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[REVIEW]

Intrapituitary Regulatory System of Proliferation of Mammotrophs 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 mammotroph cell proliferation. The regulation of mammotroph functions is a suitable system for understanding the intrapituitary regulatory system operated by growth factors and cytokines, since mammotrophs are the most actively proliferating cells in female pituitary glands. This review discusses the possible intrapituitary regulation of mammotroph differentiation and proliferation in rat and mouse pituitaries.

Key words: pituitary, mammotroph, proliferation, rat, mouse

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 hormones and hormones from the target organs (Schwartz and Cherny, 1992; Denef, 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 mammotrophs differs between males and females (Takahashi and Kawashima, 1982), and changes during pregnancy and lactation (Haggi *et al.*, 1986). In addition, the regulation of mammotroph proliferation has been well studied compared to other types of anterior pituitary cells; therefore this study focuses on the proliferation and differentiation of mammotrophs.

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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-

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-α (TGF-α)

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

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

TGF-α 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-α mRNA expression was detected in somatotrophs, gonadotrophs and mammotrophs (Fan and Childs, 1995) while in mouse pituitaries TGF-α 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-α mRNA-expressing cells are medium-sized and either round or oval. In adult male and female mouse pituitaries, TGF-α mRNA-expressing cells account for 65 and 55% of all pituitary cells, respectively. To determine TGF-α mRNA-expressing cell types in mouse pituitaries, serial sections were studied by non-radioisotopic *in situ* hybridization using cDNA probes for TGF-α mRNA, GH mRNA and PRL mRNA. Most of the GH mRNA-expressing cells contained TGF-α mRNA (79–83%), whereas only a small population of PRL mRNA-expressing cells contained TGF-α mRNA (1–3%) (Sharma *et al*., 2003). An immunocytochemical study also showed that somatotrophs express TGF-α mRNA (Takahashi *et al*., 2002). This discrepancy between TGF-α-mRNA expressing cell types might be partly based upon the different animal species studied. These findings indicate that the main source of TGF-α is somatotroph populations, since somatotrophs are the most abundant cells in anterior pituitary glands. In rat and mouse pituitaries, for example, somatotrophs and mammotrophs are distributed evenly throughout the anterior lobes of the pituitary glands. Based on the morphological analysis of TGF-α expression in mouse pituitaries, it is likely that TGF-α produced in the somatotrophs acts on mammotrophs 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-α production. To study whether TGF-α mediates the estrogen-induced proliferation of mammotrophs, a specific inhibitor of EGF receptors, 3,4-dimethoxy-a- (3-pyridyl)-(Z)-cinnamonitrile (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 mammotrophs and corticotrophs (Honda *et al*., 2000; Oomizu *et al*., 2000). EGF also stimulates the differentiation of mammotrophs 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 corticotrophs and thyrotrophs (Fan and Childs, 1995). In mouse pituitaries, EGF mRNA expression was observed in somatotrophs and mammotrophs, 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 mammotroph proliferation.

Transforming growth factor β (TGF-β)

TGF-β is a member of the cytokine family that regulates the differentiation and proliferation of various tissues. TGF-β1, -β2, and -β3 are synthesized in mammalian tissues. TGF-β1 inhibits PRL gene expression (Abraham *et al*., 1998) and the proliferation of mammotrophs (Sarkar *et al*., 1992). At low concentrations, it slightly stimulates the DNA replication of mammotrophs (Qian *et al*., 1996). TGF-β1 also acts in G1 arrest during the cell cycle as a paracrine inhibitor of mammotroph proliferation, while p15 and p27, which are Cdk (cyclin dependent kinase) inhibitors, are functional mediators of TGF-β-induced cell cycle arrest (Qian *et al*., 1996; Frost *et al*., 2001). In human pituitary tumor cell lines, TGF-β1 induces apoptosis (Oka *et al*., 1999). Mammotrophs synthesize TGF-β1, and TGF-β1 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-β1 production, resulting in the reduced TGF-β1-induced inhibition of mammotroph proliferation. The expression of TGF-β type II receptors in pituitary cells is also reduced by estrogen treatment (De *et al*., 1996) while TGF-α expression is inhibited by TGF-β1 (Mueller and Kudlow, 1991), leading to a reduced TGF-α growth stimulatory signal.

TGF-β2 is produced in rat pituitary glands, but is not localized in mammotrophs. It exerts no significant effect on the proliferation of mammotrophs (Hentges *et al*., 2000). TGF-β3, on the other hand, is produced in mammotrophs and stimulates mammotroph proliferation (Hentges *et al*., 2000). Its synthesis is stimulated by estrogen. The immunoneutralization of TGF-β3 with anti-TGF-β3 antibodies nullified the estrogen-induced proliferation of mammotrophs. This mitogenic action of TGF-β3 on mammotrophs 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 mammotrophs in neonatal rat pituitary glands (Porter *et al*., 1994). bFGF stimulates mammotroph proliferation in the presence of estrogen, indicating that it is an estrogen-dependent mitogenic factor for pituitary cells. Estrogen treatment stimulates TGF-β3 production, and TGF-β3 increases the release of bFGF from FS cells. The immunoneutralization of bFGF in a FS cell-conditioned medium inhibited its growth stimulatory action on mammotrophs (Hentges *et al*., 2000). It can be concluded therefore that bFGF is located downstream of the estrogen-TGF-β3 signaling cascade as described above, acting as a mediator of TGF-β3-induced mammotroph proliferation.

Estradiol and TGF-β3 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 mammotrophs 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 mammotrophs might be indirect, since mammotrophs 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 mammotrophs. 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 mammotrophs through VIP production, since VIP receptors are expressed in mammotrophs (Wanke and Rorstad, 1990).

Nerve growth factor (NGF)

Nerve growth factor (NGF) is localized in rat mammotrophs and, together with PRL, its secretion is stimulated by VIP (Missale *et al*., 1996). The NGF receptor, gp^{140trk}, is expressed in mammosomatotrophs and mammotrophs (Patterson and Childs, 1994a). NGF secretion is stimulated by interleukin-1β (IL-1β), and inhibited by GH releasing hormone, tumor necrosis factor-α (TNF-α) 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 mammotrophs, and NGF treatment was seen to stimulate the appearance of mammotrophs and increase the number of mammotrophs in rat pituitary cells (Missale *et al*., 1995). NGF treatment also stimulated the DNA replication of mammotrophs, 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 mammotrophs (Missale *et al*., 1994). NGF might therefore be involved in the functioning of mammotrophs 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 mammotrophs, somatotrophs, and thyrotrophs contain galanin, whereas male anterior pituitary gland mammotrophs 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 mammotrophs, and serum PRL levels (Perumal and Vrontakis, 2003). These results suggest that galanin regulates PRL secretion and the proliferation of mammotrophs.

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 mammotrophs (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 mammotrophs and TGF-β1 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 mammotrophs (Shah *et al*., 1999). This inhibitory action of calcitonin on mammotroph proliferation was attenuated by the immunoneutralization of TGF-β1 with anti-TGF-β1 serum. Calcitonin stimulates TGF-β1 synthesis, and increases the number of TGF-β1-expressing cells in female rat pituitaries. This finding indicates that the antiproliferative action of calcitonin on the mammotrophs is mediated by TGF-β1 (Wang *et al*., 2003), since TGF-β1, which is produced in mammotrophs, inhibits the proliferation of mammotrophs 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-β1 expression as well as calcitonin expression, and both are involved in the inhibition of mammotroph proliferation. On the other hand, estrogen stimulates the production of stimulatory factors for mammotroph proliferation such as TGF-α and bFGF, leading to an increased number of mammotrophs.

Tumor necrosis factor-α (TNF-α)

TNF-α is synthesized in somatotrophs and intermediate cells in rabbit pituitaries (Arras *et al*., 1996), and TNF-α receptors have been detected in mouse pituitary cells (Kobayashi *et al*., 1997). TNF-α induces apoptosis in somatotrophs and, in an estrogen-dependent manner, mammotrophs (Candolfi *et al*., 2002). It also decreases PRL release (Theas *et al*., 1998). TNF-α release from pituitary glands was higher during proestrus (Theas *et al*., 2000), thus TNF-α inhibits PRL secretion and mammotroph growth. This apoptotic effect of TNF-α plays a role in the turnover of mammotrophs during the estrous cycle in female rats and mice. As mentioned earlier, mammotrophs are the most actively proliferating cells in rat and mouse pituitaries (Takahashi, 1992). In adult female rats, the high mitotic activity of mammotrophs during estrus might lead to mammotroph growth (Takahashi *et al*., 1984). To maintain the number of pituitary cells, particularly mammotrophs, apoptotic regulation is necessary to reduce an increasing number of mammotroph cells during the estrous cycle and lactating period. Pituitary cell apoptosis might be regulated by TGF-β1 as well as TNF-α, since TGF-β1 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 (α, β-, and γ-MSH), adrenocorticotropic hormone (ACTH) and three endorphins (α-, β-, and γ-endorphins) in the anterior and intermediate lobes of pituitary glands. α-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). α-MSH stimulated PRL secretion and the proliferation of mammotrophs 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 α-MSH and β-endorphin (Ellerkmann *et al*., 1992a,b). Suckling-induced acute PRL release is also mediated by α-MSH probably secreted from the intermediate lobes (Hill *et al*., 1991). These results indicate that α-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 α-MSH binding study of rat anterior pituitaries showed that α-MSH binding is restricted to a subset of pituitary cells (10.5%) and that all cells that bind α-MSH are mammotrophs (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, TSHβ 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 mammotrophs 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). α-MSH released from the intermediate lobe can therefore reach mammotrophs in the anterior pituitary and stimulate PRL release and cell proliferation. In rat pituitaries, mammotrophs in the central region of the anterior pituitary stimulate PRL secretion and cell proliferation in response to α-MSH (Porter and Frawley, 1992). During the postnatal ontogeny period, α-MSH is clearly localized in the mouse anterior pituitary (Marcinkiewicz *et al*., 1993), while corticotroph subpopulations in adult female rat pituitaries produce α-MSH as described above (Tanaka and Kurosumi, 1986). Therefore, it is possible that α-MSH produced in the anterior pituitary controls the functioning of mammotrophs in a paracrine manner.

Tilemans *et al.* (1997) showed that γ3-MSH stimulated the proliferation of mammotrophs in aggregate immature rat pituitary cell cultures, and concluded that the mitogenic action of γ3-MSH is mediated by MC3-R. On the other hand, rat recombinant POMC (1-74) also stimulated the proliferation of mammotrophs, but was reportedly not mediated by MC3-Rs (Bert *et al*., 1999). New γ3-MSH receptors are known to be involved in the proliferation of mammotrophs (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, mammotrophs 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 mammotrophs. Growth factors whose synthesis are up-regulated by estrogen directly promote DNA replication and the mitosis of mammotrophs, whereas growth factors whose synthesis are down-regulated by estrogen inhibit the mammotroph proliferation. In rat and mouse pituitaries, somatotrophs produce TGF-α, EGF, IGF-I and TNF-α, while mammotrophs produce TGF-β3, NGF, galanin and VIP. Calcitonin is synthesized in the gonadotrophs. Receptors for most of those growth factors can be detected in mammotrophs. These findings suggest that these growth factors regulate the function and proliferation of mammotrophs (Fig. 1). In addition, TGF-α, EGF, TGF-β3, bFGF, IGF-I, and galanin stimulate the DNA replication and proliferation of mammotrophs. The effect of TGF-β3 might be indirect, and mediated through bFGF. TGF-β3, TNF-α and calcitonin inhibit the DNA replication and proliferation of mammotrophs. TNF-α is also an apoptotic factor for mammotrophs. The anti-proliferative action of calcitonin on mammotrophs might be mediated by TGF-β3.

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 mammotroph 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).

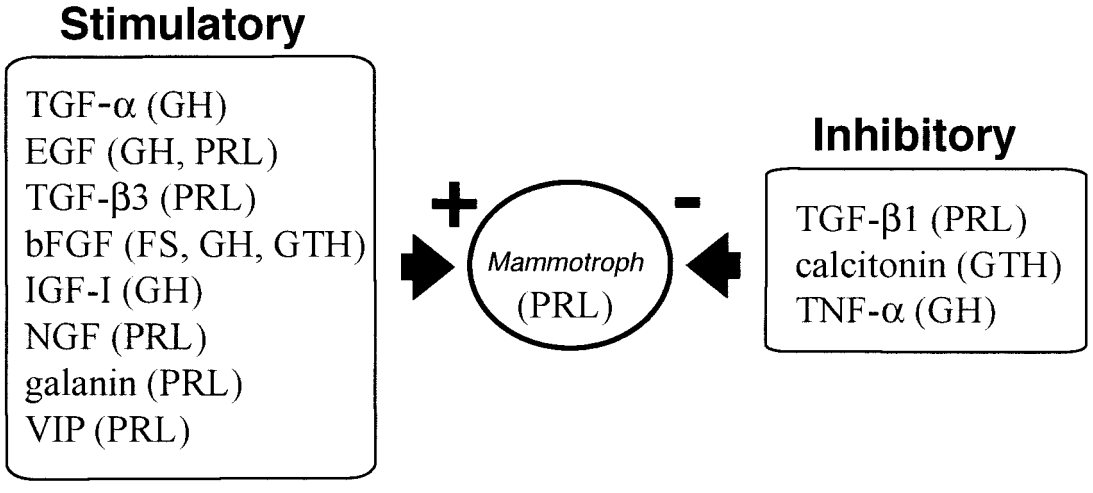

Fig. 1. Summary of stimulatory and inhibitory factors for mammotroph proliferation in rat and mouse pituitaries. Cell types of pituitary cells expressing each factor are shown in parentheses. GH, somatotroph; PRL, mammotroph, FS, folliculostellate cell; GTH, gonadotroph. VIP stimulates prolactin release,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

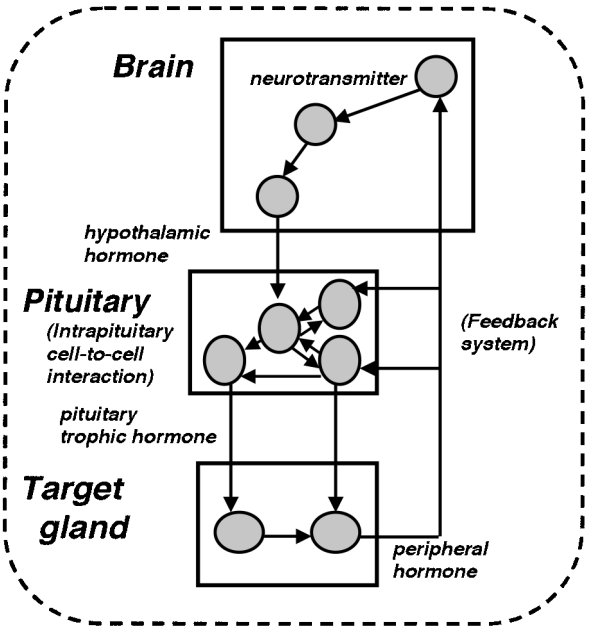

Fig. 2. Regulatory system of proliferation and function of anterior pituitary cells. Anterior pituitary cells are controlled by hypothalamic hormones and peripheral hormones secreted from peripheral target glands. Circles in *Brain* indicate neurons, and those in *Pituitary* and *Target gland* indicate 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). Mammotroph proliferation is regulated by various growth factors synthesized in pituitary glands (Fig. 1) as well as hypothalamic dopamine and ovarian estrogen.

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