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COMMENTARY

HORMONE RECEPTOR REGULATED PROOPIOMELANOCORTIN GENE EXPRESSION

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Proopiomelanocortin (POMC) is a polyprotein found mainly in the pituitary gland [1], although it is also present in brain, placenta and intestine [2]. While POMC itself has little biological activity, adrenocorticotropin (ACTH) and beta-endorphin, opioid peptides within the structure of the POMC molecule, are physiologically important hormones [3]. ACTH and beta-endorphin are freed from POMC by a series of proteolytic cleavage steps. This processing has been investigated most extensively in anterior and intermediate lobes of the pituitary where some of the peptidases involved in POMC processing have been characterized [4, 5]. The degradation of POMC in these two lobes is different since ACTH is converted to alpha melanocyte stimulating hormone (alpha-MSH) in the intermediate but not the anterior pituitary. As a result, the main biologically active secreted POMC products from the intermediate lobe are alpha-MSH and beta-endorphin, whereas ACTH and beta-endorphin are released from the adenohypophysis. Differential expression of processing enzymes, therefore, is the crucial determinant for the tissue-selective appearance of various POMC-derived peptides.

ACTH and beta-endorphin have numerous physiological actions including mediating the response of the body to stress, regulation of pain mechanisms, and modification of the immune system. While the expression of different POMC peptides varies according to the presence of the processing enzymes, the levels of these opioids are directly dependent on the activity of the POMC gene. Within the last decade, the structure of the POMC gene has been identified, and many of the humoral and molecular factors regulating its activity have been elucidated. This commentary will discuss recent advances in our knowledge of the control of the POMC gene and how modifications in its activity may have important physiological consequences.

POMC gene

The human POMC gene consists of three exons separated by two non-coding intervening sequences [6]. The biologically active POMC peptides, beta-endorphin and ACTH, are exclusively coded by exon 3, whereas the signal peptide and amino terminal region of the precursor protein results from expression of exon 2 [7]. Exon 1 is not known to code for any proteins. The structure of the POMC gene has many similarities to that of the gene for preproenkephalin, another opioid peptide [2]. Furthermore, human preproenkephalin, the precursor of met- and leu-enkephalin, and POMC have the same number of amino acid residues and the biologically active peptides in each precursor polyprotein are flanked by dibasic amino acid residues. These similarities may allow the opioid peptide precursor molecules to be processed to mature hormone by common proteolytic mechanisms [2].

Glucocorticoid regulation

The synthesis of POMC-derived peptides in the pituitary is regulated by hormones both of peripheral and central origin. Glucocorticoids, whose production and release from the adrenal cortex is stimulated by ACTH [8], inhibits POMC gene activity in the anterior pituitary [9–12]. In a series of studies that examined the mechanisms by which glucocorticoids regulate POMC expression, it was shown that removal of the adrenal gland, the major source of glucocorticoids in the body, raised POMC mRNA levels and the transcription rate of the POMC gene in the adenohypophysis [11, 12]. Injection of glucocorticoid analogues such as dexamethasone into adrenalectomized animals returned POMC gene transcriptional activity back to normal levels. As a result, it was suggested that glucocorticoids tonically inhibit POMC gene activity in anterior pituitary.

Glucocorticoids suppress POMC gene expression through at least two mechanisms. By modifying the synthesis and release of corticotropin releasing factor (CRF), a potent central stimulant of ACTH and beta-endorphin secretion and production, glucocorticoid could indirectly control POMC expression [11, 12]. In support of this proposal, adrenalectomy was shown to raise the levels of CRF immunoreactivity in the paraventricular nucleus of the hypothalamus [13, 14]. Glucocorticoids can also act directly on corticotrophs to block POMC gene

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activity. This was shown by Roberts *et al.* [10], who observed that glucocorticoids applied to a tumor cell line (AtT-20) of the anterior pituitary, consisting of a homogeneous population of corticotrophs, reduced the levels of POMC mRNA.

The molecular mechanisms by which glucocorticoids directly inhibit POMC gene activity have been reviewed recently by Roberts [15]. Using the 5′ flanking region of the transcriptional initiation site of the rat POMC gene, it was possible to show binding of purified glucocorticoid receptor (activated with agonist) to the POMC gene. Nucleotide sequences within this binding region corresponded to those found in other genes regulated by glucocorticoids. If this binding region is the site at which glucocorticoids inhibit POMC gene activity, then the interaction of the receptor protein with this upstream element could induce conformation changes in the POMC gene so that transcribing regions are hindered from binding to RNA polymerase or stimulatory factors may be prevented from activating the gene. Beato [16] has shown that binding of the glucocorticoid receptor to regions upstream from the MMTV gene transcriptional initiation site could induce pronounced changes in the topology of the DNA and increase the superhelical structure (the construct was incorporated into a circularized plasmid DNA) of the upstream regulatory elements. Interestingly, Roberts [15] has shown that, following insertion of the POMC gene fragment containing the glucocorticoid receptor binding site into a transient expression assay system (AtT-20 cells transfected with a plasmid containing the gene coding for thymidine kinase), glucocorticoids acted as if they stimulated gene activity. Glucocorticoids are known to stimulate the transcription of genes such as those coding for enkephalin [17] and growth hormone [18, 19]. The preliminary results of Roberts [15] may indicate that some regulatory element downstream from the glucocorticoid binding site, but 5′ to the initiation site, is altered by the glucocorticoid receptor interaction with the POMC gene, thereby causing an inhibition of transcription. Sequence analysis of the 5′ flanking region of the rat POMC gene revealed a cAMP regulatory site 3′ to the glucocorticoid binding site and upstream from the initiation site of transcription [15]. cAMP activates the POMC gene [20]. Therefore, glucocorticoids could induce a conformation change in the POMC gene to diminish the ability of cAMP regulatory factors to stimulate the POMC gene. The presence of the glucocorticoid binding site in the POMC gene upstream from a cAMP regulatory element may explain the inhibitory action of glucocorticoids on POMC gene expression and their stimulatory action on other genes such as the growth hormone gene [21, 22], possibly lacking a cAMP regulatory element.

Whereas glucocorticoids inhibit POMC gene expression in the anterior pituitary, they have only limited influence on the activity of this gene in the intermediate lobe [23]. This differential action may be due to the absence of glucocorticoid receptors in the intermediate lobe cells [24, 25]. Radiolabeled glucocorticoids are not taken up into this tissue, and Antakly and Eisen [26] showed that immunoreactive glucocorticoid receptor, while detectable in anterior pituitary, was not present in the intermediate lobe. The lack of expression of the glucocorticoid receptor in the intermediate lobe has been proposed to be due to the action of an inhibitory factor released from the hypothalamus. Pituitary stalk-transection, which cuts the brain's connection with the pituitary, neurointermediate lobe grafts into kidney capsules, and culturing of intermediate lobe cells cause the expression of immunologically detectable receptor [27]. Thus, the presence of activable cytosolic receptor appears to be essential for glucocorticoids to regulate POMC gene expression, and differential expression of this receptor confers tissue selectivity to the actions of this steroid.

Dopamine

Although glucocorticoids do not regulate POMC gene expression in the intermediate lobe, the hypothalamic neurotransmitter dopamine is an effective inhibitor of POMC synthesis in this tissue [28]. Both dopamine and dopamine receptor agonists reduce POMC mRNA levels and POMC gene transcriptional activity in the intermediate lobe [28–30]. Reverse effects occur *in vivo* if dopamine receptor antagonists are administered to rats. Dopamine regulates POMC gene expression in this tissue by preventing the synthesis of cAMP in response to norepinephrine and beta-adrenergic receptor agonists, stimulants of POMC gene expression in the intermediate lobe [30]. Dopamine blockade of adenylate cyclase activity in the intermediate lobe is mediated by a guanine nucleotide inhibitory protein (N_i) [31]. This was established in experiments employing pertussis toxin, an agent that catalyzes the ADP-ribosylation of a subunit of N_i and prevents hormones from blocking cAMP formation. Pertussis toxin treatment of intermediate lobe cells abolishes the inhibitory effects of dopamine on adenylate cyclase activity and its ability to lower POMC mRNA levels [30, 31]. Dopamine does not regulate the POMC gene in the anterior pituitary [29], indicating that different hormones and molecular mechanisms negatively regulate POMC gene expression in the two pituitary lobes.

Stimulation of POMC gene activity

CRF, in addition to stimulating ACTH and beta-endorphin release from the anterior pituitary, increases the synthesis of POMC. Continuous administration of CRF to rats for 3 days raised POMC mRNA levels over 2-fold in the anterior pituitary [32, 33]. Direct application of CRF at AtT-20 cells increased POMC mRNA levels after 4 hr of treatment [20]. This effect was time-dependent, reversible and specific, since beta-actin mRNA levels were not altered by CRF stimulation. Both Herbert *et al.* [34] and Roberts [15] have shown that CRF stimulates POMC gene transcription, indicating that the peptide activates the POMC gene.

cAMP appears to mediate the stimulatory effect of CRF on POMC gene activity. CRF enhances both adenylate cyclase and cAMP-dependent protein kinase activities in anterior pituitary and AtT-20-cells [35–37]. Furthermore, forskolin, a diterpene which greatly facilitates adenylate cyclase activity, as well as cell membrane permeable analogues of

cAMP (e.g. 8-bromo-cAMP) raise POMC mRNA levels in AtT-20 cells and stimulate POMC gene transcription [15, 20, 34]. That cAMP-dependent protein kinase mediates the effects of CRF on POMC gene expression was supported by studies in which the inhibitor of cAMP-dependent protein kinase (PKI), a protein of approximately 10 kilodaltons (kD) which inhibits the catalytic subunit of the kinase, was inserted into AtT-20 cells using a liposome technique [38]. This manipulation prevented CRF and 8-bromo-cAMP from elevating POMC mRNA levels in these tumor cells. In these same PKI-loaded cells, phorbol ester, the activator of protein kinase C, was still able to increase POMC mRNA levels, indicating that the PKI treatment specifically blocked cAMP-dependent protein kinase activation. These studies suggest that stimulation of cAMP-dependent protein kinase activity is an essential step for CRF to induce POMC gene expression. In addition, other second messenger systems, such as protein kinase C, appear to independently regulate the POMC gene as well.

The link between cAMP-dependent protein kinase and biological responses such as POMC gene activity may involve the phosphorylation of nuclear proteins. Waterman *et al.* [39] showed in a clonal rat pituitary cell line (GH_4) that cAMP induces the phosphorylation of a nuclear protein of 19 kD. Phosphorylation of this protein was speculated to lead to an increase in the transcriptional activity of the growth hormone gene. In the same cell line, cAMP also induces the phosphorylation of a different 23 kD basic nuclear protein named BPR. This phosphorylation event was blocked by calcium channel blockers. Similarly, the ability of cAMP to stimulate the prolactin gene in these cells was calcium dependent. Thus, it was proposed that in the same pituitary cells cAMP-dependent protein kinase catalyzed the phosphorylation of different nuclear proteins to activate different sets of genes coding for distinct hormones.

In AtT-20 cells, forskolin enhances the phosphorylation of a 25 kD nuclear protein [40]. This effect is blocked by prior insertion of PKI into the cells. Whether this phosphoprotein is related to cAMP stimulation of POMC gene expression is not established. Roberts [15] noted that in a region 275 nucleotides upstream from the initiation site of transcription of the rat POMC gene is a segment similar in sequence to the cAMP regulatory element of other genes. Conceivably, the 25 kD nuclear protein in AtT-20 cells whose phosphorylation is cAMP dependent may either bind to this cAMP regulatory element or alter the binding of other cellular factors to this region to stimulate the POMC gene. Interestingly, phorbol esters induce the phosphorylation of a similar nuclear phosphoprotein in AtT-20 cells as cAMP [40]. It is possible that both cAMP-dependent protein kinase and protein kinase C act through a similar phosphorylation event to modify opioid peptide gene expression. This is supported by the recent studies of Herbert *et al.* [34], who using the chloramphenicol acetyltransferase (CAT) transient expression assay (AtT-20 cells transfected with a plasmid containing the CAT gene) showed that cAMP and phorbol esters stimulate the same upstream element to enhance preproenkephalin gene transcription.

Synthesis–secretion coupling

For a number of hormones and neurotransmitters, there appear to be cellular mechanisms linking the process of secretion to the synthesis of the bioactive substance. In adrenal chromaffin cells, a variety of secretagogues (cAMP and depolarizing agents such as nicotine and veratridine) increase enkephalin release and activate the preproenkephalin gene [41–44]. Roberts [15] has proposed that all physiological stimulants of POMC gene expression in the pituitary evoke the secretion of opioid peptides, implying that POMC synthesis and release are coordinately controlled. This contention is supported by the finding that CRF, forskolin, 8-bromo-cAMP and phorbol ester increase both POMC gene expression and ACTH release from anterior pituitary cells [15, 20, 34], and beta-adrenergic agonists elevate POMC mRNA levels and evoke alpha-MSH and beta-endorphin release from the neurointermediate lobe [30, 31].

Whether or not changes in POMC peptide release act as a trigger for POMC gene activation is not known. *In vivo*, in rats, adrenalectomy rapidly raises ACTH and beta-endorphin plasma levels before changes in POMC mRNA levels are detected in the anterior pituitary [11, 25]. Glucocorticoid administration to these animals reduces ACTH release much quicker than it inhibits POMC gene expression. Furthermore, in AtT-20 cells, forskolin can maximally stimulate ACTH release within 2 min of application [45], whereas stimulation of POMC gene transcription or the induction of POMC mRNA requires substantially longer periods of treatment. Thus, the hormone release process itself may turn on the POMC gene.

However, in other endocrine cells, hormone release and synthesis are controlled by separate mechanisms. Barinaga *et al.* [46] showed that treatment of anterior pituitary cells with the depolarizing agent K^+ or phorbol esters stimulated growth hormone release but did not affect the transcriptional activity of the growth hormone gene. cAMP promoting agents and growth hormone releasing factor increased both growth hormone release and synthesis from these same cells. However, the stimulatory effects on synthesis and secretion could be pharmacologically dissected.

In AtT-20 cells, forskolin induces the phosphorylation of nuclear proteins through an activation of cAMP-dependent protein kinase. While these phosphorylation events may be important for the stimulation of the POMC gene, forskolin also induces the phosphorylation of cytoplasmic and membrane proteins which could have a role in evoking ACTH secretion [40]. In fact, recent studies by Luini *et al.* [47] have shown that cAMP increases the activity of a calcium conductance channel in AtT-20 cells. Such an activation could lead to calcium influx, which serves as a stimulus for hormone release in other endocrine cells. Furthermore, Nowycky and Reisine [48] have shown in excised patches of AtT-20 cells that cAMP suppresses the activity of a K^+ conductance channel. This effect may be due to

the phosphorylation of this ionic conductance channel in the cell membrane. These findings would indicate that the molecular mechanisms mediating the control of POMC gene activity and ACTH release are different. Since the precise events regulating POMC gene activity and ACTH release have not been identified, the question of whether some coordinating process is still involved in modifying POMC gene activity and POMC peptide release remains to be answered.

Summary

The activity of the POMC gene is regulated by both stimulatory and inhibitory hormones. Presumably, some balance exists in the influence of these hormones in order to maintain a steady-state level of activity of this gene. Physiological insults, such as stress, may upset this balance and change the rate of production of POMC and its biologically active peptides. The relative strength of these different hormones may therefore determine the long-term expression of this gene.

Chronic administration of CRF to rats, primates and humans produces prolonged increases in plasma ACTH levels [49]. This long-term effect is most likely due to an activation of the POMC gene. Interestingly, chronic treatment of anterior pituitary cells with CRF desensitizes CRF receptors [50]. Thus, despite the corticotrophs becoming refractory to the acute stimulatory actions of CRF, the POMC gene remains stimulated. These findings suggest that corticotrophs do not have to be continuously activated by CRF to maintain a long-term increase in POMC gene expression. This contrasts with the actions of glucocorticoids whose effects are abruptly terminated following their removal from the target tissue [11, 12]. The molecular basis of this form of cellular memory to the actions of CRF may involve cAMP regulatory phosphoproteins binding to and activating the POMC gene. If this phenomenon is shown to occur and the phosphorylation state of these nuclear proteins is found to govern their level of interaction with POMC gene, then it would represent a novel mechanism of gene regulation. Proof for this mechanism and the elucidation of how other second messengers such as protein kinase C and calcium regulate the POMC gene will greatly aid our understanding of the precise molecular mechanisms controlling opioid peptide expression.

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