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Crosstalk between intra- and extracellular factors in the development of prolactinomas in the anterior pituitary

Review Article

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Summary

Prolactinomas are non invasive neoplasms resulting from an abnormal proliferation of the lactotroph cells in the anterior pituitary. Although prolactinomas have clearly been shown to be monoclonal tumors, the seek for the initial causal mutation has not been successful yet. In parallel to the recent advances made in the mutation analyses, this review aims at presenting the main extracellular factors, such as hypothalamic factors, estrogens, growth factors and cytokines, that could participate to the overproliferation of the lactotroph cells. In addition to their role in the development and maintenance of prolactinomas, it is proposed that the initial causal mutation could take place in the transduction pathways of such extracellular factors. Taking in account the complex regulation of the anterior pituitary could help in designing more specific and efficient treatments, especially for patients resistant to the most commonly used bromocriptine therapy.

I. Introduction

The anterior pituitary is composed of five different cell types which are defined by the hormones they produce and secrete into the bloodstream. These cell types (and their characteristic hormone) are: corticotrophs (adrenocorticotropic hormone or ACTH), gonadotrophs (luteinizing and follicle-stimulating hormone or LH and FSH), thyrotrophs (thyroid-stimulating hormone or TSH), somatotrophs (growth hormone or GH) and lactotrophs (prolactin). These cells receive, decode and transfer to peripheral endocrine organs the signals coming from the brain. The anterior pituitary thus plays a central and unique role in controlling the neuroendocrine interactions in the body. Any disregulation of the proliferation/differentiation of the cells or in the hormone production can have dramatic effects. This review focuses on prolactinomas, resulting from an overproliferation of the lactotroph cells, which produce and secrete in the bloodstream the hormone prolactin.

Prolactin (PRL) was first isolated by its ability to stimulate mammary development and lactation in rabbits (Riddle et al, 1933). Since, prolactin has been shown to exhibit a very wide range of physiological functions, including lactation, reproduction, osmoregulation, adaptation to stress, immunoregulation, parental behaviors, growth and development (reviewed in Bole-Feysot, 1998). Prolactin has been identified in all vertebrates and sequence comparisons suggest that it could share a common ancestral gene with two closely related hormones: growth hormone (GH) also secreted in the anterior pituitary, and placenta lactogen (PL) secreted by mammalian placenta (Miller and Eberhardt, 1983; Goffin et al, 1996). Prolactin acts via its binding to a specific membrane receptor (PRLR), which belongs to the large class 1 cytokine receptor family (reviewed in Kelly et al, 1991). Consistent with the variety of effects of prolactin, PRLR is expressed in a wide number of tissues, including skin, lung, heart, liver, gastrointestinal tract, reproductive organs, central nervous system and pituitary itself (Bole-Feysot et al, 1998). Structural variants of both PRL (Sinha, 1995) and PRLR (Kelly et al, 1991), resulting from alternative splicing and/or posttranslational modifications, have been described, and could also participate to the pleiotropic physiological effects of prolactin. The regulation of prolactin synthesis and

secretion involves numerous factors, including dopamine, VIP (vasointestinal peptide), TRH (thyrotropin-releasing hormone), estrogens and growth factors (Brown, 1994), which also appear to regulate the differenciation and proliferation of the lactotroph cells (see below, and **Figure 1**).

Among all the dysregulations leading to hyperprolactinemia, prolactinomas, the most frequent anterior pituitary tumors (Thapar et al, 1993), derive from an abnormal proliferation of the lactotroph cells. They are, in most of the case, noninvasive neoplasms. The characteristic symptoms of prolactinomas, although not always present in the same patient, can be elevated prolactin levels, amenorrhea, galactorrhea, infertility, loss of libido, headaches or visual defects due to the compression of the optic chiasma by the tumor (Katznelson and Klibanski, 1997). Prolactinomas are more frequent in women and are often microadenomas in this case (< 10 mm), whereas in men they are more likely macroadenomas (Faglia, 1993). It is however not clear whether this reveal a true gender difference and an influence of sex hormones, or if it is due to the fact that women consult more quickly a clinician, especially because of the menstrual disturbance (Katznelson and Klibanski, 1997). Most of the prolactinomas can be shrinked by dopamine agonists such as bromocriptine, although 8-15% of patients are resistant to this treatment, or by trans-phenoidal surgery aiming at removing the tumor, but the choice between pharmacology and surgery as a first treatment, as well as the addition of radiotherapy, is still discussed and depends on many factors, including the size of the adenoma, resistance/sensitivity to bromocriptine and its side-effects, or particular situation like pregnancy (see Besser, 1993).

The etiology of prolactinomas remains still poorly understood. It has first been thought that an overproduction of stimulating factors or a defect in inhibiting factors from the hypothalamus could be the origin of pituitary adenomas. However, the discovery of the monoclonality of prolactinomas suggests a intrinsic pituitary cellular defect such as a mutation in transduction mechanisms rather than an extracellular dysfunction (Herman et al, 1990). A number of such mutations in signal transduction pathways have already been identified in sommatotroph adenomas, such as a truncated GHRH receptor (Hashimoto et al, 1995) or the *gsp* somatic mutation in the G protein alpha-s subunit leading to a constitutively activation of adenylyl cyclase (Landis et al, 1989). Such pituitary molecular alterations have not yet been fully characterized in prolactinomas, but, like for sommatotroph adenomas, if an hormonal dysregulation would cause prolactinomas, polyclonal tumor would also ensue.

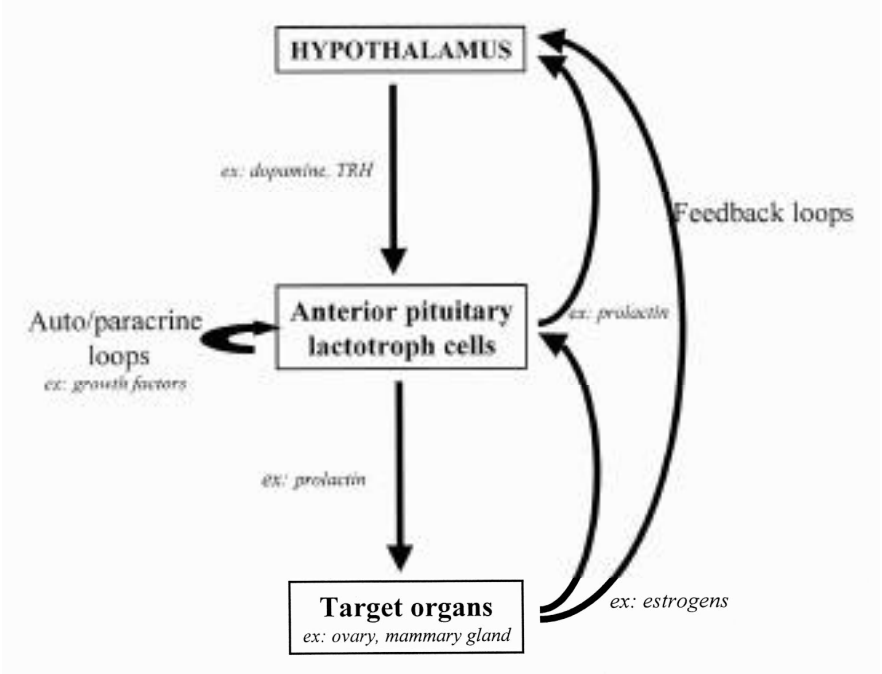

Figure 1. **The organization of the hypothalamo-pituitary axis**. The pituitary gland regulates target organs such as ovaries and mammary glands. Threee groups of factors can control the activity of the pituitary: (**i**) signals coming from the hypothalamus in the central nervous system; (**ii**) auto/paracrine loops of pituitary cytokines and growth factors; and (**iii**) feedback by hormones secreted by the target organs.

Nowadays, beside the antagonism between the "hypothalamic origin" and the "pituitary mutation origin", a more multifactorial view of the development of prolactinomas, and pituitary adenomas in general, can be drawn, considering first the appearance of a somatic mutation leading to monoclonal expansion of a single transformed cell, and in a second step extracellular factors could favor the development of the mutated clones into large or invasive tumor. The best demonstration of the importance of extracellular factors is obviously the possibility to shrink most of prolactinomas with molecules that mimic the inhibitory action of hypothalamic dopamine. In this respect, it has to be stressed that prolactinomas are a very potent model for other endocrine or brain tumors.

Parallely to the identification of mutated genes in prolactinomas, a better understanding of how the differenciation/proliferation and hormonal production of the lactotroph cells can be regulated, on both physiological and molecular levels, is thus needed to identify candidate molecules for the elaboration of more specific therapeutical drugs. With this perspective, this review presents the efforts that have being made to identify mutations in prolactinomas, and discusses the potential implication of different extracellular factors which regulate prolactin production and lactotrophs differentiation/ proliferation, since a dysfunction of their signaling pathways could be as well involved in the development/maintenance of the pituitary tumor.

II. Mutations in prolactinomas

Based on study demonstrating the monoclonality of prolactinomas (Herman et al, 1990), it can be assumed that a somatic mutation is the first step of the development of prolactinomas, as it has already been shown in other pituitary tumors. For example, mutations in the G-protein subunit alpha-s gene (*gsp* mutations) are found in up to 40 % of screened human GH-secreting adenomas, and lead to a constituvely stimulation of the cAMP signaling pathway (Vallar et al, 1987; Landis et al, 1989). However those mutations seem specific to GH cell tumorigenesis, and are absent in prolactinomas (Tordjman et al, 1993; Williamson et al, 1994). Similarly, mutations in the subunit alpha of Gi2 protein, although found in other endocrine tumors (Lyons et al, 1990), have not been found in pituitary tumors (Lyons et al, 1990; Tordjman et al, 1993).

Three homologous ras protooncogenes H-ras, K-ras and N-ras are structurally related to G proteins (Lochrie et al, 1985). Missense mutations which convert ras protooncogenes into active oncogenes are commonly identified in a variety of different human cancer (Bos, 1989) and both benign and malignant endocrine tumors (Namba et al, 1990). Four studies (Karga et al, 1992; Herman et al, 1993; Boggild et al, 1994; Cai et al, 1994) examining more than 200 secreting and non-secreting pituitary tumors identified only one H-ras mutation in an aggressive prolactinoma (Karga et al, 1992). Thus ras oncogene point mutations and activation are uncommon event in pituitary tumor initiation, but may be important in aggressive tumors and in the very rare pituitary metastasis formation and growth. Similarly, mutations in two common genes associated with cancer, p53 and Rb, have not been detected yet in prolactinomas (Hollstein et al, 1991; Herman et al, 1993, Pei et al, 1994, 1995; Cryns et al, 1993; Zhu et al, 1994; Woloschak et al, 1994, 1996).

Some studies have also started to investigate the signaling pathways of hypothalamic regulatory factors, without much success, since no mutation have been identified neither in the D2 dopaminergic receptor gene (Friedman et al, 1994), nor in the TRH receptor gene (Dong et al, 1996; Faccenda et al, 1996).

Thus, in prolactinomas, mutations usually associated with cancer are rare and sporadic, suggesting that they have probably little or no role in pituitary tumorigenesis. They seem more likely involved in the rare cases of invasive and aggressive tumors. Thus, more subtile or tissue-specific mutations in protooncogenes, tumor suppressor genes, or in molecules involved in the transduction pathways of extracellular regulating factors have still to be identified. In the following parts, the physiological regulators of the lactotroph cells are presented, and their potential role in the induction/maintenance of prolactinomas is discussed.

III. Early development of the anterior pituitary

Using the expression of Ki-67 antigen as a marker of the mitotic phase, it has been shown that pituitary tumors have a doubling time ranging from 100 to 700 days (Landolt et al, 1988; Knosp et al, 1989), prolactinomas having one of the most rapid growth and being also more frequently encountered in young patients (Haddad et al, 1991). Because of their slow growth, it can be assumed that the initial mutation take place early in life. One can thus ask whether factors regulating the embryonic development of the anterior pituitary could be at the origin of prolactinomas.

The embryonic development of the anterior pituitary is a complex multi-step process. The Rathke's pouch first appears as an invagination of an ectodermal layer of cells and will then give rise to the anterior pituitary, whereas the neural lobe of the pituitary originates from the ventral hypothalamus (reviewed in Treier and Rosenfeld, 1996, 1998; Watkins-Chow and Camper, 1998; Sheng and Westphal, 1999). During the embryonic development of the anterior pituitary, five endocrine cell types arise from a common pluripotent precursor in a specific spatial and temporal pattern (corticotrophs, gonadotrophs, thyrotrophs, somatotrophs and lactotrophs) (Voss and Rosenfeld, 1992). The expression of the gene encoding alpha GSU, the common alpha subunit of glycoprotein hormones (luteinizing, follicle-stimuling and

thyroid-stimuling hormones) represents the first discernible steps in the development of the anterior pituitary gland at e11 (Simmons et al, 1990). Subsequently, the ontogeny of the five cell types can be followed by the appearance of each cell type-specific secreted hormone (**Figure 2**). The first committed cells to appear at e12 are the corticotrophs, revealed by the expression of the proopiomelanocortin gene (Therrien and Drouin, 1993). The thyrotrophs expressing TSH (e14) and the gonadotrophs (e15) expressing LH and FSH emerge in turn, followed by the somatotrophs (e16) and finally the lactotrophs proliferating mainly after birth (Voss and Rosenfeld, 1992).

The appearance and maintenance of these specfic cell types from a common precursor result from positive or negative control exerted by cell-type specific transcription factors. The POU domain factor Pit-1 (also called GHF-1), specific of the pituitary gland, was initially identified and cloned as a transactivator of the GH and PRL genes (Ingraham et al., 1988; Bodner et al., 1988), and later as a regulator of the TSH beta gene (Steinfelder et al., 1991). Analyses of Pit-1 expression pattern reveal that initiation of its expression correlated both spatially and temporally to the activation of the transcription of its target genes (Dollé et al, 1990, Simmons et al, 1990). Later studies indicate that, in addition to activation of the GH, PRL and TSH genes, Pit-1 might be competent to initiate other programs of gene activation required for cell differentiation/proliferation of the thyrosomatolactotroph lineage (reviewed in Andersen and Rosenfeld, 1994). Indeed, it has been shown that Pit-1 antisense oligonucleotides not only block GH and PRL transcription but also inhibit the proliferation of pituitary somatotropic (GC) and lactotropic (235-1) cell lines (Castrillo et al, 1991). Moreover, Pit-1 defective mice and humans are not only characterized by a lack in hormone genes expression but also by the failure for the three cell types to proliferate (Li et al, 1990; Pfäffle et al, 1992; Radovick et al, 1992). Regarding the crucial role of Pit-1 in pituitary cell differentiation, one may ask if this transcription factor might be associated with abnormal cell proliferation or development of anterior pituitary tumors. Pit-1 mRNA and protein have been found in human normal and tumorous pituitaries, in lactotrophs, sommatotrophs and thyrotrophs cells (Pellegrini et al, 1994; Asa et al, 1993; Delhase et al, 1993). However, no mutations of Pit-1 gene have been detected in human GH, PRL or TSH secreting adenomas, and Pit-1 transcripts, correlated with the presence of the Pit-1 protein, are identical in size and sequence to those observed in normal pituitary (reviewed in Pellegrini-Bouiller et al, 1997). The results concerning the expression level of Pit-1 in pituitary adenomas are less clear.

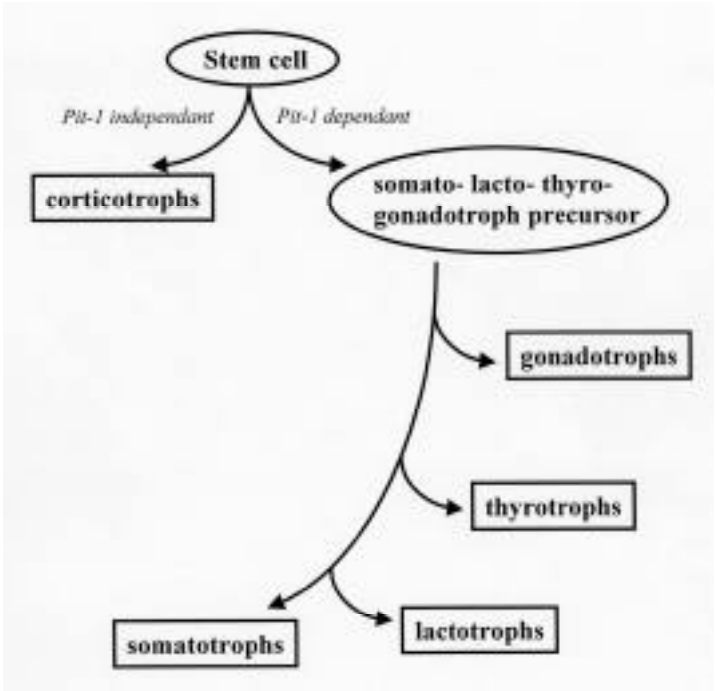

Figure 2. Ontogeny of anterior pituitary cell types. Theh five cell types of the antrior pituitary derive from a common precursor in a specific spatial and temporal pattern rregulated by transcription factors.

The transcripts of Pit-1 are more abondant in lactotrophs and somatotrophs adenomas than in normal pituitary, but one must be careful in interpreting those results obtained by northern blot on whole pituitaries since the level of expression of Pit-1 has to be balanced with the predominant cell type in the specific adenoma (Pellegrini-Bouiller et al, 1997). Since there is no difference in Pit-1 expression between somatotroph and lactotroph adenomas, other cell type-specific factors, with or without interaction with Pit-1, should be specifically involved in the abnormal proliferation of each cell type. But those mechanisms remain to be elucidated (see Treier and Rosenfeld, 1996). For example, it has been shown that Pit-1 can heterodimerize with another factor, Oct-1, to stimulate the transcription of the prolactin gene (Voss et al, 1991). On the other hand, the hormone production and cell fate in lactotrophs are regulated by many other factors and hormones in adult, as we will see below. For exemple, there is evidence that the prolactin distal enhancer requires the estrogen receptor in addition to Pit-1 for full activity (d'Emben et al, 1992; Waterman et al, 1988).

The recent discovery in the pituitary of other homeobox genes, such as OTX, Prop-1 or members of the LIM family (see Treier and Rosenfeld, 1996; Sheng and Westphal, 1999), should offer in the next few years a better understanding of processes that direct organogenesis, specific cell commitment as well as regulation of cell proliferation in the anterior pituitary. Strengthened by the fact that the mutation at the origin of the prolactinoma is thought to appear early in life (Landolt et al, 1988; Knosp et al, 1989), it will have to be shown whether those multi-step events of the anterior pituitary development could also be involved in abnormal proliferation of cells in adenomas.

IV. Extracellular regulating factors

In adult, regarding either hormone production or cell differentiation/proliferation, lactotrophs are controled by many factors from different origins. Based on the organization of the hypothalamo-pituitary axis, three levels of control by extracellular factors have to be distinguished, and will be followed in this review (**Figure 1**):

(**i**) regulation by central signals, including classic hypothalamic stimulatory (mainly VIP and TRH) and inhibitory (mainly dopamine). These factors, via the portal veinous system, acts as classical endocrine factors, stimulating their distal specific receptors on the membrane of pituitary cells.

(**ii**) regulation by the intrapituitary network of cytokines and growth factors. It is more and more clear that locally produced cytokines and growth factors could mediate cell division and hormone production, either directly or by regulating other specific trophic hormone expression.

(**iii**) regulation by the circulating hormones produced by target organs, that could act as an endocrine feeback mechanism.

A. Hypothalamic factors

Like other pituitary hormones, prolactin synthesis/secretion is under the control of dual stimulatory and inhibitory factors from the hypothalamus. However, in contrast to other pituitary hormones, prolactin is predominantly under tonic inbition of dopamine, that is produced in the neurons of the tegmental ventral area of the hypothalamus, and transported to the anterior pituitary via the hypophyseal stalk circulation. Stimulatory factors, like VIP, TRH, serotonin or opioid peptides provide thus a flexible regulation of prolaction production under diverse physiological conditions (Ben-Jonathan et al, 1996). It has also to be mentionned that an intrapituitary production of hypothalamic releasing hormones (TRH, GHRH, CRH, VIP), which are able to elicit hormone secretion, induce c-fos expression and facilitate cell replication, have been documented (Hsu et al, 1989; Castro et al, 1991; Pagesy et al, 1992; Wakabayashi et al, 1992).

1. Stimulatory factors

Thyrotropin- releasing hormone (TRH) is one of the most potent stimulator of prolactin synthesis and secretion *in vivo* (Bowers, 1971). Via its specific membrane G-protein coupled receptor TRHR, TRH increases intracellular concentration of calcium by stimulating both the entry of extracellular calcium and calcium release from intracellular store (Gershengorn and Thaw, 1985; Ashworth and Hinkle, 1996). However, no evidence of an important role of TRH in the regulation of lactotroph differentiation/proliferation has been described yet. In pituitary adenomas, TRH signalling appears intact, as evidenced by normal receptor expression level, binding and induced-PRL release from lactotrophs (Le Dafniet et al, 1987; Yamada et al, 1997). Although some splice variants of the TRH receptor have been described recently in some pituitary tumors (Yamada et al, 1997), studies failed to find any mutation in the TRH receptor gene or any direct link between the TRH signaling and prolactinomas (Dong et al, 1996; Faccenda et al, 1996).

Both VIP (vasointestinal peptide) and PACAP (adenylate cyclase activating polypeptide), which share receptors (Harmar and Lutz, 1994), has also been shown to have *in vivo* stimulatory effects on prolactin synthesis and secretion in rat (Onali et al, 1983; Rawlings and Hezareh, 1996). However, like for TRH, their implication in the control of lactotroph proliferation and in the development of prolactinomas remain to be shown.

2. Inhibiting factors

Although other prolactin inhibitory factors have been described (Schally et al, 1991), dopamine remain the major

inhibitor of prolactin *in vivo*. Dopamine is a widely distributed catecholaminergic neurotransmitter in the brain. Its pleiotropic effects are mediated by receptors, belonging to the G-protein-coupled seven transmembrane receptor family and subdivised in five different subtypes from D1 to D5 (reviewed in Jackson and Westlind-Danielsson, 1994). Dopaminergic neurons of the tegmental ventral area of the hypothalamus project on the hypophyseal stalk, as well as directly to the intermediate lobe of the pituitary. It is well characterized for many years that, in normal lactotroph cells, PRL synthesis and secretion are under the tonic inhibitory control of dopamine (Ben-Jonathan, 1985). Therefore, damage to the hypothalamus or the hypophyseal stalk, by compression of a tumor other than prolactinoma for example, can impede dopaminergic inhibition and lead to hyperprolactinemia (Besser, 1993). Hyperprolactinemia can also be caused by medications interacting with dopamine secretion or effect, such as antihypertensive drugs (e.g. reserpine or alpha-methyldopa) or psychotropic agents (e.g. haloperidol) (see Katznelson and Klibanski, 1997).

In the anterior pituitary, the effects of dopamine are mediated by the receptor subtype D2 (D2R). D2R are present in the anterior (lactotroph cells) and in the intermediate (melanotroph cells) lobe of the pituitary. D2 receptors have been first characterized by their ability, via Gi/G0 proteins, to inhibit ADc and the cAMP/protein kinase A signaling pathway, but it appears now that they can be coupled to other transduction pathways. D2R can hyperpolarize cells via the stimulation of K+ currents, stimulate the synthesis of arachidonic acid by phospholipase A2, decrease intracellular Ca2+ concentration, by modulating membrane Ca2+ channels, as well as the release of Ca2+ from intracellular store through the inhibition of IP3 levels (see Jackson and Westlind-Danielsson, 1994). The D2R gene contain eight exons, seven of which are coding exons. An alternative splicing mechanism, including or not the exon 6 in the mRNA, produces two different isoforms of the receptors: a short one D2S (415 AA) and a long one D2L (444 AA) (Dal Toso et al, 1989; Eidne et al, 1989; Giros et al, 1989; Monsma et al, 1989). These two isoforms differ in their third intracellular loop, that specify the coupling to G-protein, suggesting that they could activate different second messengers (Picetti et al, 1997).

No mutation in the D2R gene could be detected in human prolactinomas (Friedman et al, 1994), but there is evidence that dopamine can modulate not only prolactin production but also the lactotroph differentiation/proliferation. Although, this is known, empirically, for about thirty years since prolactinomas can be shrinked by dopamine agonists, little is known about the mechanisms involved in the dopaminergic control of lactotroph proliferation. Interestingly, animal models with a dopaminergic defect show abnormal phenotypes of lactotroph cells. Knock-out mice for D2R, resulting in the blockade of the dopaminergic inhibition, show hyperprolactinemia coupled to hypoestrogenism and unaffected expression of hypothalamic stimulatory factors (VIP and TRH), as well as an hyperplasia of the anterior pituitary lobe, leading to lactotroph adenomas after about 6 months of life (Saiardi et al, 1997; Kelly et al, 1997). These results clearly show that removing the dopaminergic inhibition is sufficient to induce an overproliferation of lactotroph cells. On the other hand, knock-out mice for the dopamine transporter show low blood levels of prolactin and a reduced number of lactotrophs, since, in this case, released dopamine is no more removed, leading to an overstimulation of dopamine receptors (Bossé et al, 1997). Thus, even if D2R does not seem to be directly involved in prolactinomas (excepted for resistant prolactinomas, see below), it is clear that the transduction pathway activated by dopamine plays a key role in the regulation of prolactin production and lactotrophs differentiation/proliferation.

3. Resistance to bromocriptine

In most prolactinomas, the dopaminergic regulation of PRL production, via the D2R, is maintained. This explains that most prolactinomas are successfully treated with bromocriptine, that mimics the action of dopamine. However, 8-15% of prolactinomas are resistant to this treatment, even with high doses of bromocriptine (15 mg daily). The resistance is not due to difference in drug metabolism, but several elements of the dopaminergic transduction pathway have been shown to be affected in the bromocriptine-resistant tumors (reviewed in Barlier et al, 1997). First a decrease of the level of both D2R mRNA and protein has been shown. The density of the binding sites for D2R agonists in the resistant tumor was half of that in the responsive tumors, without any change in the affinity of the receptor. Interestingly the alternative splicing of the D2R mRNA is also modified in resistant tumors (and not in sensitive tumors) compared to normal pituitaries, leading to a preferential decrease of the D2R short isoform (Caccavelli et al, 1994). Although both isoforms show comparative binding and are coupled to ADc via G proteins, they seem to be coupled to different second messenger systems (Picetti et al, 1997). On the level of G proteins, a marked decrease in Gi2alpha transcripts was found in resistant prolactinomas, compared to the sensitive ones, leading to a 60% decrease in the Gi2alpha/G0alpha ratio (Caccavelli et al, 1996). This change in the balance between the two isoform transduction pathways could shift the D2R coupling from ADc to Ca^{2+} channels, and contribute to the dopamine resistance. Interestingly, a recent study has shown that the level of Pit-1 mRNA is highly correlated to the D2R mRNA level in 15 prolactinomas with variable response to bromocriptine (Pellegrini-Bouiller et al, 1996). Thus, in resitant prolactinomas, expression of both D2R and Pit-1 are decreased, which suggests a crossregulation of the two genes and which could explain the cell-specific role of Pit-1 in this case. Pit-1 promoter has already been shown to be negatively regulated by dopamine (Elsholtz et al, 1991; Lew and

Elsholtz, 1995), but, on the other way, a direct regulation of Pit-1 on the transcription of the D2R gene seems unlikely since no Pit-1 consensus binding site has been found in the promoter region of the D2R (Minowa et al, 1992; Valdenaire et al, 1994). One can thus postulate either an indirect regulation of D2R gene by Pit-1 that remain to be elucidated, or a common regulatory factor acting on both D2R and Pit-1 gene. In the latter case, it has to be noted that a retinoic acid receptor response element has been described in the mouse Pit-1 gene upstream enhancer as well as in the rat promoter of the D2R gene (Rhodes et al, 1993; Valdenaire et al, 1994). Retinoic acid and its coregulator NZF 1 has also been shown to increase the expression of Pit-1 and co-regulate target genes with Pit-1 (Sanchez-Pacheco et al, 1995; Jiang et al, 1996). Recently, it has been shown that retinoic acid can regulate D2R expression and binding sites during embryologic development (Valdenaire et al, 1998) and that retinoic acid receptor deficient mice exhibit reduced expression of D2R (Samad et al, 1997). Knowing the key role of retinoic acid in development (Chambon, 1996), this could be a very seducing hypothesis to explain, at least part of the lactotroph-specific action of Pit-1 during development, and maybe in resistant prolactinomas.

In conclusion, contrary to bromocriptine-sensitive prolactinomas, in bromocriptine-resistant prolactinomas, a decrease in the number of D2R could lead to a less efficient inhibition of dopamine on lactotroph differentiation/proliferation. The molecular mechanisms, that could involved Pit-1, dopamine and the ratio of the two isoforms of the D2 receptor, leading to an abnormal proliferation of these cells should be clarified in such an interesting model.

B. Cytokines and growth factors

Although their action on specific cell type and their physiological relevance remain to be precised in most of the cases, there is now evidence that many intrapituitary cytokines and growth factors can modulate both hormone production and cell fate in the anterior pituitary. Only the cytokines and growth factors whose actions on lactotrophs has been documented will be described in this review. For further informations about their other actions on the anterior pituitary, the recent review from Ray and Melmed (1997) is recommended.

1. Interleukins

Although classically involved in hematopoietic and inflammatory cell functions, interleukin 6 (IL6) is also expressed in the hypothalamus and the pituitary (Rezai et al, 1994). Heterotrimerization between the specific subunit of IL6 and two gp130 signal transducer subunits (Hibi et al, 1990) is needed to activate the IL6 receptor that can be either soluble, or membrane-anchored. The presence of the IL6 receptor in the anterior pituitary (Ohmichi et al, 1992), even if its specific expression on lactotroph cells remain to be shown, suggests a paracrine or autocrine role of the cytokine. Although such a role of IL6 has not been extensively studied, it has been shown that IL6 could stimulate the release of PRL, GH, FSH and LH from cultured rat pituicytes in vitro (Spangelo et al, 1989; Yamaguchi et al, 1990), and that IL6 levels are increased in pituitary tumors (Jones et al, 1994; Rezai et al, 1994).

2. Nerve Growth Factor (NGF)

The neurotrophic NGF family includes NGF, brain-derived neurotrophic factor (BDNF), and neurotrophin-3 and 4. NGF action is mediated by the Trk family of tyrosine kinase receptors (Trk A, B, C, and the low-affinity p75 receptor), which are closely related to the transforming *trk* oncogenes (Saltiel et al, 1994).

NGF expression has been described in lactotroph cells, where it could be co-secreted with prolactin (Missale et al, 1996a). It has been shown that NGF could induce prolactin secretion and increase the number of lactotroph cells in postnatal rat pituitary cultures (Missale et al, 1995a) and in mice overexpressing a pituitary-directed NGF transgene (Borrelli et al, 1992). Interestingly, in bromocriptine-resistant human pituitary PRL-secreting tumor cells, NGF can restore dopamine responsivness, presumably by inducing D2 receptor availability (Missale et al, 1995b). Such an interaction between NGF and D2R has also been shown by the use of NGF antisense nucleotides in prolactinomas cells that result in loss of D2R expression and an increase in cell proliferation rate (Missale et al, 1996b).

3. Epidermal Growth Factor (EGF)

EGF is a single-chained polypeptide (53 AA) acting via a receptor with intrinsic tyrosine kinase activity and that need dimerization to be active (Groenen et al, 1994). Both mRNA and protein as well as the receptor have been described in the rat pituitary (Halpern and Hinkle, 1983; Chabot et al, 1986). EGF can enhance the number of lactotroph cells and induce PRL production (Felix et al, 1995), presumably via its direct action on the prolactin gene promoter (Supowit et al, 1984). Its expression in pituitary cell cultures is modulated by estrogens (Mouihate and Lestage, 1995). It has also to be noted that, like NGF, EGF can induce D2R expression confering dopamine responsivness to prolactinoma cells resistant to bromocriptine (Missale et al, 1991).

4. Transforming Growth Factors (TGF-alpha and TGF-beta)

TGF-alpha has been implicated in a number of malignencies (Groenen et al, 1994) and its expression is sufficient to induce fibroblasts transformation in culture (Rosenthal et al, 1986). It acts via the same receptor than EGF (Groenen et al, 1994). TGF-alpha is also expressed in the pituitary, predominantly in lactotroph cells (Kobrin et al,

1987). Interestingly, female transgenic mice, carrying a lactotroph-targeted TGF-alpha transgene, develop selective pituitary lactotrophs hyperplasia and PRL-containing adenoma formation (Mc Andrew et al, 1995). This sex specificity in adenoma development could be explained by the fact that TGF-alpha expression is regulated by estogens (Bates et al, 1988) and activators of protein kinase C (Mueller et al, 1989). Indeed, it has been shown that estrogen treatment both induce lactotroph hyperplasia and expression of TGF-alpha, and that this estrogen effect can be reverse by bromocriptine (Bordundvaag et al, 1992).

Expression of TGF-beta1, the predominant form of TGF-beta, has been described in lactotroph cells where it inhibits prolactin gene expression (Sarkar et al, 1992*;* Abraham et al, 1998). TGF-beta, contrary to TGF-alpha, inhibits cell growth (Massague and Polyak, 1995) and its expression is decreased during estrogen treatment that induce lactotroph hyperplasia (Pastorcic et al, 1995). Interestingly, two putative TGF-beta inhibitory response elements in the 5'-flanking regions of the rat PRL gene have been reported (Delidow et al, 1991). Thus, TGF-beta, has to be considered as an inhibitor of tumor ell proliferation, and has effects similar to those of dopamine on lactotroph cells. In this sense, it has to be noted that TGF-beta can inhibit TGF-alpha expression (Mueller and Kudlow, 1991). A balance between the opposite effects of the two TGF could thus be regulated by estrogens.

5. Fibroblasts Growth Factors (FGFs)

The family of FGF consists of peptides that share the capacity to bind heparin (Mason, 1994). Basic FGF (bFGF) is found in abundance in normal pituitary tissue as well as in human pituitary adenomas (Gospodarowicz et al, 1987; Li et al, 1992). Similarly FGF receptor, that possess an intrinsic tyrosine kinase domain, is also detected in pituitary endocrine cells and in human pituitary adenomas (Li et al, 1992; Gonzalez et al, 1994). Although bFGF can enhance prolactin secretion in GH4C1 (Yajima et al, 1984) and GH3 cell lines (Black et al, 1990) as well as in lactotroph tumors (Atkin et al, 1993), the role of bFGF in pituitary cell proliferation is discussed since some studies suggest a proliferative role in pituitary adenomas (Zimering et al, 1990) whereas others show no effect or an inhibitory effect on cell proliferation (Schweigerer et al, 1987; Atkin et al, 1993).

FGF-4, the protein product of the *hst* gene (Sakamoto et al, 1986), is a potent in vitro and in vivo mitogen for PRL-secreting cells. FGF-4 stimulates PRL synthesis and secretion and potentiate lactotrophs tumorigenesis in rat (Shimon et al, 1996). DNA sequences from human prolactinomas have been demonstrated to be transforming in the NIH-3T3 cell focus assay, and to contain part of the coding region of *hst* gene, suggesting indirectly that this growth factor gene may be associated with pituitary tumorigenesis in human (Gonsky et al, 1991). Abnormal *hst* mRNA and protein levels have reported in some prolactinomas, mainly invasive ones (Gonsky et al, 1991; Herman et al, 1993; Shimon et al, 1998).

6. Galanin

Galanin is a small peptide (29 AA) with a limited sequence homology with other characterized peptides (Takemoto et al, 1983), although it acts though G protein-coupled receptors (Habert-Ortoli et al, 1994; Howard et al, 1997; Wang et al, 1997). It co-localizes with PRL and can be released by lactotroph cells in the pituitary (Steel et al, 1989; Wynick et al, 1993). Its stimulation of prolactin release has been documented in rodents as well as in humans (Wynick et al, 1993; 1998). Galanin seems extremely sensitive to the estrogen status of the animal. It has also been shown that its expression is increased during pregnancy (Vrontakis et al, 1992) and that it can mediate the estrogen-induced stimulation of prolaction production and lactotroph proliferation (Wynick et al, 1993; reviewed in Hyde et al, 1998). On the contrary, pituitary galanin content is dramatically decreased in ovariectomized animals (O'Halloran et al, 1990). In addition to its action on prolactin production, galanin has also been shown to be a potent mitogen to the 235-1 clonal lactotroph cell line (Wynick et al, 1993). Recently, it has been reported that mice, whose galanin gene has been disrupted, exhibit low prolactin levels associated, without changes in the pituitary content of VIP and TRH, and fail to develop hyperproliferation of lactotroph cells in response to estrogen (Wynick et al, 1998). Interestingly, an estrogen-induced increase of the galanin mRNA has been shown in several breast cancer cell lines (Ormandy, 1998). It would thus be interesting to see whether galanin could be directly involved in human prolactinoma development.

7. Prolactin

Consistant with its homology with GH and with the fact that PRLR belongs to the cytokine receptor family, prolactin has been reported to influence growth and development in a wide range of species, in addition to its well-known effects in lactation and reproduction. In amphibians, PRL is best known for its antimetamorphic effects and is thus considered as a larval form of GH (Tata, 1993) Although no genetic desease has been directly associated with the gene encoding PRL or PRLR in mammals, prolactin has been associated with autoimmune deseases, as well as with certain forms of cancers and may be directly or indirectly involved in tumor growth (recently reviewed in Bole-Feysot et al, 1998). PRL is thought to increase colorectal tumor aggressivity, induce the proliferation of several lines of human breast cancer, active B lymphocytes and lymphoma cells (see Bole-Feysot et al, 1998). PRL has been shown to modulate proliferation of many cell types, including smooth muscle, pancreatic beta-cells, prostate epithelial cells, astrocytes and various cells of the immune system (see Bole-Feysot et al, 1998). PRL has also been suggested to have some functional activity in various

developmental processes, such as the maturation of the lung, the differentiation of preadipocytes and maturation of germ cells (see Bole-Feysot et al, 1998). PRL influences the development of the tuberoinfundibular hypothalamic dopamine system, that will latter participate to the lactotroph regulation (Shyr et al, 1986). PRL could thus play a role in the development of the hypothalamo-pituitary axis, from which it is secreted and regulated.

Based on the effects of PRL on the proliferation of various cell types and on the development of hypothalamic neurons,as well as the presence of its receptor on lactotrophs as well as other anterior pituitary cell types (Ouhtit et al, 1993; Morel et al, 1994), one can postulated that prolactin could have autocrine/paracrine effects on the lactotroph cells. However, such an hypothesis, emphasized by the fact that most of the factors stimulating prolactin production, stimulate also the proliferation of the lactotrophs, has not been much explored yet. Treatments with prolactin has been shown to inhibits prolactin secretion in 2B8 pituitary cell line (Herbert et al, 1979) and PRLR expression in lymphocyte cells (Di Carlo et al, 1995), but prolactin has been shown to act as an autocrine growth factor in somatolactotroph (GH3) cell line (Krown et al, 1992) and lymphocytes (Hartmann et al, 1989). Since there is more and more evidence for a para/autocrine role of prolactin in breast cancer (Fuh and Wells, 1995; Mershon et al, 1995; Vonderhaar, 1998), many studies remain to be done to investigate the potential hyperproliferative role of prolactin on lactotroph cells and in prolactinomas. The development of PRLR antagonists should help those investigations (Fuh and Wells, 1995; Kuo et al, 1998).

C. Circulating hormones

1. Estrogens

Estrogens are highly important physiologic stimulators of prolactin release and are responsible for the elevation of prolactin levels during gestation (Raymond et al, 1978). Estrogen positively regulate lactotroph proliferation after birth (Lieberman et al, 1983; Elias and Weiner, 1987). Exogenous administration of estrogens induces prolactin gene transcription and secretion (Lloyd, et al, 1991; Song et al, 1989; Chernavsky, 1993; Perez, 1986) and stimulates lactotroph proliferation and adenoma formation (Lloyd, 1983; Asscheman et al, 1988; Gooren et al, 1988). Recent studies have shown that only a small pool of estrogens is required to stimulate the proliferation of lactotrophs (Chun et al, 1998), and that estrogens are crucial for prolactin production and lactotroph proliferation,but not for the specification of the lactotroph cell type in mice lacking the estrogen receptor alpha (Scully et al, 1997). Estrogens can act directly on the level of the promoter of the prolactin gene via their specific nuclear receptors (Maurer and Notides, 1987; Waterman et al, 1988) or stimulate other growth factors such as galanin (Ormandy, 1998) or TGF-alpha (Bates et al, 1988).

2. Extrapituitary PRL

PRL can be found in several fluid compartments, such as blood, amniotic fluid, milk tears, sweat and cerebrospinal fluid. Indeed, in addition to being synthesized and secreted by lactotroph cells of the anterior pituitary, PRL is also produced by a wide number of other tissues, including brain, thymus, spleen, skin and mammary gland, lacrima and sweat glands, and cells, such as lymphocytes (reviewed in Ben-Jonathan, 1996). The importance of the extrapituitary PRL has been demonstrated by Nagy and Berczi (1991). They showed that, in hypophysectomized rats, biologically active PRL blood levels can return up to 50% of normal levels with time. Moreover, neutralization of circulating PRL with anti-PRL antibodies, in the same hypophysectomized rats, results in immune dysfunctions and death, showing that extrapituitary PRL is important and, at least in part, can compensate for pituitary PRL. Extrapituitary PRL can act as a growth factor, a neurotransmitter or an immune modulator, via a classic endocrine pathway or in a paracrine or autocrine manner, and could, in particular, influence also the lactotroph cells.

V. Concluding remarks

Prolactinomas are, in most of the cases, non invasive neoplasms, caused by an overproliferation of the lactotroph cells. The etiology of prolactinomas remains poorly understood, and, although prolactinomas are monoclonal, no original mutation has yet been identified. In physiological conditions, the development and maintenance of lactotroph cells as well as the regulation of prolactin requires the coordination of the action of many extracellular factors from different origins and natures (hypothalamic factors, estrogens, intrapituitary growth factors and cytokines). In this respect, prolactinomas are a very potent model for the better understanding of other endocrine tumors. In this review, it is proposed that the original mutation could be part of the transduction pathways used by these extracellular factors, and/or that a dysregulation of one or several of those factors could facilitate the expansion of the tumor.

Among all the regulator factors, dopamine is the major inhibitor one, since the blockade of its effects is sufficient to induce the development of lactotroph tumors. A better understanding of the target genes activated by dopamine signaling in lactotroph cells could highlight the etiology of prolactinomas, and it has to be mentionned that the study of the effect of such a neurotransmitter on cell differentiation/proliferation is certainly a very promising field in neurosciences. On the other hand, since most of the factors stimulate both lactotroph cell proliferation and prolactin production, and since prolactin is known to have strong mitogenic effects in other cell types, one could also postulate that prolactin could play a crucial role in prolactinomas.

More than the wide number of physiological regulator factors (the list given in this review being not exhaustive), it has to be stressed that those factors are from different natures and origins. Altogether, they activate most, if not all, the possible intracellular signaling pathways, giving thus a high level of possible effects and regulations. For the sake of simplicity, each factor has been described independantly in this review, but many interactions among them occur. Much work remain to be done to understand how those factors can interact on extra- and intracellular levels, and by which molecular processes they can co-regulate lactotroph differentiation and/or proliferation. It appears to be a crucial point for therapeutical research since a combination of different drugs could be more efficient, for example for the treatment of prolactinomas resistant to bromocriptine (Missale et al, 1995b).Two levels of interactions can be distinguished: first, a physiological level representing the interactions between the factors outside the lactotroph cells, and second, an intracellular level in the lactotroph cells representing the cross-talk between the different signaling pathways. On the physiological level, interactions are numerous, and are far from being fully understood. Some pituitary growth factors, such as galanin, TGF-alpha and TGF-beta, could mediate the action of estrogens on the lactotroph, in addition to the direct transcriptional estrogenic signal (Bates et al, 1988; Hyde et al, 1998). On the other hand, prolactin, EGF and NGF can have a direct effect on the hypothalamic dopaminergic neurons and on the expression of the D2R by lactotrophs (Shyr et al, 1986; Missale et al, 1991; Missale et al, 1996b). EGF has also been shown to regulate the expression of TRH receptors in rat pituitary cells (Monden et al, 1995).

It has also to be stressed that the other cell types of the anterior pituitary secrete growth factors and that such paracrine regulation between cells could participate, in great part, to the control of prolactin production and lactotroph proliferation (reviewed in Schwartz and Cherny, 1992). Abraham et al (1996) have shown, for example, that the level of prolactin gene expression was influenced by the phenotype of the adjacent cells. Such a complex organization of the hypothalamo-pituitary axis allows multi-step and subtile regulations of hormone production and cell proliferation in the anterior pituitary, according to signals coming from inside as well as outside the body. Although not fully characterized yet, they have to be taken in account in the seek for novel therapeutic drugs.

Figure 3 represents a very schematic view of the main intracellular signaling pathways activated by hypothalamic factors, estrogens, and pituitary growth factors. It is also clear now that those signaling pathways are not independant and that cross-regulations can occur. For example, TRH has been shown to be able to activate MAP kinase activity, in addition to PLC, in GH3 cells, and, furthermore, this effect can be attenuated by dopamine, suggesting complex cross-talk (Ohmichi et al, 1994). Much work remain to be done to show and understand the different intracellular interactions occuring in lactotroph cells to regulate their differentiation/proliferation. The challenge will be to show how molecules from different natures and activating different signaling pathways can interact and coordinate to regulate cell fate. Very interesting findings are arising in this field (reviewed in Weiss et al, 1998). In fibroblasts, it has been shown that serotonin, alpha 2 adrenergic agonists and bFGF synergize to promote proliferation, via a common G-protein pathway (Seuwen et al, 1990). More recently, Lev et al (1995) have shown that the G protein-mediated activation of PLC by bradykinin could lead to the activation of the Ras/MAPK pathway via PYK2, a non-receptor tyrosine kinase.

Very little is also known about the transcription factors that are finally activated by those different pathways. In this respect, the pituitary-specific factor Pit-1 has long been thought to be the crucial determinant of the control of prolactin production and lactotroph differentiation/ proliferation, since it is the final effectors of dopamine, TRH, VIP and growth factors signalings (Andersen and Rosenfeld, 1994). However clear role of Pit-1 in prolactinomas failed to be proved. It would have to be shown wether all those signaling networks activate finally the same or different transcription factor(s) and which can be involved in the development of prolactinomas.

Finally, especially because of the wide number of molecules which could potentially participate in the developpment of prolactinomas, application to prolactinomas of new technologies like differential display or microarrays DNA chips could be very promising. Indeed, a new pituitary-specific gene, PTTG (pituitary tumor-transforming gene), which can induce tumor development, has recently been identified by using differential display on rat pituitary tumor cells and normal pituitary tissue (Pei and Melmed, 1997; Zhang et al, 1999). Such studies could help in understanding the genes whose expression is altered in prolactinomas, but also in identifiing the initial mutation since prolactinomas are monoclonal tumors.

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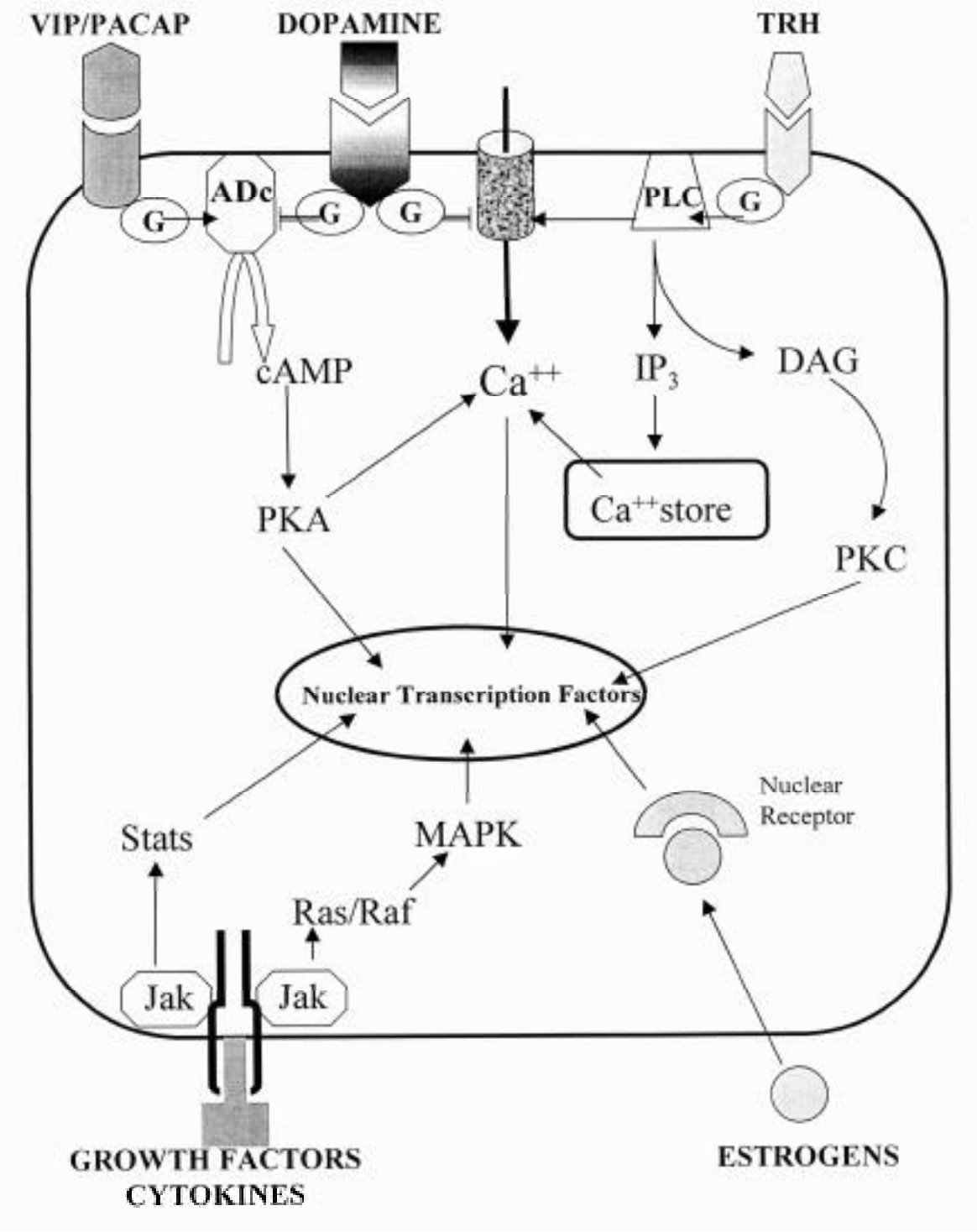

Figure 3. Schematic representation of the intracellular pathways activated by dopamine, VIP, TRH, growth factors, and estrogens. Adc: adenyl cyclase; Ca^{++}: calcium; cAMP: cyclic AMP; DAG:diacyl glycerol; G: protein G; IP3: inositol triphosphate; Jak: janus kinase; MAPK: MAP kinase; PKA: protein kinase A; PKC: protein kinase C; PLC: phospholipase C; →: activation; ==½ inhibition

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