

## [REVIEW]

# Intrapituitary Regulatory System of Proliferation of Mammothrophs in the Pituitary Gland

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**ABSTRACT**—Anterior pituitary cells produce growth factors plus cytokines and their receptors. Although some of these pituitary growth factors and cytokines are known to be involved in the control of cell differentiation, proliferation and hormone production in the pituitary gland, their physiological roles remain unknown. Lots of evidence indicates that they are involved in the regulation of prolactin-secreting mammothroph cell proliferation. The regulation of mammothroph functions is a suitable system for understanding the intrapituitary regulatory system operated by growth factors and cytokines, since mammothrophs are the most actively proliferating cells in female pituitary glands. This review discusses the possible intrapituitary regulation of mammothroph differentiation and proliferation in rat and mouse pituitaries.

**Key words:** pituitary, mammothroph, proliferation, rat, mouse

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## INTRODUCTION

Pituitary glands in mammals consist of neurohypophysis and adenohypophysis, the latter of which can be further divided into anterior and intermediate lobes. These glands constitute a functional link between the nervous system and endocrine system, regulating various functions including growth, energy metabolism, osmoregulation, reproduction and behavior. Organogenesis and initial cytodifferentiation of pituitary glands are regulated by factors produced by two opposing signaling gradients. One signal is generated by the ventral floor cells of the diencephalons, while the other is generated by oral ectodermal cells (Dasen *et al.*, 1999; Scully and Rosenfeld, 2002). After the initial differentiation of different cell types, the cell populations of each secretory cell type expand by proliferation. Thus, pituitary cells appear to proliferate by self-duplication, however, other types of growth cannot be ruled out.

The proportions of each hormone secretory cell type vary with age or alterations in physiological status. Secretory cell numbers are determined by the balance between the proliferation and apoptosis of pituitary secretory cells, which is partly regulated by growth factors and cytokines produced in the pituitary gland as well as hypothalamic hor-

mones and hormones from the target organs (Schwartz and Cherny, 1992; Deneff, 1994; Takahashi, 1995; Renner *et al.*, 1996; Ray and Melmed, 1997; Schwartz, 2000). This review describes the actions of growth factors produced within the pituitary gland and shows the intrapituitary regulatory system involved in controlling pituitary functions. Of the several types of pituitary cells, the proportion of mammothrophs differs between males and females (Takahashi and Kawashima, 1982), and changes during pregnancy and lactation (Haggi *et al.*, 1986). In addition, the regulation of mammothroph proliferation has been well studied compared to other types of anterior pituitary cells; therefore this study focuses on the proliferation and differentiation of mammothrophs.

## DEVELOPMENT OF PITUITARY GLANDS AND MAMMOTROPH DIFFERENTIATION

Pituitary gland development is regulated by extrinsic and intrinsic signals that control the expression of several transcription factors. Two highly related paired-like homeodomain factors, *Hesx1/Rpx* and an activator prophet of Pit-1 (*Prop-1*), are thought to play essential roles in the morphogenesis of pituitary glands (review, Olson *et al.*, 2003). *Hesx1/Rpx* appears to be important for the initial progression of pituitary development, while its subsequent down-regulation leads to the emergence of *Prop-1*-dependent lineages (Gage *et al.*, 1996; Sornson *et al.*, 1996). *Prop-1* is required for the initial proliferation of *Pit-1*-dependent thy-

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rotrophs, somatotrophs, mammotrophs and gonadotrophs (Gage *et al.*, 1996; Sornson *et al.*, 1996). Pit-1, the POU domain protein, is expressed in thyrotrophs, somatotrophs and mammotrophs, and is required for the differentiation of these cell lineages (Li *et al.*, 1990). The Pit-1-related cell lineage is a clearly understood model system of cell differentiation. The differentiation of thyrotrophs, somatotrophs and mammotrophs is mediated via the reciprocal interactions of two transcription factors, Pit-1 and GATA2 (Dasen *et al.*, 1999). In thyrotrophs, both Pit-1 and GATA-2 are expressed, and Pit-1 is required for the activation of growth hormone (GH) and prolactin (PRL) genes.

Several reports suggest that mammotrophs are transdifferentiated from somatotrophs under estrogen stimulation and/or other factors (Boockfor *et al.*, 1986; Behringer *et al.*, 1988; Borrelli *et al.*, 1989; Inoue and Sakai, 1991; Kineman *et al.*, 1992; Kakeya *et al.*, 2000), and that the transdifferentiation of pre-existing somatotrophs into mammotrophs is a post-mitotic event (Goda *et al.*, 1998; Kakeya *et al.*, 2002). This transdifferentiation seems to contradict the self-duplication of mammotrophs described above. However, several reports suggest that transdifferentiation into mammotrophs without mitosis occurs during the prenatal and early postnatal period as well as during late pregnancy and lactation in rats allowing generation of a large number of mammotrophs in a short period of time (Frawley and Boockfor, 1991; Takahashi, 1992).

### MAMMOTROPH PROLIFERATION

Mammotrophs are the most actively proliferating cells in rat and mouse pituitaries (Shirasawa and Yoshimura, 1982; Takahashi and Kawashima, 1982; Takahashi, 1992; Takahashi, 1995). In adult female rats the mitotic activity of mammotrophs is higher during estrus than during any other stage of the estrous cycle (Takahashi *et al.*, 1984; Oishi *et al.*, 1993). This high proliferation of mammotrophs depends upon ovaries or ovarian estrogen, since ovariectomy decreased the high mitotic activity observed during estrus, while estrogen replacement increased mitotic activity. This sexual difference in mitotic activity is thought to lead to the sexual difference in mammotroph number (Takahashi and Kawashima, 1982; Sasaki and Iwama, 1988).

A sex-difference in mammotroph development has been observed in rats and mice. In mouse pituitaries, for example, the total number of mammotrophs did not differ between sexes at 14 and 21 days of age, but at 35 days of age female pituitaries contained more mammotrophs than male pituitaries by approximately three-fold. At 60 days of age the number of mammotrophs in the female mice was twice that of the male mice (Takahashi, 1995). This difference in the growth pattern of mammotroph populations is thought to be due to a difference in the proliferation of mammotrophs between male and female mice. Maternal estrogens may be involved in the growth of mammotrophs during the perinatal period. The factors that enhance mammotroph

proliferation during the neonatal period remain to be clarified, although the involvement of a milk-borne factor of maternal origin in mammotroph differentiation had been already reported (Porter *et al.*, 1993).

Pituitary gland growth is stimulated by estrogen, and an increased number of mammotrophs can be observed in estrogen-treated rats and mice. The proliferation of mammotrophs is regulated by estrogen (Lloyd *et al.*, 1975; Takahashi *et al.*, 1984; Takahashi and Kawashima, 1987; Oomizu and Takahashi, 1996). This estrogenic effect might be mediated directly through changes in the expression of genes essential to the cell cycle. Estrogen stimulates the expression of cell-cycle-regulatory proteins such as cyclins and cyclin-dependent kinase inhibitors, which lead to the progression of the cell cycle (review: Pestell *et al.*, 1999; Foster *et al.*, 2001). On the other hand, several studies on estrogen-responsive tissues have suggested that the effect of estrogen on cell proliferation is mediated by growth factors whose production is stimulated by estrogen in an autocrine or paracrine manner (Sirbasku, 1978; Sutherland *et al.*, 1988). The following sections discuss some of the growth factors involved in mammotroph growth.

### Transforming growth factor- $\alpha$ (TGF- $\alpha$ )

TGF- $\alpha$ , an epidermal growth factor (EGF), binds to EGF receptors (Massague, 1990) and stimulates DNA-replication of mammotrophs in serum-free primary cultures of mouse anterior pituitary cells (Oomizu *et al.*, 2000). TGF- $\alpha$  gene expression is stimulated by estrogen in ovariectomized mice (Sharma *et al.*, 2003). Borgundvaag *et al.* (1992) showed a concurrent increase in TGF- $\alpha$  mRNA and pituitary weights in chronic estrogen-treated rats. Treatment of mouse pituitary cells with a combination of estradiol (E2) and anti-TGF- $\alpha$  antibodies did not increase the number of DNA-replicating cells (Sharma *et al.*, 2003). Thus, immunoneutralization with anti-TGF- $\alpha$  antibodies blocked the estrogen-induced proliferation of mammotrophs. Moreover, the blockade of TGF- $\alpha$  message translation was attempted by TGF- $\alpha$ -antisense oligodeoxynucleotide treatment resulting in the inhibition of estrogen-induced mammotroph proliferation (Oomizu *et al.*, 2000). These findings suggest that TGF- $\alpha$  acts as an estrogen-induced growth factor in the anterior pituitary glands, stimulating DNA replication and mammotroph mitosis.

The overexpression of human TGF- $\alpha$  in transgenic mice accelerated the development of pituitary mammotrophic adenomas (McAndrew *et al.*, 1995). Furthermore, in pituitary tumor cells, TGF- $\alpha$  affected cell proliferation in either a stimulatory or inhibitory manner (Ramsdell, 1991; Finley *et al.*, 1994). TGF- $\alpha$  is therefore also involved in the growth of pituitary tumor cells.

TGF- $\alpha$  is produced in the pituitary glands of several species (Kudlow and Kobrin, 1984; Kobrin *et al.*, 1987; Lazar and Blum, 1992). In rat pituitary cells, TGF- $\alpha$  mRNA expression was detected in somatotrophs, gonadotrophs and mammotrophs (Fan and Childs, 1995) while in mouse pituitaries TGF- $\alpha$  mRNA-expressing cells are evenly distrib-

uted throughout the anterior pituitary gland, but not in the intermediate or posterior lobes (Sharma *et al.*, 2003). TGF- $\alpha$  mRNA-expressing cells are medium-sized and either round or oval. In adult male and female mouse pituitaries, TGF- $\alpha$  mRNA-expressing cells account for 65 and 55% of all pituitary cells, respectively. To determine TGF- $\alpha$  mRNA-expressing cell types in mouse pituitaries, serial sections were studied by non-radioisotopic *in situ* hybridization using cDNA probes for TGF- $\alpha$  mRNA, GH mRNA and PRL mRNA. Most of the GH mRNA-expressing cells contained TGF- $\alpha$  mRNA (79–83%), whereas only a small population of PRL mRNA-expressing cells contained TGF- $\alpha$  mRNA (1–3%) (Sharma *et al.*, 2003). An immunocytochemical study also showed that somatotrophs express TGF- $\alpha$  mRNA (Takahashi *et al.*, 2002). This discrepancy between TGF- $\alpha$ -mRNA expressing cell types might be partly based upon the different animal species studied. These findings indicate that the main source of TGF- $\alpha$  is somatotroph populations, since somatotrophs are the most abundant cells in anterior pituitary glands. In rat and mouse pituitaries, for example, somatotrophs and mammothrophs are distributed evenly throughout the anterior lobes of the pituitary glands. Based on the morphological analysis of TGF- $\alpha$  expression in mouse pituitaries, it is likely that TGF- $\alpha$  produced in the somatotrophs acts on mammothrophs in a paracrine manner.

Immunoreactive EGF receptors have been observed in all subsets of rat pituitary secretory cells, but are only present in a fraction of these cells (Fan and Childs, 1995; Honda *et al.*, 2000). EGF receptor expression changes with various conditions such as stress and the estrous cycle (Fan and Childs, 1995; Armstrong and Childs, 1997a, b). Similarly, estrogen treatment with E2 increases EGF receptor mRNA in mouse pituitaries (Oomizu *et al.*, 2000). Estrogen appears to stimulate pituitary growth at the level of EGF receptor production as well as TGF- $\alpha$  production. To study whether TGF- $\alpha$  mediates the estrogen-induced proliferation of mammothrophs, a specific inhibitor of EGF receptors, 3,4-dimethoxy-*a*-(3-pyridyl)-(Z)-cinnamionitrile (RG-13022) has been used (Yoneda *et al.*, 1991). RG-13022 ( $10^{-7}$  M) was seen to significantly inhibit the EGF (10 ng/ml)-induced increase in DNA-replicating cells. E2-induced pituitary cell proliferation was also inhibited by RG-13022. Therefore, EGF receptor signaling is thought to be involved in the proliferation of pituitary cells, and to be required for pituitary cell differentiation during early pituitary organogenesis (Roh *et al.*, 2001).

### Epidermal growth factor (EGF)

EGF treatment increases PRL release (Aanestad *et al.*, 1993), and stimulates the proliferation of mammothrophs and corticotrophs (Honda *et al.*, 2000; Oomizu *et al.*, 2000). EGF also stimulates the differentiation of mammothrophs in normal pituitary cells (Felix *et al.*, 1995) and pituitary tumor cell lines (Inoue and Sakai, 1991; Kakeya *et al.*, 2000). In rat pituitaries, somatotrophs and gonadotrophs express EGF mRNA, while cold stress induces EGF mRNA expression in corti-

cotrophs and thyrotrophs (Fan and Childs, 1995). In mouse pituitaries, EGF mRNA expression was observed in somatotrophs and mammothrophs, but not detected in corticotrophs, thyrotrophs or gonadotrophs (Honda *et al.*, 2000). Estrogen has been shown to stimulate EGF release from rat pituitary cells (Mouihate and Lestage, 1995). Therefore, EGF might also be involved in estrogen-induced mammothroph proliferation.

### Transforming growth factor $\beta$ (TGF- $\beta$ )

TGF- $\beta$  is a member of the cytokine family that regulates the differentiation and proliferation of various tissues. TGF- $\beta$ 1, - $\beta$ 2, and - $\beta$ 3 are synthesized in mammalian tissues. TGF- $\beta$ 1 inhibits PRL gene expression (Abraham *et al.*, 1998) and the proliferation of mammothrophs (Sarkar *et al.*, 1992). At low concentrations, it slightly stimulates the DNA replication of mammothrophs (Qian *et al.*, 1996). TGF- $\beta$ 1 also acts in G1 arrest during the cell cycle as a paracrine inhibitor of mammothroph proliferation, while p15 and p27, which are Cdk (cyclin dependent kinase) inhibitors, are functional mediators of TGF- $\beta$ -induced cell cycle arrest (Qian *et al.*, 1996; Frost *et al.*, 2001). In human pituitary tumor cell lines, TGF- $\beta$ 1 induces apoptosis (Oka *et al.*, 1999). Mammothrophs synthesize TGF- $\beta$ 1, and TGF- $\beta$ 1 synthesis is inhibited by estrogen (Burns and Sarkar, 1993; Qian *et al.*, 1996). Estrogen-induced pituitary growth might therefore be associated with the estrogen-induced inhibition of pituitary TGF- $\beta$ 1 production, resulting in the reduced TGF- $\beta$ 1-induced inhibition of mammothroph proliferation. The expression of TGF- $\beta$  type II receptors in pituitary cells is also reduced by estrogen treatment (De *et al.*, 1996) while TGF- $\alpha$  expression is inhibited by TGF- $\beta$ 1 (Mueller and Kudlow, 1991), leading to a reduced TGF- $\alpha$  growth stimulatory signal.

TGF- $\beta$ 2 is produced in rat pituitary glands, but is not localized in mammothrophs. It exerts no significant effect on the proliferation of mammothrophs (Hentges *et al.*, 2000). TGF- $\beta$ 3, on the other hand, is produced in mammothrophs and stimulates mammothroph proliferation (Hentges *et al.*, 2000). Its synthesis is stimulated by estrogen. The immunoneutralization of TGF- $\beta$ 3 with anti-TGF- $\beta$ 3 antibodies nullified the estrogen-induced proliferation of mammothrophs. This mitogenic action of TGF- $\beta$ 3 on mammothrophs is indirect and mediated by basic fibroblast growth factor (bFGF) secreted from folliculostellate (FS) cells (Hentges *et al.*, 2000).

### Basic fibroblast growth factor (bFGF)

bFGF belongs to the fibroblast growth factor (FGF) family, and is the most abundant growth factor in normal pituitary glands (Gospodarowicz and Ferrara, 1989; Amano *et al.*, 1993). bFGF is produced in FS cells (Ferrara *et al.*, 1987; Amano *et al.*, 1993), gonadotrophs (Schechter and Weiner, 1991; Schechter *et al.*, 1995), and somatotrophs (Marin and Boya, 1995), and is involved in the regulation of PRL synthesis and secretion (Larson *et al.*, 1990; Mallo *et al.*, 1995). A reverse hemolytic plaque assay also revealed

that bFGF promotes the differentiation of mammothrophs in neonatal rat pituitary glands (Porter *et al.*, 1994). bFGF stimulates mammothroph proliferation in the presence of estrogen, indicating that it is an estrogen-dependent mitogenic factor for pituitary cells. Estrogen treatment stimulates TGF- $\beta$ 3 production, and TGF- $\beta$ 3 increases the release of bFGF from FS cells. The immunoneutralization of bFGF in a FS cell-conditioned medium inhibited its growth stimulatory action on mammothrophs (Hentges *et al.*, 2000). It can be concluded therefore that bFGF is located downstream of the estrogen-TGF- $\beta$ 3 signaling cascade as described above, acting as a mediator of TGF- $\beta$ 3-induced mammothroph proliferation.

Estradiol and TGF- $\beta$ 3 stimulated bFGF production and release in FS cells obtained from F344 rats, but not in FS cells obtained from Sprague-Dawley (SD) rats (Oomizu *et al.*, 2004). It is thought that the higher responsiveness of pituitary cells derived from Fisher 344 rats to estrogen in terms of pituitary growth is related to the difference in FS cell populations between Fisher 344 and SD rats.

#### **Insulin-like growth factor (IGF)**

IGF-I and -II are produced in a number of tissues including the pituitary glands, and regulate the proliferation and differentiation of various cells in an autocrine and/or paracrine manner (Fagin *et al.*, 1988; Bach and Bondy, 1992; Ren *et al.*, 1994; Yokoyama *et al.*, 1997; Gonzalez-Parra *et al.*, 2001). In human pituitary glands, IGF-I-expressing cells are not hormone-secreting cells (Ren *et al.*, 1994) while in rat pituitaries IGF-I mRNA-expressing cells were detected, but their cell types were not determined (Bach and Bondy, 1992). *In situ* hybridization and immunocytochemistry revealed that IGF-I is produced in the somatotrophs of mouse pituitaries (Honda *et al.*, 1998). In normal human and rat pituitaries, IGF-II-expressing cells have not been determined (Haselbacher *et al.*, 1985; Bach and Bondy, 1992).

Pituitary cells express type 1 IGF receptors (IGFR1) and type 2 IGF-I receptors (IGFR2) (Bach and Bondy, 1992; Ren *et al.*, 1994; Gonzalez-Parra *et al.*, 2001). In mouse pituitaries, IGFR1 is expressed in somatotrophs and some corticotrophs (Honda *et al.*, 1998), while in rat pituitaries it is found in the gonadotrophs (Unger and Lange, 1997). IGFR2 is localized in somatotrophs as well as other types of cells in rat pituitaries (Ocrant *et al.*, 1989). IGF-I was seen to stimulate the proliferation of anterior pituitary cells, in particular mammothrophs and corticotrophs, indicating that anterior pituitary cell proliferation is stimulated by IGF-I produced in the anterior pituitary cells (Oomizu *et al.*, 1998). The mitogenic activity of IGF-I on mouse mammothrophs might be indirect, since mammothrophs in mouse pituitaries do not express IGFR1 (Honda *et al.*, 1998). In rat pituitaries, IGF-I also stimulated vasoactive intestinal peptide (VIP) gene expression (Lara *et al.*, 1994), which stimulates PRL release (Hagen *et al.*, 1986; Nagy *et al.*, 1988). Therefore, it is possible that VIP might mediate the effects of IGF-I on the mammothrophs. In addition, there are many reports showing

that IGF-I regulates GH expression and secretion at the pituitary (Goodyer *et al.*, 1984; Yamashita and Melmed, 1986) and/or hypothalamic level (Abe *et al.*, 1983; Tannenbaum *et al.*, 1983). IGF-I treatment was seen to decrease GH mRNA levels in mouse pituitaries (Honda *et al.*, 2003).

Somatotrophs are the main source of pituitary IGF-I, while IGF-I gene expression was enhanced in GH-secreting tumor-bearing rats compared to control animals (Fagin *et al.*, 1988). GH treatment also increased IGF-I mRNA levels in pituitary tumor GH3 cells (Fagin *et al.*, 1989) and mouse pituitary cells (Honda *et al.*, 2003). In addition, estrogen treatment for 54 days stimulated IGF-I expression in rat pituitaries (Michels *et al.*, 1993), however, E2 treatments failed to stimulate IGF-I expression in mouse pituitaries. These discrepancies might be due to the different animal species studied, their sex, and/or the experimental protocols. The up-regulation of IGF-I transcription in the pituitary glands probably requires chronic E2 treatment. It is possible therefore, that GH and estrogen augment IGF-I production in somatotrophs, while enhanced IGF-I release stimulates the proliferation of mammothrophs through VIP production, since VIP receptors are expressed in mammothrophs (Wanke and Rorstad, 1990).

#### **Nerve growth factor (NGF)**

Nerve growth factor (NGF) is localized in rat mammothrophs and, together with PRL, its secretion is stimulated by VIP (Missale *et al.*, 1996). The NGF receptor, gp<sup>140trk</sup>, is expressed in mammosomatotrophs and mammothrophs (Patterson and Childs, 1994a). NGF secretion is stimulated by interleukin-1 $\beta$  (IL-1 $\beta$ ), and inhibited by GH releasing hormone, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and bFGF (Patterson and Childs, 1994b). These results suggest that NGF is involved in the neuroendocrine-immune system. NGF promotes the differentiation and proliferation of mammothrophs, and NGF treatment was seen to stimulate the appearance of mammothrophs and increase the number of mammothrophs in rat pituitary cells (Missale *et al.*, 1995). NGF treatment also stimulated the DNA replication of mammothrophs, corticotrophs and non-hormone containing cells (Proesmans *et al.*, 1997). In pituitary tumor GH3 cells, NGF treatment decreased cell proliferation and GH secretion, but stimulated PRL secretion and dopamine receptor expression, suggesting that NGF induces the transdifferentiation of mammosomatotrophs into mammothrophs (Missale *et al.*, 1994). NGF might therefore be involved in the functioning of mammothrophs in an autocrine manner.

#### **Galanin**

Galanin is synthesized in the central and peripheral nervous system as well as other tissues including anterior pituitary glands. Immunocytochemical studies of female rats at the light microscope level have shown that mammothrophs, somatotrophs, and thyrotrophs contain galanin, whereas male anterior pituitary gland mammothrophs do not (Kaplan *et al.*, 1988; Hyde *et al.*, 1991). Estrogen treatment

is known to increase galanin mRNA production (Kaplan *et al.*, 1988; Cai *et al.*, 1998; Wynick *et al.*, 1998), and galanin receptor (galanin-2 receptors) expression has been observed in rat anterior pituitary glands (Waters and Krause, 2000). Therefore, it is possible that galanin plays a paracrine role within the pituitary gland. The targeted over-expression of galanin in mouse pituitary cells increased the number of somatotrophs and mammothrophs, and serum PRL levels (Perumal and Vrontakis, 2003). These results suggest that galanin regulates PRL secretion and the proliferation of mammothrophs.

#### Vasoactive intestinal peptide (VIP)

VIP is synthesized in the jejunum and colon as a gastrointestinal hormone. It is also synthesized in the anterior pituitary gland, and is localized in subpopulations of mammothrophs (Morel *et al.*, 1982; Koves *et al.*, 1990; Chew *et al.*, 1996) or other cell types (Lam *et al.*, 1989; Carrillo and Phelps, 1992). VIP controls PRL secretion possibly in an autocrine manner (Nagy *et al.*, 1988; Wanke and Rorstad, 1990; Escalada *et al.*, 1996), and is probably involved in estrogen-induced changes in pituitary glands such as PRL secretion, the proliferation of mammothrophs and TGF- $\beta$ 1 synthesis, since estrogen stimulates VIP synthesis and release (Gomez and Balsa, 2003).

#### Calcitonin

Calcitonin is synthesized in the anterior pituitary gland, and localized in the gonadotrophs of rat pituitaries (Ren *et al.*, 2001). Calcitonin receptors can also be detected in rat anterior pituitary glands (Sun *et al.*, 2002). Calcitonin inhibits PRL secretion (Shah *et al.*, 1988, 1996), and the proliferation of mammothrophs (Shah *et al.*, 1999). This inhibitory action of calcitonin on mammothroph proliferation was attenuated by the immunoneutralization of TGF- $\beta$ 1 with anti-TGF- $\beta$ 1 serum. Calcitonin stimulates TGF- $\beta$ 1 synthesis, and increases the number of TGF- $\beta$ 1-expressing cells in female rat pituitaries. This finding indicates that the antiproliferative action of calcitonin on the mammothrophs is mediated by TGF- $\beta$ 1 (Wang *et al.*, 2003), since TGF- $\beta$ 1, which is produced in mammothrophs, inhibits the proliferation of mammothrophs as described above. In rats, calcitonin synthesis is highest during the diestrus and lowest during the evening of proestrus, indicating that calcitonin gene expression is controlled by ovarian steroid hormones. Moreover, estrogen inhibits calcitonin expression, while progesterone does not, however, estrogen plus progesterone stimulates expression (Sun *et al.*, 2002). Thus, estrogen inhibits TGF- $\beta$ 1 expression as well as calcitonin expression, and both are involved in the inhibition of mammothroph proliferation. On the other hand, estrogen stimulates the production of stimulatory factors for mammothroph proliferation such as TGF- $\alpha$  and bFGF, leading to an increased number of mammothrophs.

#### Tumor necrosis factor- $\alpha$ (TNF- $\alpha$ )

TNF- $\alpha$  is synthesized in somatotrophs and intermediate

cells in rabbit pituitaries (Arras *et al.*, 1996), and TNF- $\alpha$  receptors have been detected in mouse pituitary cells (Kobayashi *et al.*, 1997). TNF- $\alpha$  induces apoptosis in somatotrophs and, in an estrogen-dependent manner, mammothrophs (Candolfi *et al.*, 2002). It also decreases PRL release (Theas *et al.*, 1998). TNF- $\alpha$  release from pituitary glands was higher during proestrus (Theas *et al.*, 2000), thus TNF- $\alpha$  inhibits PRL secretion and mammothroph growth. This apoptotic effect of TNF- $\alpha$  plays a role in the turnover of mammothrophs during the estrous cycle in female rats and mice. As mentioned earlier, mammothrophs are the most actively proliferating cells in rat and mouse pituitaries (Takahashi, 1992). In adult female rats, the high mitotic activity of mammothrophs during estrus might lead to mammothroph growth (Takahashi *et al.*, 1984). To maintain the number of pituitary cells, particularly mammothrophs, apoptotic regulation is necessary to reduce an increasing number of mammothroph cells during the estrous cycle and lactating period. Pituitary cell apoptosis might be regulated by TGF- $\beta$ 1 as well as TNF- $\alpha$ , since TGF- $\beta$ 1 induces apoptosis in human pituitary tumor cells (Kulig *et al.*, 1999; Oka *et al.*, 1999).

#### Proopiomelanocortin (POMC) peptides

POMC is synthesized and processed by proteolytic enzymes to produce three melanocyte-stimulating hormones ( $\alpha$ ,  $\beta$ -, and  $\gamma$ -MSH), adrenocorticotrophic hormone (ACTH) and three endorphins ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -endorphins) in the anterior and intermediate lobes of pituitary glands.  $\alpha$ -MSH is mainly produced in the intermediate lobes, while a light and electron microscopic study revealed that it is also produced in the corticotrophs of adult female rat pituitaries (Tanaka and Kurosumi, 1986).  $\alpha$ -MSH stimulated PRL secretion and the proliferation of mammothrophs through melanocortin-3 receptor (MC3-R) (Morooka *et al.*, 1998; Matsumura *et al.*, 2003). Estrogen-induced acute PRL secretion is dependent on the neurointermediate lobe both *in vivo* (Murai and Ben-Jonathan, 1990) and *in vitro* (Ellerkmann *et al.*, 1991). It was revealed that the associated active substances are acetylated forms of  $\alpha$ -MSH and  $\beta$ -endorphin (Ellerkmann *et al.*, 1992a,b). Suckling-induced acute PRL release is also mediated by  $\alpha$ -MSH probably secreted from the intermediate lobes (Hill *et al.*, 1991). These results indicate that  $\alpha$ -MSH augments the release of PRL, acting as a PRL-releasing factor. However, PRL-releasing factors other than POMC peptides might be involved in PRL secretion, since other PRL-releasing factors have been found in the intermediate lobes of rats (Laudon *et al.*, 1990; Allen *et al.*, 1995).

A radiolabeled  $\alpha$ -MSH binding study of rat anterior pituitaries showed that  $\alpha$ -MSH binding is restricted to a subset of pituitary cells (10.5%) and that all cells that bind  $\alpha$ -MSH are mammothrophs (Zheng *et al.*, 1997). On the other hand, in immature rat pituitaries, MC3-R mRNA-expressing cells are found in cells expressing GH mRNA alone or with PRL mRNA, TSH $\beta$  mRNA or POMC mRNA (Roudbaraki *et al.*, 1999). These results suggest that MC3-R-expressing cells

vary with postnatal development of the pituitary glands. The difference between these results regarding MC3-R mRNA-expressing cell types and the proportional abundance of each cell type might be due to differences in the ages of animals used. In mice, MC3-R mRNA is localized in most mammothrophs and some somatotrophs (Matsumura *et al.*, 2003). Blood from the rat intermediate lobe to the anterior lobe flows through the portal link between the vascular network of the intermediate lobe and the sinusoidal capillaries of the anterior pituitary (Murakami *et al.*, 1985).  $\alpha$ -MSH released from the intermediate lobe can therefore reach mammothrophs in the anterior pituitary and stimulate PRL release and cell proliferation. In rat pituitaries, mammothrophs in the central region of the anterior pituitary stimulate PRL secretion and cell proliferation in response to  $\alpha$ -MSH (Porter and Frawley, 1992). During the postnatal ontogeny period,  $\alpha$ -MSH is clearly localized in the mouse anterior pituitary (Marcinkiewicz *et al.*, 1993), while corticotroph subpopulations in adult female rat pituitaries produce  $\alpha$ -MSH as described above (Tanaka and Kurosumi, 1986). Therefore, it is possible that  $\alpha$ -MSH produced in the anterior pituitary controls the functioning of mammothrophs in a paracrine manner.

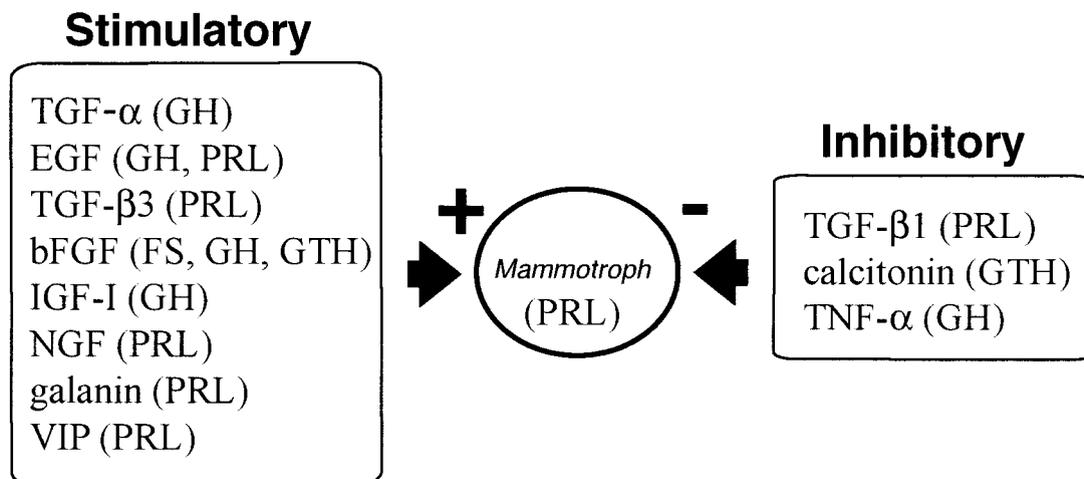
Tilemans *et al.* (1997) showed that  $\gamma$ 3-MSH stimulated the proliferation of mammothrophs in aggregate immature rat pituitary cell cultures, and concluded that the mitogenic action of  $\gamma$ 3-MSH is mediated by MC3-R. On the other hand, rat recombinant POMC (1-74) also stimulated the proliferation of mammothrophs, but was reportedly not mediated by MC3-Rs (Bert *et al.*, 1999). New  $\gamma$ 3-MSH receptors are known to be involved in the proliferation of mammothrophs (Langouche *et al.*, 2002; Denef *et al.*, 2003).

### CONCLUSIONS

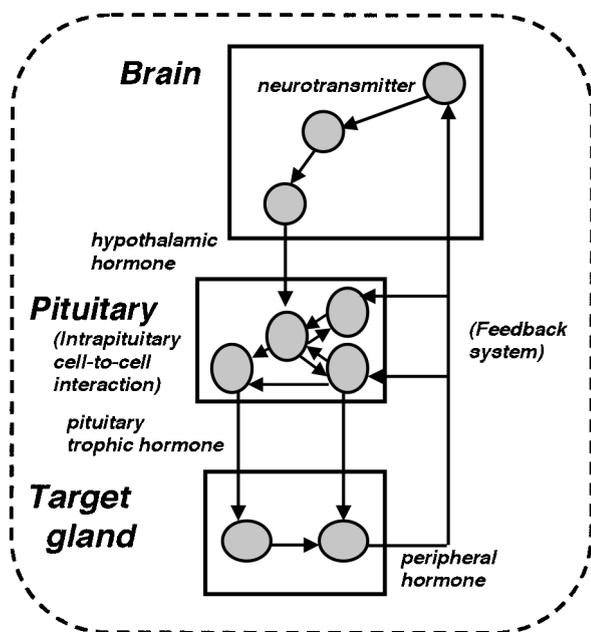
With aging and under various physiological conditions,

pituitary secretory cells change in the number and proportion of each cell type. Regulation of pituitary cell proliferation and apoptosis is essential for the dynamic maintenance of pituitary cell populations. Of the pituitary cells, mammothrophs are the most actively proliferating in rats and mice (Takahashi, 1992). Estrogen controls the synthesis and release of growth factors and the expression of cell cycle associated genes, which in turn stimulate the proliferation of mammothrophs. Growth factors whose synthesis are up-regulated by estrogen directly promote DNA replication and the mitosis of mammothrophs, whereas growth factors whose synthesis are down-regulated by estrogen inhibit the mammothroph proliferation. In rat and mouse pituitaries, somatotrophs produce TGF- $\alpha$ , EGF, IGF-I and TNF- $\alpha$ , while mammothrophs produce TGF- $\beta$ 3, NGF, galanin and VIP. Calcitonin is synthesized in the gonadotrophs. Receptors for most of those growth factors can be detected in mammothrophs. These findings suggest that these growth factors regulate the function and proliferation of mammothrophs (Fig. 1). In addition, TGF- $\alpha$ , EGF, TGF- $\beta$ 3, bFGF, IGF-I, and galanin stimulate the DNA replication and proliferation of mammothrophs. The effect of TGF- $\beta$ 3 might be indirect, and mediated through bFGF. TGF- $\beta$ 3, TNF- $\alpha$  and calcitonin inhibit the DNA replication and proliferation of mammothrophs. TNF- $\alpha$  is also an apoptotic factor for mammothrophs. The anti-proliferative action of calcitonin on mammothrophs might be mediated by TGF- $\beta$ 3.

The growth factors produced in pituitary glands act on pituitary cells as local mediators of estrogenic actions, and are involved in the regulation of pituitary cell turnover. It is not clear which is the primary factor involved in the regulation of mammothroph proliferation, however, these findings suggest that intrapituitary cell-to-cell interactions as well as hypothalamic and peripheral target tissue inputs play an important role in the control of pituitary secretory cells (Fig. 2).



**Fig. 1.** Summary of stimulatory and inhibitory factors for mammothroph proliferation in rat and mouse pituitaries. Cell types of pituitary cells expressing each factor are shown in parentheses. GH, somatotroph; PRL, mammothroph, FS, folliculostellate cell; GTH, gonadotroph. VIP stimulates prolactin release, but its stimulatory role for mammothroph proliferation has not been clarified. References are cited and discussed in the text.



**Fig. 2.** Regulatory system of proliferation and function of anterior pituitary cells. Anterior pituitary cells are controlled by hypothalamic hormones and peripheral hormones secreted from peripheral target glands. Circles in *Brain* indicate neurons, and those in *Pituitary* and *Target gland* indicate endocrine cells. Arrows indicate flows of signaling molecules (neurotransmitters, hormones and growth factors etc.). The anterior pituitary cells are also controlled by growth factors secreted from neighboring pituitary cells in a paracrine manner (intrapituitary cell-to-cell interaction). Mammothroph proliferation is regulated by various growth factors synthesized in pituitary glands (Fig. 1) as well as hypothalamic dopamine and ovarian estrogen.

## REFERENCES

- Aanestad M, Røtnes JS, Torjesen PA, Haug E, Sand O, Bjørø T (1993) Epidermal growth factor stimulates the prolactin synthesis and secretion in rat pituitary cells in culture (GH<sub>4</sub>C<sub>1</sub> cells) by increasing the intracellular concentration of free calcium. *Acta Endocrinol* 128: 361–366
- Abe H, Molitch ME, Van Wyk J, Underwood LE (1983) Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats. *Endocrinology* 113: 1319–1324
- Abraham EJ, Faught WJ, Frawley LS (1998) Transforming growth factor  $\beta$ 1 is a paracrine inhibitor of prolactin gene expression. *Endocrinology* 139: 5174–5181
- Allen DL, Low MJ, Allen RG, Ben-Jonathan N (1995) Identification of two classes of prolactin-releasing factors in intermediate lobe tumors from transgenic mice. *Endocrinology* 136: 3093–3099
- Amano O, Yoshitake Y, Nishikawa K, Iseki S (1993) Immunocytochemical localization of basic fibroblast growth factor in the rat pituitary gland. *Arch Histol Cytol* 56: 269–276
- Armstrong J, Childs GV (1997a) Changes in expression of epidermal growth factor receptors by anterior pituitary cells during the estrous cycle: cyclic expression by gonadotropes. *Endocrinology* 138: 1903–1908
- Armstrong JL, Childs GV (1997b) Regulation of expression of epidermal growth factor receptors in gonadotropes by epidermal growth factor and estradiol: studies in cycling female rats. *Endocrinology* 138: 5434–5441
- Arras M, Hoche A, Bohle R, Eckert P, Riedel W, Schaper J (1996)

- Tumor necrosis factor- $\alpha$  in macrophages of heart, liver, kidney, and in the pituitary gland. *Cell Tissue Res* 285: 39–49
- Bach MA, Bondy CA (1992) Anatomy of the pituitary insulin-like growth factor system. *Endocrinology* 131: 2588–2594
- Behringer RR, Mathews LS, Palmiter RD, Brinster RL (1988) Dwarf mice produced by genetic ablation of growth hormone-expressing cells. *Genes Dev* 2: 453–461
- Bert C, Vande Vijver V, Andries M, Verhaert P, Proost P, De Vreese B, Van Beeumen J, Vankelecom H, Deneef C (1999) Production of recombinant rat proopiomelanocortin 1–74 and characterization of its mitogenic action on pituitary lactotrophs. *Mol Cell Endocrinol* 154: 111–122
- Boockfor FR, Hoeffler JP, Frawley LS (1986) Estradiol induces a shift in cultured cells that release prolactin or growth hormone. *Am J Physiol* 250: E103–E105
- Borgundvaag B, Kudlow JE, Mueller SG, George SR (1992) Dopamine receptor activation inhibits estrogen-stimulated transforming growth factor- $\alpha$  gene expression and growth in anterior pituitary, but not in uterus. *Endocrinology* 130: 3453–3458
- Borrelli E, Heyman RA, Arias C, Sawchenko PE, Evans RM (1989) Transgenic mice with inducible dwarfism. *Nature* 339: 538–541
- Burns G, Sarkar DK (1993) Transforming growth factor  $\beta$ 1-like immunoreactivity in the pituitary gland of the rat: effect of estrogen. *Endocrinology* 133: 1444–1449
- Cai A, Bowers RC, Moore J, Hyde JF (1998) Function of galanin in the anterior pituitary of estrogen-treated Fischer 344 rats: autocrine and paracrine regulation of prolactin secretion. *Endocrinology* 139: 2452–2458
- Candolfi M, Zaldivar V, De Laurentis A, Jaita G, Pisera D, Seilicovich A (2002) TNF- $\alpha$  induces apoptosis of lactotropes from female rats. *Endocrinology* 143: 3611–3617
- Carrillo AJ, Phelps CJ (1992) Quantification of vasoactive intestinal peptide immunoreactivity in the anterior pituitary glands of intact male and female, ovariectomized, and estradiol benzoate-treated rats. *Endocrinology* 131: 964–969
- Chew LJ, Seah V, Murphy D, Carter D (1996) Anterior pituitary vasoactive intestinal peptide mRNA is colocalised with prolactin mRNA in hyperoestrogenised rats. *J Mol Endocrinol* 16: 211–220
- Dasen JS, O'Connell SM, Flynn SE, Treier M, Gleiberman AS, Szeto DP, Hooshmand F, Aggarwal AK, Rosenfeld MG (1999) Reciprocal interactions of Pit1 and GATA2 mediate signaling gradient-induced determination of pituitary cell types. *Cell* 97: 587–598
- De A, Morgan TE, Speth RC, Boyadjieva N, Sarkar DK (1996) Pituitary lactotrope expresses transforming growth factor  $\beta$  (TGF  $\beta$ ) type II receptor mRNA and protein and contains 125I-TGF  $\beta$  1 binding sites. *J Endocrinol* 149: 19–27
- Deneef C (1994). Paracrine mechanisms in the pituitary. In "The Pituitary Gland" Ed by H Imura, Raven Press, Ltd., New York, pp 351–378
- Deneef C, Lu J, Swinnen E (2003)  $\gamma$ -MSH peptides in the pituitary: effects, target cells, and receptors. *Ann NY Acad Sci* 994: 123–132
- Ellerkmann E, Nagy GM, Frawley LS (1991) Rapid augmentation of prolactin cell number and secretory capacity by an estrogen-induced factor released from the neurointermediate lobe. *Endocrinology* 129: 838–842
- Ellerkmann E, Nagy GM, Frawley LS (1992a)  $\alpha$ -melanocyte-stimulating hormone is a mammothrophic factor released by neurointermediate lobe cells after estrogen treatment. *Endocrinology* 130: 133–138
- Ellerkmann E, Porter TE, Nagy GM, Frawley LS (1992b) N-acetylation is required for the lactotrope recruitment activity of  $\alpha$ -melanocyte-stimulating hormone and  $\beta$ -endorphin. *Endocrinology* 131: 566–570

- Escalada J, Cacicedo L, Ortego J, Melian E, Sanchez-Franco F (1996) Prolactin gene expression and secretion during pregnancy and lactation in the rat: role of dopamine and vasoactive intestinal peptide. *Endocrinology* 137: 631–637
- Fagin JA, Brown A, Melmed S (1988) Regulation of pituitary insulin-like growth factor-I messenger ribonucleic acid levels in rats harboring somatomammotropic tumors: implications for growth hormone autoregulation. *Endocrinology* 122: 2204–2210
- Fagin JA, Fernandez-Mejia C, Melmed S (1989) Pituitary insulin-like growth factor-I gene expression: regulation by triiodothyronine and growth hormone. *Endocrinology* 125: 2385–2391
- Fan X, Childs GV (1995) Epidermal growth factor and transforming growth factor- $\alpha$  messenger ribonucleic acids and their receptors in the rat anterior pituitary: localization and regulation. *Endocrinology* 136: 2284–2293
- Felix R, Meza U, Cota G (1995) Induction of classical lactotropes by epidermal growth factor in rat pituitary cell cultures. *Endocrinology* 136: 939–946
- Ferrara N, Schweigener L, Neufeld G, Mitchell R, Gospodarowicz D (1987) Pituitary follicular cells produce basic fibroblast growth factor. *Proc Natl Acad Sci USA* 84: 5773–5777
- Finley EL, King JS, Ramsdell JS (1994) Human pituitary somatotropes express transforming growth factor- $\alpha$  and its receptor. *J Endocrinol* 141: 547–554
- Foster JS, Henley DC, Ahamed S, Wimalasena J (2001) Estrogens and cell-cycle regulation in breast cancer. *Trends Endocrinol Metab* 12: 320–327
- Frawley LS, Boockfor FR (1991) Mammototropes: presence and functions in normal and neoplastic pituitary tissue. *Endocr Rev* 12: 337–355
- Frost SJ, Simpson DJ, Farrell WE (2001) Decreased proliferation and cell cycle arrest in neoplastic rat pituitary cells is associated with transforming growth factor- $\beta$ 1-induced expression of p15/INK4B. *Mol Cell Endocrinol* 176: 29–37
- Gage PJ, Brinkmeier ML, Scarlett LM, Knapp LT, Camper SA, Mahon KA (1996) The Ames dwarf gene, *df*, is required early in pituitary ontogeny for the extinction of Rpx transcription and initiation of lineage-specific cell proliferation. *Mol Endocrinol* 10: 1570–1581
- Goda H, Sakai T, Kurosumi M, Inoue K (1998) Prolactin-producing cells differentiate from G0/G1-arrested somatotrophs *in vitro*: an analysis of cell cycle phases and mammotroph differentiation. *Endocr J* 45: 725–735
- Gomez O, Balsa JA (2003) Autocrine/paracrine action of pituitary vasoactive intestinal peptide on lactotroph hyperplasia induced by estrogen. *Endocrinology* 144: 4403–4409
- Gonzalez-Parra S, Argente J, Chowen JA, van Kleffens M, van Neck JW, Lindenbeigh-Kortleve DJ, Drop SL (2001) Gene expression of the insulin-like growth factor system during postnatal development of the rat pituitary gland. *J Neuroendocrinol* 13: 86–93
- Goodyer CG, De Stephano L, Guyda HJ, Posner BI (1984) Effects of insulin-like growth factors on adult male rat pituitary function in tissue culture. *Endocrinology* 115: 1568–1576
- Gospodarowicz D, Ferrara N (1989) Fibroblast growth factor and the control of pituitary and gonad development and function. *J Steroid Biochem* 32: 183–191
- Hagen TC, Arnaout MA, Scherzer WJ, Martinson DR, Garthwaite TL (1986) Antisera to vasoactive intestinal polypeptide inhibit basal prolactin release from dispersed anterior pituitary cells. *Neuroendocrinology* 43: 641–645
- Haggi ES, Torres AI, Maldonado CA, Aoki A (1986) Regression of redundant lactotrophs in rat pituitary gland after cessation of lactation. *J Endocrinol* 111: 367–373
- Haselbacher GK, Schwab ME, Pasi A, Humbel RE (1985) Insulin-like growth factor II (IGF II) in human brain: regional distribution of IGF II and of higher molecular mass forms. *Proc Natl Acad Sci USA* 82: 2153–2157
- Hentges S, Boyadjieva N, Sarkar DK (2000) Transforming growth factor- $\beta$ 3 stimulates lactotrope cell growth by increasing basic fibroblast growth factor from folliculo-stellate cells. *Endocrinology* 141: 859–867
- Hentges S, Pastorcic M, De A, Boyadjieva N, Sarkar DK (2000) Opposing actions of two transforming growth factor- $\beta$  isoforms on anterior lactotropic cell proliferation. *Endocrinology* 141: 1528–1535
- Hill JB, Nagy GM, Frawley LS (1991) Suckling unmasks the stimulatory effect of dopamine on prolactin release: possible role for  $\alpha$ -melanocyte-stimulating hormone as a mammotrope responsiveness factor. *Endocrinology* 129: 843–847
- Honda J, Manabe Y, Matsumura R, Takeuchi S, Takahashi S (2003) IGF-I regulates pro-opiomelanocortin and GH gene expression in the mouse pituitary gland. *J Endocrinol* 178: 71–82
- Honda J, Oomizu S, Kiuchi Y, Komatsu N, Takeuchi S, Takahashi S (2000) Identification of epidermal growth factor mRNA-expressing cells in the mouse anterior pituitary. *Neuroendocrinology* 71: 155–162
- Honda J, Takeuchi S, Fukamachi H, Takahashi S (1998) Insulin-like growth factor-I and its receptor in mouse pituitary glands. *Zool Sci* 15: 573–579
- Hyde JF, Engle MG, Maley BE (1991) Colocalization of galanin and prolactin within secretory granules of anterior pituitary cells in estrogen-treated Fischer 344 rats. *Endocrinology* 129: 270–276
- Inoue K, Sakai T (1991) Conversion of growth hormone-secreting cells into prolactin-secreting cells and its promotion by insulin and insulin-like growth factor-1 *in vitro*. *Exp Cell Res* 195: 53–58
- Takeuchi S, Takahashi S (2000) Epidermal growth factor, insulin, and estrogen stimulate development of prolactin-secreting cells in cultures of GH3 cells. *Cell Tissue Res* 299: 237–243
- Takeuchi S, Takahashi S (2002) Induction of mammotroph development by a combination of epidermal growth factor, insulin, and estradiol-17 $\beta$  in rat pituitary tumor GH3 cells. *Zool Sci* 19: 789–795
- Kaplan LM, Gabriel SM, Koenig JI, Sunday ME, Spindel ER, Martin JB, Chin WW (1988) Galanin is an estrogen-inducible, secretory product of the rat anterior pituitary. *Proc Natl Acad Sci USA* 85: 7408–7412
- Kineman RD, Faught WJ, Frawley LS (1992) Steroids can modulate transdifferentiation of prolactin and growth hormone cells in bovine pituitary cultures. *Endocrinology* 130: 3289–3294
- Kobayashi H, Fukata J, Murakami N, Usui T, Ebisui O, Muro S, Hanaoka I, Inoue K, Imura H, Nakao K (1997) Tumor necrosis factor receptors in the pituitary cells. *Brain Res* 758: 45–50
- Kobrin MS, Asa SL, Samsouandar J, Kudlow JE (1987)  $\alpha$ -transforming growth factor in the bovine anterior pituitary gland: secretion by dispersed cells and immunohistochemical localization. *Endocrinology* 121: 1412–1416
- Koves K, Gottschall PE, Gorcs T, Scammell JG, Arimura A (1990) Presence of immunoreactive vasoactive intestinal polypeptide in anterior pituitary of normal male and long term estrogen-treated female rats: a light microscopic immunohistochemical study. *Endocrinology* 126: 1756–1763
- Kudlow JE, Kobrin MS (1984) Secretion of epidermal growth factor-like mitogens by cultured cells from bovine anterior pituitary glands. *Endocrinology* 115: 911–917
- Kulig E, Jin L, Qian X, Horvath E, Kovacs K, Stefanescu L, Scheithauer BW, Lloyd RV (1999) Apoptosis in nontumorous and neoplastic human pituitaries: expression of the Bcl-2 family of proteins. *Am J Pathol* 154: 767–774
- Lam KS, Lechan RM, Minamitani N, Segerson TP, Reichlin S (1989) Vasoactive intestinal peptide in the anterior pituitary is increased in hypothyroidism. *Endocrinology* 124: 1077–1084

- Langouche L, Pals K, Deneff C (2002) Structure-activity relationship and signal transduction of  $\gamma$ -MSH peptides in GH3 cells: further evidence for a new melanocortin receptor. *Peptides* 23: 1077–1086
- Lara JI, Lorenzo MJ, Cacicedo L, Tolón RM, Balsa JA, López-Fernández J, Sánchez-Franco F (1994) Induction of vasoactive intestinal peptide gene expression and prolactin secretion by insulin-like growth factor I in rat pituitary cells: evidence for an autocrine regulatory system. *Endocrinology* 135: 2526–2532
- Larson GH, Koos RD, Sortino MA, Wise PM (1990) Acute effect of basic fibroblast growth factor on secretion of prolactin as assessed by the reverse hemolytic plaque assay. *Endocrinology* 126: 927–932
- Laudon M, Grossman DA, Ben-Jonathan N (1990) Prolactin-releasing factor: cellular origin in the intermediate lobe of the pituitary. *Endocrinology* 126: 3185–3192
- Lazar LM, Blum M (1992) Regional distribution and developmental expression of epidermal growth factor and transforming growth factor- $\alpha$  mRNA in mouse brain by a quantitative nuclease protection assay. *J Neurosci* 12: 1688–1697
- Li S, Crenshaw EB, 3rd, Rawson EJ, Simmons DM, Swanson LW, Rosenfeld MG (1990) Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. *Nature* 347: 528–533
- Lloyd HM, Meares JD, Jacobi J (1975) Effects of oestrogen and bromocriptine on *in vivo* secretion and mitosis in prolactin cells. *Nature* 225: 497–498
- Mallo F, Wilson E, Whorwood CB, Singh S, Sheppard MC (1995) Basic and acidic fibroblast growth factor increase prolactin mRNA in a dose-dependent and specific manner in GH3 cells. *Mol Cell Endocrinol* 114: 117–125
- Marcinkiewicz M, Day R, Seidah NG, Chretien M (1993) Ontogeny of the prohormone convertases PC1 and PC2 in the mouse hypophysis and their colocalization with corticotropin and  $\alpha$ -melanotropin. *Proc Natl Acad Sci USA* 90: 4922–4926
- Marin F, Boya J (1995) Immunocytochemical localization of basic fibroblast growth factor in the human pituitary gland. *Neuroendocrinology* 62: 523–529
- Massague J (1990) Transforming growth factor- $\alpha$ : A model for membrane-anchored growth factors. *J Biol Chem* 265: 21393–21396
- Matsumura R, Takagi C, Takeuchi S, Okuda K, Takeuchi S, Takahashi S (2003)  $\alpha$ -Melanocyte-stimulating hormone stimulates prolactin secretion through melanocortin-3 receptors expressed in mammothrophs in the mouse pituitary. *Neuroendocrinology* 78: 96–104
- McAndrew J, Paterson AJ, Asa SL, McCarthy KJ, Kudlow JE (1995) Targeting of transforming growth factor- $\alpha$  expression to pituitary lactotrophs in transgenic mice results in selective lactotroph proliferation and adenomas. *Endocrinology* 136: 4479–4488
- Michels KM, Lee W-H, Seltzer A, Saavedra JM, Bondy CA (1993) Up-regulation of pituitary [ $^{125}$ I]insulin-like growth factor-I (IGF-I) binding and IGF binding protein-2 and IGF-I gene expression by estrogen. *Endocrinology* 132: 23–29
- Missale C, Boroni F, Frassine M, Caruso A, Spano P (1995) Nerve growth factor promotes the differentiation of pituitary mammothroph cells *in vitro*. *Endocrinology* 136: 1205–1213
- Missale C, Boroni F, Sigala S, Buriani A, Fabris M, Leon A, Dal Toso R, Spano P (1996) Nerve growth factor in the anterior pituitary: localization in mammothroph cells and cosecretion with prolactin by a dopamine-regulated mechanism. *Proc Natl Acad Sci USA* 93: 4240–4245
- Missale C, Boroni F, Sigala S, Zanellato A, Toso RD, Balsari A, Spano P (1994) Nerve growth factor directs differentiation of the bipotential cell line GH-3 into the mammothroph phenotype. *Endocrinology* 135: 290–298
- Morel G, Besson J, Rosselin G, Dubois PM (1982) Ultrastructural evidence for endogenous vasoactive intestinal peptide-like immunoreactivity in the pituitary gland. *Neuroendocrinology* 34: 85–89
- Morooka Y, Oomizu S, Takeuchi S, Takahashi S (1998) Augmentation of prolactin release by  $\alpha$ -melanocyte stimulating hormone is possibly mediated by melanocortin 3-receptors in the mouse anterior pituitary cells. *Zool Sci* 15: 567–572
- Mouihate A, Lestage J (1995) Estrogen increases the release of epidermal growth factor from individual pituitary cells in female rats. *J Endocrinol* 146: 495–500
- Mueller SG, Kudlow JE (1991) Transforming growth factor- $\beta$  (TGF  $\beta$ ) inhibits TGF  $\alpha$  expression in bovine anterior pituitary-derived cells. *Mol Endocrinol* 5: 1439–1446
- Murai I, Ben-Jonathan N (1990) Acute stimulation of prolactin release by estradiol: mediation by the posterior pituitary. *Endocrinology* 126: 3179–3184
- Murakami T, Ohtsuka A, Taguchi T, Kikuta A, Ohtani O (1985) Blood vascular bed of the rat pituitary intermediate lobe, with special reference to its development and portal drainage into the anterior lobe. A scanning electron microscope study of vascular casts. *Arch Histol Jpn* 48: 69–87
- Nagy G, Mulchahey JJ, Neill JD (1988) Autocrine control of prolactin secretion by vasoactive intestinal peptide. *Endocrinology* 122: 364–366
- Ocran I, Valentino KL, Hoffman AR, Hintz RL, Wilson DM, Rosenfeld RG (1989) Structural characterization and immunohistochemical localization of receptors for insulin-like growth factor II in the rat pituitary gland. *Neuroendocrinology* 49: 248–254
- Oishi Y, Okuda M, Takahashi H, Fujii T, Morii S (1993) Cellular proliferation in the anterior pituitary gland of normal adult rats: influence of sex, estrous cycle, and circadian change. *Anat Rec* 235: 111–120
- Oka H, Jin L, Kulig E, Scheithauer BW, Lloyd RV (1999) Pituitary adenylate cyclase-activating polypeptide inhibits transforming growth factor- $\beta$ 1-induced apoptosis in a human pituitary adenoma cell line. *Am J Pathol* 155: 1893–1900
- Olson LE, Dasen JS, Ju BG, Tollkuhn J, Rosenfeld MG (2003) Paired-like repression/activation in pituitary development. *Recent Prog Horm Res* 58: 249–261
- Oomizu S, Chaturvedi K, Sarkar DK (2004) Folliculostellate cells determine the susceptibility of lactotrophs to estradiol's mitogenic action. *Endocrinology* 145: 1473–1480
- Oomizu S, Honda J, Takeuchi S, Takeuchi S, Masui T, Takahashi S (2000) Transforming growth factor- $\alpha$  stimulates proliferation of mammothrophs and corticotrophs in the mouse pituitary. *J Endocrinol* 165: 493–501
- Oomizu S, Takahashi S (1996) Insulin stimulates the proliferation of mouse anterior pituitary cells *in vitro*. *Biomed Res* 17: 365–371
- Oomizu S, Takeuchi S, Takahashi S (1998) Stimulatory effect of insulin-like growth factor I on proliferation of mouse pituitary cells in serum-free culture. *J Endocrinol* 157: 53–62
- Patterson JC, Childs GV (1994a) Nerve growth factor and its receptor in the anterior pituitary. *Endocrinology* 135: 1689–1696
- Patterson JC, Childs GV (1994b) Nerve growth factor in the anterior pituitary: regulation of secretion. *Endocrinology* 135: 1697–1704
- Perumal P, Vrontakis ME (2003) Transgenic mice over-expressing galanin exhibit pituitary adenomas and increased secretion of galanin, prolactin and growth hormone. *J Endocrinol* 179: 145–154
- Pestell RG, Albanese C, Reutens AT, Segall JE, Lee RJ, Arnold A (1999) The cyclins and cyclin-dependent kinase inhibitors in hormonal regulation of proliferation and differentiation. *Endocr Rev* 20: 501–534
- Porter TE, Frawley LS (1992) Neurointermediate lobe peptides

- recruit prolactin-secreting cells exclusively within the central region of the adenohypophysis. *Endocrinology* 131: 2649–2652
- Porter TE, Wiles CD, Frawley LS (1993) Lactotrope differentiation in rats is modulated by a milk-borne signal transferred to the neonatal circulation. *Endocrinology* 133: 1284–1291
- Porter TE, Wiles CD, Frawley LS (1994) Stimulation of lactotrope differentiation in *in vitro* by fibroblast growth factor. *Endocrinology* 134: 164–168
- Proesmans M, Van Bael A, Andries M, Deneff C (1997) Mitogenic effects of nerve growth factor on different cell types in reaggregate cell cultures of immature rat pituitary. *Mol Cell Endocrinol* 134: 119–127
- Qian X, Jin L, Grande JP, Lloyd RV (1996) Transforming growth factor- $\beta$  and p27 expression in pituitary cells. *Endocrinology* 137: 3051–3060
- Ramsdell JS (1991) Transforming growth factor- $\alpha$  and - $\beta$  are potent and effective inhibitors of GH<sub>4</sub> pituitary tumor cell proliferation. *Endocrinology* 128: 1981–1990
- Ray D, Melmed S (1997) Pituitary cytokine and growth factor expression and action. *Endocr Rev* 18: 206–228
- Ren P, Schelthauer B, W, Halper J (1994) Immunohistological localization of TGF $\alpha$ , EGF, IGF-I, and TGF $\beta$  in the normal human pituitary gland. *Endocr Pathol* 5: 40–48
- Ren Y, Chien J, Sun YP, Shah GV (2001) Calcitonin is expressed in gonadotropes of the anterior pituitary gland: its possible role in paracrine regulation of lactotrope function. *J Endocrinol* 171: 217–228
- Renner U, Pagotto U, Arzt E, Stalla GK (1996) Autocrine and paracrine roles of polypeptide growth factors, cytokines and vasogenic substances in normal and tumors pituitary function and growth: a review. *Eur J Endocrinol* 135: 515–532
- Roh M, Paterson AJ, Asa SL, Chin E, Kudlow JE (2001) Stage-sensitive blockade of pituitary somatotroph development by targeted expression of a dominant negative epidermal growth factor receptor in transgenic mice. *Mol Endocrinol* 15: 600–613
- Roudbaraki M, Lorisignol A, Langouche L, Callewaert G, Vankelecom H, Deneff C (1999) Target cells of  $\gamma$ 3-melanocyte-stimulating hormone detected through intracellular Ca<sup>2+</sup> responses in immature rat pituitary constitute a fraction of all main pituitary cell types, but mostly express multiple hormone phenotypes at the messenger ribonucleic acid level. Refractoriness to melanocortin-3 receptor blockade in the lacto-somatotroph lineage. *Endocrinology* 140: 4874–4885
- Sarkar DK, Kim KH, Minami S (1992) Transforming growth factor- $\beta$  1 messenger RNA and protein expression in the pituitary gland: its action on prolactin secretion and lactotropic growth. *Mol Endocrinol* 6: 1825–1833
- Sasaki F, Iwama Y (1988) Sex difference in prolactin and growth hormone cells in mouse adenohypophysis: stereological, morphometric, and immunohistochemical studies by light and electron microscopy. *Endocrinology* 123: 905–912
- Schechter J, Stauber C, Windle JJ, Mellon P (1995) Basic fibroblast growth factor: the neurotrophic factor influencing the ingrowth of neural tissue into the anterior pituitary of  $\alpha$ -T7 transgenic mice? *Neuroendocrinology* 61: 622–627
- Schechter J, Weiner R (1991) Changes in basic fibroblast growth factor coincident with estradiol-induced hyperplasia of the anterior pituitaries of Fischer 344 and Sprague-Dawley rats. *Endocrinology* 129: 2400–2408
- Schwartz J (2000) Intercellular communication in the anterior pituitary. *Endocr Rev* 21: 488–513
- Schwartz J, Cherny R (1992) Intercellular communication within the anterior pituitary influencing the secretion of hypophysial hormones. *Endocr Rev* 13: 453–475
- Scully KM, Rosenfeld MG (2002) Pituitary development: regulatory codes in mammalian organogenesis. *Science* 295: 2231–2235
- Shah GV, Chien J, Sun YP, Puri S, Ravindra R (1999) Calcitonin inhibits anterior pituitary cell proliferation in the adult female rats. *Endocrinology* 140: 4281–4291
- Shah GV, Epand RM, Orłowski RC (1988) Calcitonin inhibition of prolactin secretion in isolated rat pituitary cells. *J Endocrinol* 116: 279–286
- Shah GV, Pedchenko V, Stanley S, Li Z, Samson WK (1996) Calcitonin is a physiological inhibitor of prolactin secretion in ovariectomized female rats. *Endocrinology* 137: 1814–1822
- Sharma S, Oomizu S, Kakeya T, Masui T, Takeuchi S, Takahashi S (2003) Gene expression and the physiological role of transforming growth factor- $\alpha$  in the mouse pituitary. *Zool Sci* 20: 83–89
- Shirasawa N, Yoshimura F (1982) Immunohistochemical and electron microscopical studies of mitotic adenohypophysial cells in different ages of rats. *Anat Embryol* 165: 51–61
- Sirbasku DA (1978) Estrogen induction of growth factors specific for hormone-responsive mammary, pituitary, and kidney tumor cells. *Proc Natl Acad Sci USA* 75: 3786–3790
- Sornson MW, Wu W, Dasen JS, Flynn SE, Norman DJ, O'Connell SM, Gukovsky I, Carriere C, Ryan AK, Miller AP, Zuo L, Gleiberman AS, Andersen B, Beamer WG, Rosenfeld MG (1996) Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature* 384: 327–333
- Sun YP, Lee TJ, Shah GV (2002) Calcitonin expression in rat anterior pituitary gland is regulated by ovarian steroid hormones. *Endocrinology* 143: 4056–4064
- Sutherland RL, Watts CKW, Clarke CL (1988). Oestrogen actions. In "Hormones and Their actions, Part I" Ed by BA Cooke, RJB King and HJ van der Molen, Elsevier Science Publishers BV (Biomedical Division), Amsterdam, pp 197–215
- Takahashi S (1992) Heterogeneity and development of somatotrophs and mammotrophs in the rat. *Zool Sci* 9: 901–924
- Takahashi S (1995) Development and heterogeneity of prolactin cells. *Int Rev Cytol* 157: 33–98
- Takahashi S, Kawashima S (1982) Age-related changes in prolactin cell percentage and serum prolactin levels in intact and neonatally gonadectomized male and female rats. *Acta Anat* 113: 211–217
- Takahashi S, Kawashima S (1987) Proliferation of prolactin cells in the rat: effects of estrogen and bromocryptine. *Zool Sci* 4: 855–860
- Takahashi S, Okazaki K, Kawashima S (1984) Mitotic activity of prolactin cells in the pituitary glands of male and female rats of different ages. *Cell Tissue Res* 235: 497–502
- Takahashi S, Sharma S, Oomizu S, Honda J, Takeuchi S (2002) Intrapituitary regulatory system of mammotrophs in the mouse. *Arch Physiol Biochem* 110: 34–41
- Tanaka S, Kurosumi K (1986) Differential subcellular localization of ACTH and  $\alpha$ -MSH in corticotropes of the rat anterior pituitary. *Cell Tissue Res* 243: 229–238
- Tannenbaum GS, Guyda HJ, Posner BI (1983) Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. *Science* 220: 77–79
- Theas MS, De Laurentis A, Lasaga M, Pisera D, Duvilanski BH, Seilcovich A (1998) Effect of lipopolysaccharide on tumor necrosis factor and prolactin release from rat anterior pituitary cells. *Endocrine* 8: 241–245
- Theas S, Pisera D, Duvilanski B, De Laurentis A, Pampillo M, Lasaga M, Seilcovich A (2000) Estrogens modulate the inhibitory effect of tumor necrosis factor- $\alpha$  on anterior pituitary cell proliferation and prolactin release. *Endocrine* 12: 249–255
- Tillemans D, Ramaekers D, Andries M, Deneff C (1997) Effect of POMC<sub>1-76</sub>, its c-terminal fragment  $\gamma$ 3-MSH and anti-POMC<sub>1-76</sub> antibodies on DNA replication in lactotrophs in aggregate cell cultures of immature rat pituitary. *J Neuroendocrinol* 9: 627–

637

- Unger J, Lange W (1997) Insulin receptors in the pituitary gland: morphological evidence for influence on opioid peptide-synthesizing cells. *Cell Tissue Res* 288: 471–483
- Wang YQ, Yuan R, Sun Y-P, Lee T-J, Shah GV (2003) Antiproliferative action of calcitonin on lactotrophs of the rat anterior pituitary gland: evidence for the involvement of transforming growth factor  $\beta$ 1 in calcitonin action. *Endocrinology* 144: 2164–2171
- Wanke IE, Rorstad OP (1990) Receptors for vasoactive intestinal peptide in rat anterior pituitary glands: localization of binding to lactotrophs. *Endocrinology* 126: 1981–1988
- Waters SM, Krause JE (2000) Distribution of galanin-1, -2 and -3 receptor messenger RNAs in central and peripheral rat tissues. *Neurosci* 95: 265–271
- Wynick D, Small CJ, Bacon A, Holmes FE, Norman M, Ormandy CJ, Kilic E, Kerr NC, Ghatei M, Talamantes F, Bloom SR, Pachnis V (1998) Galanin regulates prolactin release and lactotroph proliferation. *Proc Natl Acad Sci USA* 95: 12671–12676
- Yamashita S, Melmed S (1986) Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* 118: 176–182
- Yokoyama S, Stefanescu L, Kovacs K (1997) Pituitary insulin-like growth factors. *Endocr Pathol* 8: 167–179
- Yoneda T, Lyall RM, Alsina MM, Persons PE, Spada AP, Levitzki A, Zilberstein A, Mundy GR (1991) The antiproliferative effects of tyrosine kinase inhibitors tyrphostins on a human squamous cell carcinoma *in vitro* and in nude mice. *Cancer Res* 51: 4430–4435
- Zheng T, Villalobos C, Nusser KD, Gettys TW, Faught WJ, Castaño JP, Frawley LS (1997) Phenotypic characterization and functional correlation of  $\alpha$ -MSH binding to pituitary cells. *Am J Physiol* 272: E282–E287

(Received March 29, 2004 / Invited Review)