Molecular and Cellular Endocrinology 345 (2011) 48–57

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Transgenic mice expressing the human growth hormone gene provide a model system to study human growth hormone synthesis and secretion in non-tumor-derived pituitary cells: Differential effects of dexamethasone and thyroid hormone

Hana Vakili, Yan Jin, James I. Nagy, Peter A. Cattini *

Department of Physiology, University of Manitoba, Canada R3E 3J9

ARTICLE INFO

Article history:
Received 27 April 2011
Received in revised form 14 June 2011
Accepted 5 July 2011
Available online 12 July 2011

Keywords:
Human growth hormone
Dexamethasone
Thyroid hormone
Pituitary
Primary cultures
Transgenic mice

ABSTRACT

Growth hormone (GH) is regulated by pituitary and hypothalamic factors as well as peripheral endocrine factors including glucocorticoids and thyroid hormone. Studies on human GH are limited largely to the assessment of plasma levels in endocrine disorders. Thus, insight into the regulation of synthesis versus secretion has come mainly from studies done on non-human GH and/or pituitary tumor cells. However, primate and non-primate GH gene loci have differences in their structure and, by extension, regulation. We generated transgenic (171hGH/CS-TG) mice containing the intact *hGH1* gene and locus control region, including sequences required for integration-independent and preferential pituitary expression. Here, we show hGH co-localizes with mouse (m) GH in somatotrophs *in situ* and in primary pituitary cells. Dexamethasone treatment increased hGH and mGH, as well as GH releasing hormone (GHRH) receptor RNA levels, and hGH release was stimulated by GHRH treatment. By contrast, triiodothyronine decreased or had no effect on hGH and mGH production, respectively, and the negative effect on hGH was also seen in the presence of dexamethasone. Thus, 171hGH/CS-TG mouse pituitary cultures represent a model system to investigate hormonal control of hGH synthesis and secretion.

1. Introduction

Growth hormone (GH) production is largely under hypothalamic control and is produced and secreted primarily by somatotrophs in the anterior pituitary in a pulsatile manner through (long-loop) feedback involving GH and peripheral insulin-like growth factor-1 (IGF-1). The hypothalamic factors involved in GH production include GH releasing hormone (GHRH) and somatostatin, which stimulate and inhibit secretion, respectively (Veldhuis et al., 2001; Robinson, 2000). Ghrelin, a second GH secretagogue (GHS) and more specifically its receptor (GHS-R) are also expressed in the human pituitary (Kedzia et al., 2009; Caminos et al., 2003). Thus, the pulsatile production of GH appears to reflect a multi-factorial interaction between forward signals from GHRH and ghrelin, with feedback signals from somatostatin, GH and IGF-1 (Veldhuis et al., 1995). As a result, GH pulses direct specific gene expression, thereby promoting skeletal and muscular growth (Werther et al., 1990; Jorgensen et al., 2006), metabolic and physical adaptations (Roemmich and Rogol, 1997), and central nervous system reactions that together help maintain health and homeostasis (Veldhuis et al., 2001). Puberty and exercise are known to increase the amount of GH released in a pulse (Ikeda et al., 1994), whereas aging, obesity and lack of exercise are examples of events that will mute the response (Ikeda et al., 1998; Surya et al., 2009; Weltman et al., 2003).

Peripheral endocrine factors such as glucocorticoids and thyroid hormone also contribute effects at multiple levels of growth and metabolism (Siebler et al., 2001). While the pituitary-specific transcription factor Pit-1 (or POU1F1) is essential for development of GH-producing somatotrophs (Li et al., 1990; Simmons et al., 1990), both adrenal glucocorticoids and thyroid hormone are required for functional maturation of these cells during fetal development (Nogami and Hisano, 2008). There is also evidence that these metabolic hormones play a significant role in determining the proportion of somatotrophs in the anterior pituitary (Porter et al., 2001). Among their targets, glucocorticoids and thyroid hormone can exert effects both on hypothalamic and pituitary sites (Miller and Mayo, 1997; Nogami et al., 2000).

Receptors for glucocorticoids and thyroid hormones are intracellular DNA-binding proteins that function as hormone-responsive transcription factors (Thompson and Evans, 1989; Barlow et al., 1986). However, our knowledge of hormonal regulation of hGH is largely limited to plasma levels in humans with endocrine

* Corresponding author. Address: Endocrine and Metabolic Diseases, Department of Physiology, University of Manitoba, 745 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 3J9.
E-mail address: peter_cattini@umanitoba.ca (P.A. Cattini).

0303-7207/$ - see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2011.07.010

disorders resulting from, for example, chronic exposure or lack of exposure to glucocorticoids and thyroid hormone (Armour et al., 1986; Magiakou et al., 1994; Agertoft and Pedersen, 1997; Pauwels et al., 2003; Wajchenberg et al., 1996; Iranmanesh et al., 1991; Williams et al., 1985). As a result, it is difficult to discriminate between primary and secondary effects of these hormones, and importantly whether an effect is at the levels of synthesis and/or secretion. Investigation into the mechanism of hGH gene (*hGH1* or *hGH-N*) regulation at the level of the pituitary has been hindered, at least in part, by the lack of a suitable *in vitro* or culture system. As a result, our knowledge of GH gene regulation has been largely derived from studies of the rat (r) GH-secreting pituitary cell lines and the endogenous *rGH* gene (Ostlund et al., 1978; Samuels and Shapiro, 1976; Leite et al., 1996). There have been multiple reports on the effects of glucocorticoids on rGH production and its gene promoter activity, but with varied results. A synthetic glucocorticoid agonist, 0.1–1 μM dexamethasone, has often been used for these studies. Endogenous rGH RNA levels were increased in rat primary pituitary cell cultures as well as pituitary tumor GC and GH3 cells (Diamond and Goodman, 1985; Martinoli et al., 1991), but there was no effect on rGH transcripts in rat pituitary MtT/S and MtT/E cells (Nogami et al., 2006). In contrast, thyroid hormone, usually 1–10 nM 3,5,3′-triiodothyronine (T3), has been associated consistently with an increase in rGH production (Diamond and Goodman, 1985; Martinoli et al., 1991; Nogami et al., 2006). However, regulation of GH by thyroid hormone is subject to species variation (Rousseau et al., 2002). Efforts have also been made to look at hormonal regulation of *hGH1* promoter activity by transfecting 0.5–5 kb (kilobase) fragments of the *hGH1* gene into rat pituitary cell lines or assessing RNA levels in human primary adenoma cells, but with variable results (Isaacs et al., 1987; Morin et al., 1990; Zhang et al., 1992; Cattini et al., 1986).

The caveat with extrapolating from rGH to hGH is that non-primate and primate GHs are structurally and functionally distinct, and differences also appear to extend beyond the coding to regulatory sequences (Strasburger, 1990; Yi et al., 2002; Nickel et al., 1990; Wells et al., 1993; Wells and de Vos, 1993; Jones et al., 1995; Lira et al., 1993). Therefore, the signals/mechanisms under which production and/or action of rodent and primate GH are subjected and regulated may also vary. Thus, in response to the lack of a model system, transgenic (TG) mice were generated containing the intact *hGH1* gene and locus control region (LCR) in a 171 kb fragment of human chromosome 17 (Jin et al., 2009); as a consequence these are referred to as 171hGH/CS-TG mice (Jin et al., 2009). The presence of the LCR ensures preferential and site of integration independent expression of hGH in the pituitary *in vivo* (Jones et al., 1995).

Here we characterize hGH-producing cells in the pituitary of 171hGH/CS-TG mice *in situ*, as well as in primary pituitary cultures derived from these mice *in vitro*. In addition, these cultures were used to assess the effects of acute glucocorticoid (dexamethasone) and T3 treatment on hGH versus mGH expression in the presence of "intact" *GH* genes and in a common pituitary cell context. Our data demonstrate expression of hGH by mouse pituitary somatotrophs, however, the proportion of hGH versus mGH among cells is variable. Furthermore, differential regulation of hGH and mGH expression was observed in pituitary cultures. These observations are discussed in relation to the results from previous studies using pituitary tumor cells.

2. Materials and methods

2.1. Transgenic mice and primary pituitary cell culture

All procedures involving animals, their tissues and cells conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and were approved by the animal Protocol Management and Review Committee at the University of Manitoba. Transgenic (TG) mice were generated containing the intact *hGH1* gene and LCR corresponding to sequences 14.5–32 kb (kilobases) upstream in 171 or 141 kb fragments of human chromosome 17. These are referred to as 171hGH/CS-TG and 141hGH/CS-TG mice, and were described previously as F-74 and F-81 mice, respectively (Jin et al., 2009). Both 171hGH/CS-TG and 141hGH/CS-TG mice express hGH specifically in the pituitary and hCS in the placental labyrinth (Jin et al., 2009). For primary pituitary cell cultures, ten adult male and female (8–10 week) mice were euthanized by cervical dislocation and anterior pituitary glands were harvested and placed in (2 ml) calcium-free phosphate buffered saline (PBS) with 0.1% bovine serum albumin (0.1% BSA/PBS). The tissue fragments were washed gently with 0.1% BSA/PBS and incubated in the same solution containing hyaluronidase (1 mg/ml), trypsin inhibitor (0.5 mg/ml), pancreatin (0.5 mg/ml) and collagenase type I (1 mg/ml) for 10 min at 37 °C in a shaking water bath. The digestate was collected in 5 ml and added to 20 ml of fetal bovine serum (FBS). The digestion of the tissue fragments was repeated two times. Digestion was continued by adding 150 units of DNase I per 50 ml of digested supernatant, with gentle mixing for 2 min at room temperature. After filtering, cells were pelleted (1200*g* for 5 min) before resuspension in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) FBS (10% FBS–DMEM). Viability of cells was assessed by Trypan blue dye exclusion, and was routinely greater than 90%. Cells were plated at 1×10^5 cells/well in 24-well plates, with or without a poly L-lysine-coated coverslip, in 1 ml of 10% FBS–DMEM with antibiotics (10 UI/ml penicillin, 10 mg/ml streptomycin), and maintained in this medium for 72 h. For glucocorticoid, thyroid hormone, growth hormone releasing hormone (GHRH) and ghrelin (GHS) treatments, cells were re-fed defined (serum-free) medium containing 20% Albumax (Invitrogen) for 24 h, and then treated with 0.2 μM dexamethasone (D8893, Sigma, Oakville, ON, Canada) and/or 1 nM triiodothyronine (T3; T5516, Sigma) and 1 μM GHRH (Aroz Technologies, Cincinnati, OH, USA) or 1 μM ghrelin (Phoneix Pharmaceuticals Inc., CA, USA) for 48, 24 h or 30 min in defined medium.

2.2. Immunohistochemistry

Human GH producing cells in pituitaries from hGH/CS-TG mice were assessed by immunocytochemistry in tissue sections. Sections through 171 and 141 hGH/CS-TG pituitary tissue from two male mice and one female adult mouse from each line were examined for hGH, mGH, Pit-1 and nuclear (DAPI) staining (for total cell number). Eight areas (~350 cells/area), selected at random, from two sections from each mouse, were assessed using antibodies shown previously to distinguish mGH and hGH (Jin et al., 2009) (and Supplementary material). Mice were deeply anesthetized with equithesin (0.3 ml, intraperitoneal) and perfused *trans*-cardially with 2 ml of cold (4 °C) pre-fixative (50 mM sodium phosphate buffer, pH 7.4, 0.1% sodium nitrite, 0.9% sodium chloride and 1 unit/ml of heparin). This was followed by perfusion with 40 ml of fixative solution containing cold 0.16 M sodium phosphate buffer, pH 7.1, 1.2% picric acid and either 1% or 2% paraformaldehyde. Animals were then flushed with 10 ml of cold 10% sucrose and 25 mM sodium phosphate buffer, pH 7.4. Pituitaries were carefully removed and stored at 4 °C for 24–48 h in cryoprotectant (25 mM sodium phosphate buffer, pH 7.4, 10% sucrose, 0.04% sodium azide). Sections (10 μm) through the anterior pituitary were cut on a cryostat, collected on gelatinized glass slides, and processed for immunofluorescence staining with primary antibodies (described in Table 1) diluted in 50 mM Tris–HCl, pH 7.6,