etc through the activation of Ca^{2+}-sensitive phosphodiesterases.

- *From the other side*, the cyclic nucleotide intracellular levels and activity of their kinases can influence membrane potential (V_m) and Ca^{2+} balance by Ca^{2+} influx via channels, and also the Ca^{2+} clearance. (Gonzalez-Iglesias 2006)

This machinery of the cyclic nucleotides and the kinases may serve as a regulator or a pacemaker for hormone secretion of pituitary cells. It was demonstrated by in vitro models, that:

- plasma membrane channels involved in action potential spiking;
- basal cyclic nucleotides could contribute to the modulation of firing activity in lactotrophs;
- both the cyclic nucleotide-dependent and -independent pathways controlling spontaneous VGCI and the level of exocytosis;
- basal cAMP production controls the VGCI and PRL release by modulating electrical activity of the cell;
- VGCI should be accountable for inhibition of intrinsic AC activity, since VGCI attenuates the intrinsic AC activity in intact cells independently of the status of PDEs.

This regulation in lactotroph pituitary cells contributes only to the control of spontaneous electrical activity and basal PRL release, whereas the AC-independent action potential secretion coupling accounts for the majority of basal PRL release. Partial dependence of basal PRL release on cyclic nucleotides and partial inhibition of AC activity by spontaneous VGCI are the findings that consistent with reciprocal modulation of cyclic nucleotides and VGCI in spontaneously firing pituitary lactotrophs. (Gonzalez-Iglesias 2006).

3.5. Role of protein phosphorylation-dephosphorylation in the extracellular signals mediated secretory function of lactotrophs

Since virtually all types of extracellular signals (either those affecting second messenger-dependent activation or inactivation of protein kinases (PKs) or those that use a direct activation or inactivation of an ion channel) converge to a final common system of fundamental importance in biological regulation, called PROTEIN PHOSPHORYLATION, one of the main objectives of this review is to summarize the role of this final messenger system. In recent years, significant advances have been made in our understanding of the role of protein phosphorylation-dephosphorylation in the extracellular signals mediating secretory function of different cells (Murányi 1997; Muranyi 1998; Lefkowitz, 1993; Shenolikar, 1988).

Given the complexity and diversity of intercellular communication between hypothalamic releasing/inhibiting factors and hormone secreting pituitary cells (neuroendocrine communication) or between different cells of the pituitary gland itself (paracrine/autocrine communication), it would not be surprising that many of these systems use reversible protein phosphorylations. In this "third messenger" system, phosphorylation of the appropriate target or substrate proteins is tightly controlled by the activities of

phosphorylating PKs and dephosphorylating protein phosphatases (PPs). While the functions of PKs in the pituitary gland have been widely explored (Beretta, 1989; Leighton, 1993; Chneiweiss, 1992), the role of the PPs has been mostly ignored. Significance of dephosphorylation in such regulatory mechanisms has been already demonstrated in the CNS (Leighton, 1993; Chneiweiss, 1992; Nestler, 1983; Shenolikar, 1994; Nestler, 1994) including control of neurotransmitter synthesis and release (Leighton, 1993), signaling through the neurotransmitter receptors (Nestler, 1983; Shenolikar, 1994) and ion channels (Shenolikar, 1994; Nestler, 1994) or various aspect of gene expression (Nestler, 1994; Mumbby, 1993; Wera 1995).

4. Overview: control mechanisms of PRL secretion

The hypophysiotrophic (inhibitory) DAergic neurons in the hypothalamus consist of three distinct groups of neurons: the tuberoinfundibular (TIDA), tuberohypophysial (THDA) and the periventricular-hypophysial (PHDA) dopaminergic systems. DA reaching the AL via short portal vessels from the neurointermediate lobe (NIL) accounts for an alternative regulatory aspect of basal and tonic PRL release besides the main physiological regulator TIDA neurons. The stimulatory and inhibitory factors influencing PRL secretion have multiple sites of action at least in two levels: *(i)* hypothalamic level as neurotransmitter on DA neurons; *(ii)* at the pituitary as a neurohormone acting on lactotrophs, or other factors may act as an autocrine or paracrine modulator. Well defined external effects (circadian patterns, noise, stress, etc) or neuronal stimuli have been identified to modulate PRL secretion, however the main components are endogenous substances that can affect the activity of neuroendocrine dopaminergic and PRF neurons and/or pre-synaptically regulate neural inputs to these neuroendocrine cells; alternatively act directly on pituitary cells.

The predominant dopamine receptor is the D2 type in pituitary lactotroph cells, which exists in two alternatively spliced D2-short (D2S) and D2-long (D2L) isoforms, co-expressed in the same cells. Due to selective coupling to G-proteins, these receptor isoforms serve different functions: *(i)* inhibition of adenylyl cyclase, *(ii)* activation of voltage-gated calcium channels, and *(iii)* inhibition of potassium channels. D2L is the main isoform present in the anterior pituitary both in rat and in human. Alterations in the proportion of D2L/D2S could be the reason of resistance in D2R agonist therapies treating prolactinomas in clinical practice.

The G-protein-coupled receptors carry the mainstream of biological responses, utilizing subunits that carry different and versatile subcellular messenger functions increasing the flexibility of the down-stream effector mechanisms according to the endocrine milieu. $G_{i/o}$ signaling pathway is involved in DA-related inhibition of PRL secretion and results in depressed cAMP production. D2 receptors trigger the voltage-sensitive potassium channels (via $G_o\alpha$) and inhibit the voltage-sensitive calcium channels. Both Ca^{2+} and cAMP play important role to trigger the hormone release. Inhibition of basal cAMP production lactotrophs does not completely abolish the spontaneous firing of APs and only partially effect the basal PRL secretion. The PTX insensitive step in agonist-induced inhibition of PRL

release parallel with an activation of the sensitive pathways are affecting the PRL release through actions blocking electrical activity.

The physiology of pituitary cells reflecting to suckling stimulus or separation from pups, controlled by various concentrations of DA: due to suckling stimulus with low dose of DA to PRL release or exhibit dose-dependent inhibitory effect. This experimental model (non-suckled *versus* suckled) serves to demonstrate manipulations on concentrations of DA or receptors of DA *in vivo* or *in vitro* moreover, able to distinguish between ligand dissociation, and stimulation-induced elevation of PRL release. The suckling-induced desensitization/sensitization of pituitary cells is manifested at the cellular level by an increase of subpopulation of those cells less/ more sensitive to high/low dose ranges of DA. The receptor mediated intracellular mechanisms leading to these suckling-induced changes in DA responsiveness of lactotrophs is still not fully understood. It is possible that DA modulates the inputs in a biphasic manner: at low concentrations DA activates D1-like receptors, but higher concentrations activate the D2-like receptors resulting in inhibition. The receptorial desensitization or "tolerance" is based on the mechanisms, in which ligands of G-protein-coupled receptors (GPCRs) can promote specific intracellular signaling adaptation mechanisms parallel with the internalization process of the receptors. Estradiol up-regulates the GIRK channel subunits and controls the functional activation of the D2R-GIRK pathway on mammotrop cells for the DA action. The negative membrane potential "primes" the lactotroph population by removing inactivation of voltage-gated Ca^{2+} channels.

The intracellular mechanisms of DA activation connected to D2 receptors and G-proteins coupled subunits to control the cAMP levels and various ion channels: triggering potassium current influences voltage-activated calcium and potassium currents. Most of these plasma membrane channels have been characterized, but the mechanism connected to Ca^{2+}-dependent action potentials and pacemaking activity is still not fully understood. The cAMP signaling pathway is probably in control of pacemaking, voltage-gated Ca^{2+} influx and PRL release is a PKA-independent mechanism. The parallel activation of PTX-sensitive and insensitive pathways are affecting the PRL release through changes in electrical activity or modulate voltage-gated calcium channels. A specialty of these „active" lactotroph cells that this voltage-gated calcium influx resulting in an organized superimposition of APs, so called „plateau-bursting" action potentials that generate high amplitude calcium signals triggers secretion in lactotropes.

The complexity and diversity of intercellular and paracellular communication suggests a common theory of a "third messenger" system: phosphorylation of the appropriate target or substrate proteins controlled by the activities of phosphorylating PKs and dephosphorylating by protein phosphatases (PPs). Significance of dephosphorylation has been already demonstrated in the CNS, signaling through the neurotransmitter receptors and ion channels, but role of PPs has been still left some uncertainty. In recent years efforts have been made to understand the role of protein phosphorylation-dephosphorylation in processing extracellular signals to mediate secretory functions.

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