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Steroid hormone mediated regulation of corticotropin-releasing hormone gene expression

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1. ABSTRACT

Corticotropin-releasing hormone (CRH), a 41 amino acid polypeptide, is expressed in many regions of central nervous system and peripheral tissues and mediates many physiological functions. Abnormal production of CRH is involved in some pathological processes. Among various endogenous factors that modulate CRH production, steroid hormones control CRH production by regulating its gene transcription. Although there is no classical steroid hormone response element in the CRH promoter, steroid hormones regulate CRH gene expression through protein-protein interaction or by binding directly to response elements.

2. INTRODUCTION

Corticotropin-releasing hormone is a 41 amino acid polypeptide that was first isolated from the hypothalamus of sheep. The primary amino acid structure of CRH is highly conserved among species, with the human peptide being identical to those in rats, and different from sheep in only 7 amino acids (1). CRH is a principal hypothalamic component of the hypothalamic-pituitary-adrenal (HPA) axis. It is synthesized in the parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVN). CRH secreted from the terminals of hypothalamic neurons acts on corticotropes of the anterior pituitary to enhance secretion of ACTH and other pro-opiomelanocortin products. ACTH, in turn, stimulates glucocorticoid secretion in the adrenal cortex. Hypothalamic secretion of CRH is stimulated by many forms of physical or psychological duress, and CRH is thought to play a critical role in the co-ordination of the neuroendocrine response to stress (2).

Although the hypothalamus is the main source of CRH, both CRH mRNA and peptide have also been found in many extrahypothalamic regions of the central nervous system (CNS) including cerebral cortex, basal ganglia, amygdale, thalamus and hippocampus. CRH acts as a neurotransmitter in some regions of the CNS, particularly those responsible for integrating the autonomic nervous system's response to stress, mediating stress-related

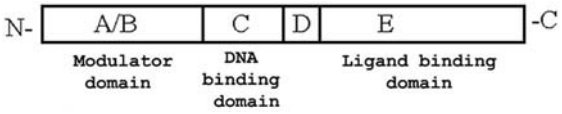

Figure 1. General structure and functional organization of steroid hormone receptors. Steroid receptors are structurally organized in different domains: 1) a variable N-terminal region, which may have modulatory effects on transactivation (region A/B). 2) a well conserved cysteine-rich central domain, which exhibits cysteine residues compatible with the formation of zinc fingers (regions C and D). 3) C-terminal domain, which is responsible for hormone binding and nuclear translocation (region E).

behavior (3, 4). The distribution of CRH outside the CNS is quite widespread; it has been reported that adrenal gland, ovary, testis, gastrointestinal tract, endocrine pancreas, lymphocyte, adipose, and placenta all contain CRH peptide and mRNA (5, 6). In accordance with the widespread distribution, considerable diversity in the biological action of CRH outside the CNS has been demonstrated. These include alterations of cardiovascular, immuno-inflammatory, gonadal, digestive, and metabolic functions of CRH. Abnormal CRH synthesis and secretion in the nervous system has been shown to be involved in depression, anxiety-related disorders, anorexia nervosa and neurodegenerative disease (7, 8). Overproduction of CRH in peripheral inflammatory sites such as synovial joints may contribute to autoimmune diseases (eg., rheumatoid arthritis) (9). During human pregnancy, excessively elevated CRH production in the placenta is associated with preterm delivery, preeclampsia and intrauterine growth retardation (10).

The steroid hormones androgen, estrogen, glucocorticoids, and progesterone are involved in a wide range of physiologic activities, including the control of development, metabolism, and reproduction as well as the stress response. Some of their functions are achieved by regulating CRH expression in CNS or in peripheral tissues. For instance, inhibition of CRH expression in PVN by glucocorticoid is crucial for maintaining basal and stress-induced HPA axis activity (11). Gender differences in stress responses may be related to the differential regulation of CRH gene expression in the hypothalamus by estrogen, progesterone and androgen (12,13). Fetal HPA development is associated with steroid regulation of CRH gene expression in placenta during pregnancy (14). Thus, understanding the mechanisms through which steroids regulate CRH in various tissues should give insights into both physiological and patho-physiological CRH processes.

3. REGULATION OF GENE EXPRESSION BY STEROID HORMONES

According to the commonly accepted theory of steroid hormone action, steroids modulate gene expression by interaction with intracellular nuclear receptors, which act as ligand-dependent transcription factors (15). All nuclear hormone receptors are structurally organized into several domains: a variable N-terminal region, a central highly conserved cysteine-rich DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD) (figure 1). Hormone-dependent transcriptional activation domains (activation function; AF-1 and AF-2), embedded within the N-terminal domain and the LBD respectively, have been identified. In the absence of ligand, steroid hormone receptors are associated with a chaperone protein complex. Upon binding of steroid hormone to the appropriate nuclear hormone receptor in the cytosol, members of the heat-shock protein family (including hsp 90, hsp70 and hsp 40) that maintain the receptors in an inactive form with high affinity for the steroid hormones, dissociate from the receptors (16). In the classical model, after translocation into the nucleus, the ligand-receptor complex binds to palindromic hormone response elements (HRE) in the promoters of target genes as either homo- or hetero-dimers, thereby stimulating, or repressing, gene expression.

However, it is now known that steroid regulation of gene expression is much more complex, and these receptors also function through protein-protein interaction with components of transcription initiation complexes and cross-talk with transcription factors functioning via other signaling pathways. Indeed, it has been suggested that many important physiological function of glucocorticoid receptors (GRs) may be reliant on protein-protein interactions. For example, glucocorticoids affect the activity of NF-kappa-B, an important modulator of cytokine–induced inflammation, in at least two ways. Glucocorticoids increase the expression levels of the inhibitor I-kappa-B, which traps NF-kappa-B in the cytoplasm. In addition, GR interacts with p65, a transcriptionally active subunit of NF-kappa-B, by protein-protein interaction (17). It has also been shown that GRs can modulate many target genes through an AP-1 transcription factor binding site by interacting with the AP-1 component proteins Fos or Jun (18).

Enhancement of the transactivation activity of the AF-1 region is potentiated after phosphorylation of the N-terminal domain of estrogen receptor-alpha (ER-alpha) by MAP kinase, which allows the interaction of the RNA helicase, and p68 with the N-terminal domain of ER-alpha and thus the opportunity for cross-talk between the ER and epidermal growth factor-dependent signaling (19).

4. COMPONENTS OF THE CRH PROMOTER

The promoter region contains short sections, called regulatory elements, which consist of specific DNA sequences that are recognized by and bind to transcriptional regulatory factor proteins. Using an internet based database (TRANSFAC) to search for potential regulatory elements in the promoter of the CRH gene from location -920 to +1 bps, no palindromic response element for steroid hormone receptors was identified. However, several short sequences similar to one arm of consensus palindromic elements, referred to as 1/2 glucocorticoid regulatory elements and 1/2 estrogen response elements, are located in the CRH promoter (figure 2). In addition, there are several other potential regulatory elements which appear to be of

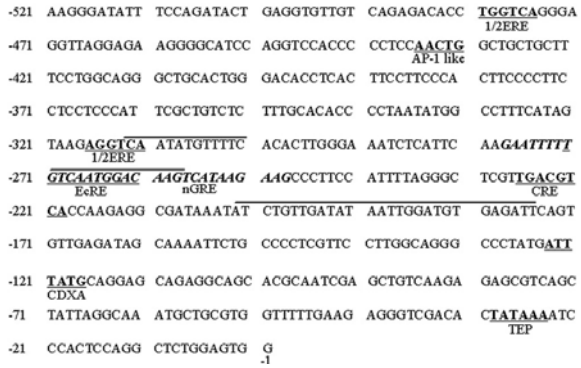

Figure 2. The DNA sequences of the promoter region of the human CRH gene. The DNA is presented for the region -1 to -521 of the human CRH gene with the consensus response/regulatory elements discussed in the text highlighted in underlined bold font, and the nGRE as bold-italic font and the *in-vitro* GR binding regions as overlined sequence.

particular importance to steroid hormone regulation of CRH gene expression, including a consensus cAMP regulatory element (CRE), a caudal-type homeobox protein (CDXA) binding site, and an ecdysone regulatory element (EcRE) and also AP-1 like sites (20) (figure 2).

5. GLUCOCORTICOID REGULATION OF CRH

Glucocorticoids are well known to restrain HPA activity via negative feedback, and this is critical for preserving homeostasis under stress conditions (11, 21). There are relatively high concentrations of GR in the CRH neurons of the PVN (22), and over the last 20 years many studies have tried to verify that CRH in the PVN is down-regulated by glucocorticoids. Immunoreactivity and gene expression based studies have shown that CRH is elevated in adrenalectomized animals, whereas the administration of glucocorticoids can result in a decrease in CRH gene expression in the PVN of adrenalectomized or intact rats (23-26). Consistent with these reports, *in vitro* hypothalamic dissociated culture systems have also shown that glucocorticoids decrease CRH expression (27,28). In NPLC-KC cells, a human hepatocellular carcinoma-derived cell line which naturally expresses the CRH gene, administration of a synthetic glucocorticoid, dexamethasone, suppresses CRH expression (29).

Negative feedback inhibition of CRH by glucocorticoids is not axiomatic, since under certain conditions glucocorticoids stop inhibiting CRH gene expression in the PVN. For example, in adrenalectomized rats, glucocorticoid administration does not prevent the increase in stress-induced CRH heteronuclear RNA (hnRNA) (30), and adrenalectomy cannot reverse the inhibitory action of dehydration on CRH expression (31). Using a restraint stress model, Shepard and coworkers (32) have recently shown that in adrenalectomized rats CRH hnRNA increased after 30 min of restraint before returning to basal levels by 90 min in the PVN while the circulating level of glucocorticoid stayed constant during stress. This suggests that stress-induced glucocorticoids do not mediate the limitation of CRH transcription in the PVN. However, Ginsberg *et al* (33) have recently demonstrated that the blocking of stress induced-glucocorticoid effects by GR antagonist Ru28362 reduced CRH gene expression in the PVN. Imaki *et al* (34) showed that treatment with dexamethasone reduced CRH gene expression only in the post-restraint period. In addition, it seems that a low level of exposure to glucocorticoid is critical for maintaining CRH expression in the PVN since Tanimura & Watts (35) observed that in adrenalectomized rats a low dose of glucocorticoids (insufficient to normalize thymus weight) augmented CRH mRNA expression in the PVN.

The effect of glucocorticoid on CRH gene expression is different in tissues outside the PVN. In the amygdala, a majority of CRH neurons contain GR (36). *In vivo*, investigators have demonstrated that glucocorticoids can either increase or have no effect on CRH gene expression in the central nucleus of amygdala depending on the concentration of glucocorticoid used (37,38), and a number of studies reported that adrenalectomy lowered CRH expression in the central nucleus of amygdala. Glucocorticoid treatment prevented the above effects of adrenalectomy, suggesting that they are glucocorticoid specific (38-40). In primary dissociated amygdala cultures, dexamethasone was not able to alter CRH gene or peptide expression. Furthermore, in those studies, dexamethasone was not able to alter forskolin-induced increases in CRH expression. These studies in the amygdala are limited however, in that they have not yet employed corticosterone, the naturally occurring glucocorticoid expressed in the rodent (41). The bed nucleus of the stria teminalis (BNST), regarded as a part of the extended amygdala, has a high density of CRH-containing neurons. High levels of circulating glucocorticoids and stress increase whereas adrenalectomy reduces CRH gene expression in the lateral BNST (42,43).

The activation of the fetal HPA axis promotes the maturation of the fetus and parturition. CRH is expressed in the placenta during human pregnancy. Placental CRH may play an important role in parturition, and in some instances may be used to predict vulnerability to preterm delivery (10,14). The differential regulation of CRH expression by glucocorticoid in hypothalamus and placenta was noted by Tropper *et al* (44), where they reported that dexamethasone treatment did not suppress levels of CRH in the plasma of pregnant women. It was subsequently demonstrated that glucocorticoids do not inhibit the production of CRH in the placenta as had been expected, but rather, they increase CRH gene expression and peptide production in placenta (45, 46). In addition, pregnant women treated with betamethasone have increased CRH levels in both plasma and placenta tissue (47).

The molecular mechanism by which glucocorticoids regulate CRH gene expression has been

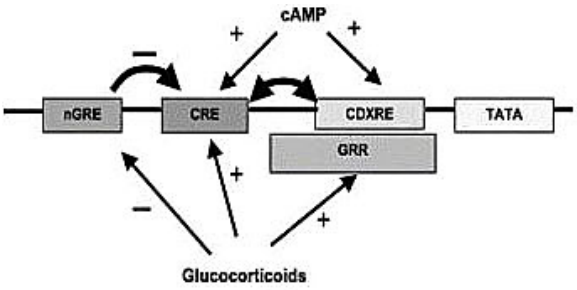

Figure 3. A schematic model of the CRH promoter with potential regulatory elements mediating the effects of cAMP and glucocorticoids in AtT-20 cells illustrated. The nGRE is a negative GRE, CRE is the cAMP regulatory element, GRR represents the region located between -213 and -99 bps that is stimulated by glucocorticoids, CDXRE is caudal-type homeobox element response element, and TATA is the TATA box. Thin arrows represent stimulatory (+) and inhibitory (-) regulatory effects by cAMP and glucocorticoids through the different elements. Thick arrows represent negative while the double headed arrow represents synergistic stimulatory interactions between sites.

the focus of much research in recent years. As noted earlier the CRH promoter does not contain a consensus glucocorticoid response element. Assessment of the CRH promoter using TRANSFAC identified a 1/2 consensus glucocorticoid regulatory element at -600 to -593 bps (figure 2). In the murine corticotroph AtT-20 cell line, not expressing endogenous CRH, reductions in CRH-CAT expression were observed upon dexamethasone treatment following transient transfection with plasmids carrying the CRH gene promoter. Guardiola-Diaz *et al* (48) demonstrated in this particular subclone of AtT-20 cells that an 18-base pair DNA fragment containing the CRE linked to a heterologous promoter conferred glucocorticoid-mediated repression of cAMP-activated CAT activity. These studies also demonstrated that the GRs do not bind directly to the CRE, suggesting that GRs repress CRH promoter activity through protein-protein interaction at the CRE. In addition, they identified 3 regions of the promoter (-313 to -133 bps) that bound GRs *in vitro* (-313/-301 bps, -270/-258 bps and -202/-175 bps; figure 2). Glucocorticoid regulatory regions in the 5' regulatory region of the CRH gene has also been studied .by Malkowski *et al* (49) in AtT20 cells. One of the putative glucocorticoid receptor binding sites, localised to base pairs -278 to –249, has been reported to be responsible for inhibitory action was and to function as a negative GRE (nGRE; figure 2).

We have also sought to identify the key elements regulating the CRH gene in AtT-20 cells. Our results showed that glucocorticoids inhibit only the component of cAMP-stimulation occurring via CRE, through an action which also involves the nGRE. We also discovered that glucocorticoids have the ability to stimulate CRH promoter activity in AtT20 cells through either the CRE or a region between -213 to -99 (the GRR in figure 3) when the nGRE is absent. The CDXRE located at -125 to -118 bps is not responsible for glucocorticoid regulation. Moreover, the GR does not bind directly to the CRE, suggesting that GRs stimulate the CRE indirectly either by affecting nuclear factors before they interact with the CRE or through protein-protein interactions with nuclear factors binding to the CRE (50). Interestingly, this CRE also confers glucocorticoid stimulation of the CRH promoter in placental cells. We have also not found any evidence that GR binds directly to the CRE in placental cells. Using nuclear run-on experiments, we have demonstrated that stimulation of the CRE by glucocorticoids does not require ongoing protein synthesis and, hence, does not occur by indirect effects such as increasing production of CBP (51). Electrophoretic mobility shift assays (EMSAs) identified binding of the transcription factors CREB and Fos at the CRE in AtT20 cells while CREB and c-Jun were detected in placental cells (50). Therefore, tissue specific expression of transcription factors may mediate regulation of the CRH gene by glucocorticoids.

6. REGULATION OF CRH BY ESTROGEN AND PROGESTERONE

The CRH promoter contains several estrogen regulatory element half sites, and therefore it has been proposed that estrogen may have direct effects on CRH gene expression. Vamvakopoulos and Chrousos (52) have demonstrated that estrogens stimulate CRH promoter activity in CV-1 cells, and that the DNA binding domain of ER could bind the 1/2 ERE. Several studies have demonstrated that CRH mRNA expression in PVN is decreased in ovariectomized animals, and is restored after estradiol replacement treatment, suggesting a stimulatory effect of estrogen on CRH (53-55). The basal and endotoxin-induced CRH levels in the PVN were both greatest on the morning of proestrus, in association with elevated 17 beta-estradiol levels (55,56). Stimulation of CRH gene expression in PVN by estrogens is also seen in male animals. Recently, Lund *et al* (57) reported that treatment of gonadectomized male rats with estradiol benzoate augmented the level of CRH hnRNA in the PVN. However, the literature is not unanimous in its support of a stimulatory effect of estrogen on CRH (58). Paulmyer-Lacroix *et al* (59) observed a decrease in CRH mRNA levels in the PVN after the implantation of estradiol capsules to ovariectomized rats, whereas administration of ovarian steroids did not have a significant affect on CRH mRNA levels in the PVN of ovariectomized ewes. In addition, the two estrogen receptors, ER-alpha and ER-beta may have different affects on CRH gene expression. Recently, Miller and coworkers (60) reported that a subset of medial parvocellular CRH neurons in the rat hypothalamus contain ER-beta but not ER-alpha. Following cotransfection of Hela cells with CRH promoter constructs, and either ER-alpha or ER-beta, ER-beta rather than ER-alpha stimulated CRH promoter activity. Additionally, ER-beta regulation of the CRH promoter was determined to occur in a manner not involving AP-1-like sites.

In human placenta, we found that estrogens inhibited CRH mRNA expression and peptide production. Placental trophoblasts express both ER-alpha and ER-beta,

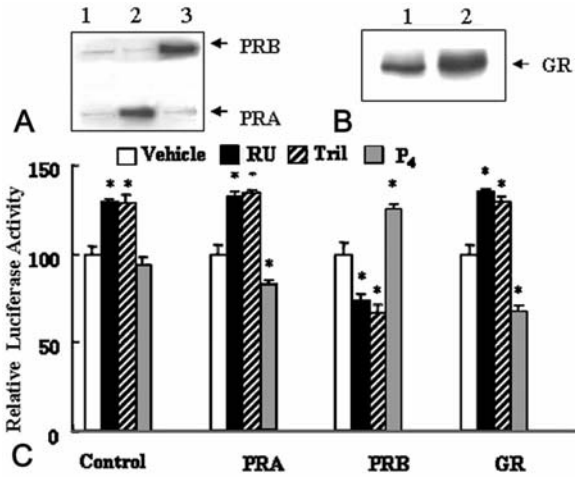

Figure 4. Effect of PR-A, PR-B and GR on CRH promoter activity in placental cells. A. Over expression of human PR-A and PR-B. Lane 1: Control; Lane 2: cells transfected with PR-A expression vector; Lane 3: cells transfected with PR-B expression vectors. B:Over expression of human GR. Lane 1: control; Lane2: primary placental cells transfected with GR expression vector. C: Primary placental trophoblasts were co-transfected with pCRH(5500)-GL3 reporter construct and control vector (control), pCRH(5500) -GL3 reporter construct and PRA vector (PRA), CRH reporter and PRB (PRB) or CRH reporter and GR (GR). Then cells were treated with 10^{-7} mol/L progesterone (P4), 10^{-6} mol/LRu38486 (RU), GR/PR antagonist, or 10^{-6} mol/L trilostane (Tril), 3 beta-hydroxysteroid dehydrogenase inhibitor (to inhibit progesterone production by placental cells), for 24h. Relative promoter activity is shown as percentage of vehicle. Values represent the mean±SEM from three independent experiments. *P<0.01 compared with vehicle.

although ER-alpha is predominant, and it is ER-alpha that mediates the down-regulation of CRH gene expression in placental trophoblasts (61). Interestingly, ER-alpha down-regulates CRH promote activity via the consensus CRE located at –248 to -213. This effect of estrogen not only occurred in primary placental trophoblasts but also in JEG-3 cells, a choricarcinima cell line (61,62). In another choriocarcinoma cell line, BeWo, estrogen has no effect on the CRH gene promoter activity (63), however in endometrial cells estrogen was also seen to inhibit CRH gene expression (64).

Progesterone, an ovarian steroid, has also been reported to regulate CRH gene expression in the PVN and in extrahypothalamic tissues. Progesterone receptors have been localized to the PVN, and the effects of progesterone on CRH expression in the PVN are usually observed together with those of estrogen. Treatment of ovariectomized animals with progesterone can reverse the effect of estrogen replacement on CRH mRNA expression in the PVN, which is consistent with the decrease in CRH mRNA observed in the stimulated luteal phase (53, 56). It has been documented that progesterone has an antagonistic effect on estrogen in many tissues including brain, uterus, and a breast cancer cell line, MCF-7. There have been some conflicting reports, however, with Li *et al* (65) demonstrating that progesterone potentiated the stimulating effect of estrogen on CRH mRNA levels in ovariectomized rats, while Broad and coworkers (66) observed that progesterone had no effect on CRH mRNA levels in the PVN of ovariectomized ewes.

Placental trophoblasts produce large amounts of progesterone during pregnancy. It has been proposed that progesterone might inhibit the glucocorticoid-mediated up-regulation of CRH gene expression in the placental cells since progesterone can also bind with one-quarter the affinity of cortisol to the GR (67). Hence when progesterone is present in very large concentrations relative to that of glucocorticoids, progesterone may have an inhibiting effect on CRH expression. In this regard, Jones *et al* (46) has demonstrated progesterone inhibits CRH release from placenta and chorion. Using primary cultured placental trophoblasts, we have explored the molecular mechanism through which progesterone may regulate CRH gene expression. Our studies have shown that progesterone down-regulates CRH mRNA expression, and also CRH gene promoter activity in transiently transfected cells. Furthermore, it was found that GR and progesterone receptors (PRs) exhibit differential action in the regulation of CRH gene expression. PRA was shown to inhibit whereas PRB stimulated CRH promoter activity (figure 4), and GRs also mediate progesterone inhibition of CRH gene expression (68). The CRE is the critical element responsible for progesterone regulation of CRH gene transcription in placenta, although it has been shown that the nuclear proteins from placental cells that bind to CRE do not include PRs or GRs (20). This is interpreted to mean that the CRE is a critical site for steroid hormone mediated regulation of CRH gene transcription in placental cells, and that the PR variants, PRA or PRB, regulate CRH transcription by interacting with different transcription factors.

CRH is also produced by human endometrial cells and is directly involved in the decidualization process as a paracrine inducer. Makrigiannakis *et al* (69) has reported that progesterone, the main decidualizing factor, increased the production and secretion of immunoreactive CRH from stromal cells and induced the activity of the CRH promoter. These effects were partially reversed by the antiprogestin RU 486 and were completely abolished in the presence of the cAMP inhibitor, Rp-cAMP. Interesting, the effect of progesterone on the CRH promoter also requires the CRE since experiments carried out with a deleted palindromic CRE at -224 bp of the CRH promoter resulted in a complete loss of progesterone effect.

7. ACTION OF ANDROGEN

Androgen receptors are sparsely distributed within CRH containing neurons in the PVN, and relatively little is known of the effect of androgen on regulation of CRH gene expression. Gender differences in stress responses may be related to differential regulation of CRH gene expression by sex steroids in the hypothalamus since,

relative to female rats, males show less resting state expression levels of CRH and less ACTH response to stress (70,71). In gonadectomized male rats, CRH hnRNA expression is increased significantly in the PVN in response to restraint stress, and this increase can be decreased by dihydrotestosterone propionate (DHTP) treatment (72). Consistent with this, it is known that androgen is a potent inhibitory regulator of HPA axis (71,73), yet Viau *et al* (74) demonstrated that gonadectomized rats exhibited a transient increase in CRH hnRNA levels after restraint stress, and the CRH expression in PVN did not correlate with plasma testosterone levels. Patchev and Almeida (75) observed that adrenalectomy in combination with gonadectomy increased CRH gene expression in PVN. Administration of testosterone to the adrenalectomy/gonadectomy rats potentiated the suppressive effect of corticosterone on CRH expression. These studies suggest that androgen exerts facilitating and "buffering" effects on glucocorticoid-mediated transcriptional regulation of CRH.

8. PERSPECTIVE

CRH was isolated from ovine hypothalamus and the amino acid sequence was determined in 1981 by Vale *et al* (76). To understand how this peptide participates in maintaining homeostasis, extensive studies have been carried out using chemical, physiological, pharmacological, histological and molecular biological methods. Dysregulation of CRH in CNS and peripheral tissues are observed in various pathologies, including affective disorder, autoimmune disease, abnormal pregnancy, etc. Elucidation of the regulatory mechanisms for CRH gene expression will help in understanding the pathophysiology of these disorders.

Steroid hormones are a group of important factors modulating CRH gene expression. Steroid hormones might exert their developmental, metabolic, and reproductive functions, at least in part, by regulation of CRH expression. The regulation of CRH gene expression by steroid hormones is dependent not only on their respective receptors being expressed in cells but also on the transcriptional machinery available in a particular cell context. Although several sites in the CRH promoter region have been identified which could be bound by steroid receptors, most studies indicate that steroids regulate CRH gene transcription by mechanisms that do not involve their receptor binding directly to the DNA. Evidence suggests that several regulatory elements might be involved in steroid mediated responses. In particular, the CRE is an essential element in mediating glucocorticoid, estrogen and progesterone regulation of CRH gene transcription in placental trophoblasts as well as other cell types (eg. AtT-20 cells). Malkoski and Dorin (49) have suggested that AP-1 may function through a site that overlaps the EcRE. Thus the steroid hormone receptors binding to the EcRE may suppress CRH expression by interfering with activation by AP-1.

Work needs to continue on many aspects of CRH gene expression by steroid hormones in various tissues. A comprehensive analysis of mRNAs and /or proteins expressed in CRH containing cells may lead to discovery of novel molecules essential for the regulation of CRH.

9. ACKNOWLEDGEMENTS

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