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Molecular Regulation of Corticotropin-Releasing Hormone Gene Expression in Parvocellular Neurons of the Hypothalamic Paraventricular Nucleus

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Adequate regulation of corticotropin releasing hormone (CRH) secretion and expression is essential for endocrine, autonomic and behavioral stress adaptation. Maintenance of messenger RNA levels for peptide synthesis requires rapid but limited activation of CRH gene transcription. Activation of CRH transcription depends on cyclic AMP/protein kinase A signaling and binding of phospho-CREB to a critical cyclic AMP response element (CRE) at −270 in the CRH promoter. DNA methylation of the CRE CpG reduces CREB binding to the promoter affecting CRH expression. CREB-dependent activation of CRH transcription requires recruitment of the CREB co-activator, Transducer Of Regulated CREB activity (TORC). Cyclic AMP activates TORC by inhibiting salt induced kinase (SIK) type 2 allowing TORC dephosphorylation and nuclear translocation. Termination of the transcriptional response is essential for preventing pathology associated with chronic elevations of CRH and HPA axis activity. Glucocorticoid feedback inhibition, mainly through modulation of afferent pathways to hypothalamic CRH neurons, plays an important role. In addition, intracellular feedback mechanisms involving, Inducible Cyclic AMP Early Repressor (ICER), and cAMP-induced SIK1 activation and consecutive TORC inactivation, contribute to limiting CRH transcription. Understanding the molecular mechanisms of regulation of CRH expression is essential for understanding the pathogenesis and developing new therapeutic approaches for stress related disorders.

KEYWORDS: corticotropin releasing hormone (CRH), CRH transcription, cyclic AMP, cyclic AMP response element binding protein (CREB), glucocorticoid feedback

Introduction

Preservation of homeostasis requires constant adaptation of the organism to internal and external disturbances or stressors. The 41-amino acid hypothalamic peptide, Corticotropin-Releasing Hormone (CRH) plays an important role in this process. CRH is the primary regulator of pituitary ACTH secretion, but in addition it regulates autonomic and behavioral effects of stress, acting as an integrator of stress responses [1–3]. The main source of CRH mediating hypothalamic pituitary adrenal (HPA) axis regulation is the hypothalamic paraventricular nucleus (PVN) but the peptide is also produced at several extrahypothalamic sites in limbic system regions of the brain [4]. CRH and CRH receptors expressed at these sites regulate behavioral and autonomic responses to stress [1] (Fig. 1).

Dysregulation of CRH has been implicated in a number of disorders, such as depression, anxiety, and post-traumatic stress disorder, as the result of alterations in circulating glucocorticoids, as well as from the neurotransmitter actions at extrahypothalamic sites [5, 6]. Therefore, elucidation of the mechanisms controlling CRH transcription is essential for a better understanding of the pathophysiology and treatment of stress-related disorders. This article reviews current knowledge on the molecular mechanisms for activation and repression of responses of CRH neuron to stress.

1. Signaling pathways regulating the CRH neuron

Afferent projections to the CRH neuron in the PVN release of a number of neurotransmitters such as norepinephrine, glutamate, GABA, serotonin, and neuropeptides such as angiotensin II, pituitary adenylate cyclase activating polypeptide (PACAP), glucagon like peptide 1 (GLP1) and CRH itself. Interaction of these regulators with cognate receptors in the CRH neuron activates intracellular signal-transduction pathways, and modifies neuron function (Fig. 2). The main neurotransmitters released in the PVN during stress are norepinephrine and glutamate [7, 8]. Norepinephrine acts on alpha adrenergic receptors which are coupled to the guanyl nucleotide binding protein q/11 and phospholipase C, thus increasing intracellular calcium and protein kinase C activity [9]. Norepinephrine can also

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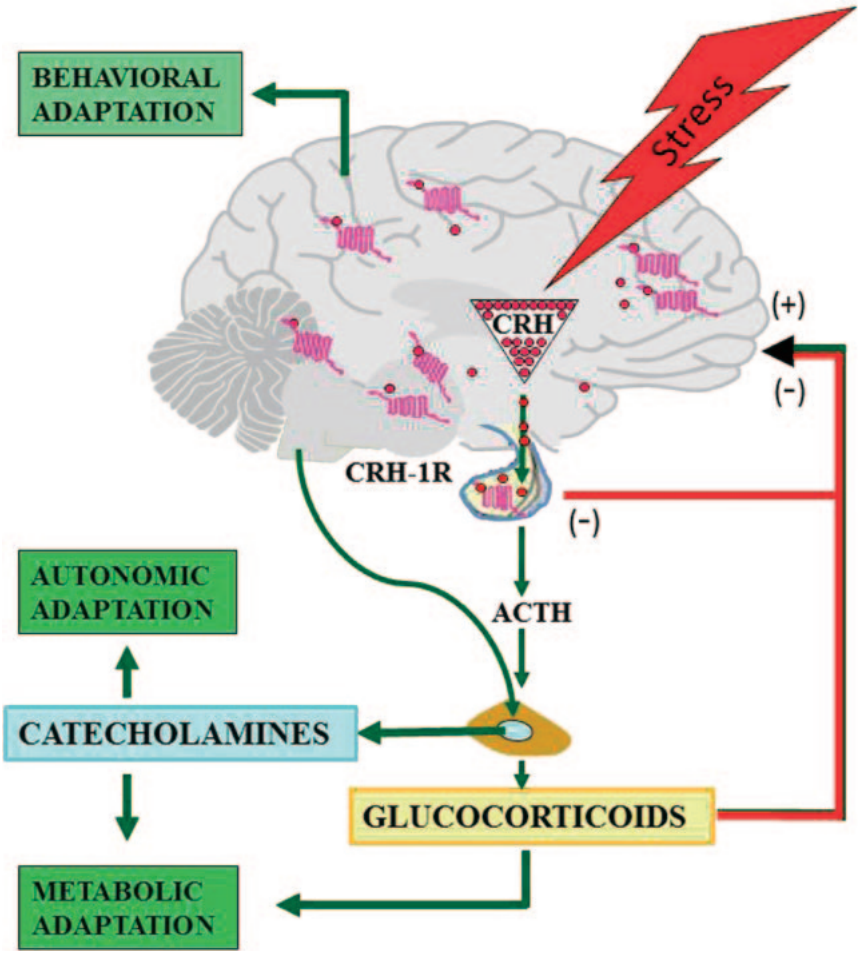

Fig. 1. **Schematic representation of the involvement of CRH on adaptive responses to stress** CRH mediates behavioral, autonomic (catecholamine secretion) and endocrine (hypothalamic pituitary adrenal axis activation) responses to stress. Stress stimulates the release of corticotrophin releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus (PVN) into the pituitary portal circulation. Binding of CRH to type 1 CRH receptors (CRHR1) in the pituitary corticotroph stimulates ACTH secretion which stimulates adrenal glucocorticoid secretion. Stimulatory pathways are shown with green arrows. Glucocorticoids, essential for metabolic adaptation, exert feedback inhibition on the HPA axis (red) and modulate brain function by positive or negative regulating a number of genes. Activation of CRHR1 in the brain (mostly limbic system) mediates behavioral and autonomic adaptation to stress.

activate beta-adrenergic receptors with lower affinity, but in vivo and in vitro evidence suggest that alpha adrenergic receptors mediate the direct stimulatory effect of norepinephrine in CRH neurons [10, 11]. Glutamate interacts with N-methyl-D-aspartate *rec*eptor (NMDA) and subunit 5 of the glutamate receptor (GluR5) located in CRH neurons and terminals in the external zone of the median eminence. Activation of these receptors increases intracellular calcium and neuronal excitability [12, 13]. Glutamate is a major excitatory neurotransmitter in the central nervous system and it is likely to mediate the rapid increases in CRH release into the pituitary portal circulation. Serotonin probably acts upon serotonin type 2C receptors ($5HT_{2C}R$), which are present in CRH neurons [14]. This 5HT receptor subtype is coupled to calcium-phospholipid-dependent pathways and their stimulation does not increase cyclic AMP production [15].

Cyclic AMP and cyclic AMP-dependent signaling are essential for transcriptional activation of the CRH neuron [16–18]. Since the major neurotransmitters mediating activation of the CRH neuron, norepinephrine and glutamate, do not stimulate cyclic AMP, neuropeptides released in the PVN during stress should be considered as potential sources of cyclic AMP [19–21]. These neuropeptides may include CRH itself, released by dendritic projections and acting upon stress inducible type 1 CRH receptors (CRHR1) in the CRH neuron [20]. Second, pituitary adenylate cyclase activating polypeptide (PACAP), shown to stimulate CRH transcription in hypothalamic neuron primary cultures. Consistent with an important role of PACAP on HPA axis activation, PACAP knockout mice display attenuated HPA axis responses to stress with blunted CRH mRNA responses in the PVN [22]. Third, glucagon-like peptide 1 (GLP-1), released in the PVN by non-catecholaminergic neurons from the nucleus of the solitary tract, is also known to stimulates CRH expression and its blockade inhibits HPA axis activity [23]. In addition, it is possible that stimulation of a calcium-dependent adenylate cyclase secondary to increases in cytosolic calcium from alpha –adrenergic, glutamate or serotonin receptor stimulation contributes to increasing intracellular cyclic AMP levels during stress. Consistently,

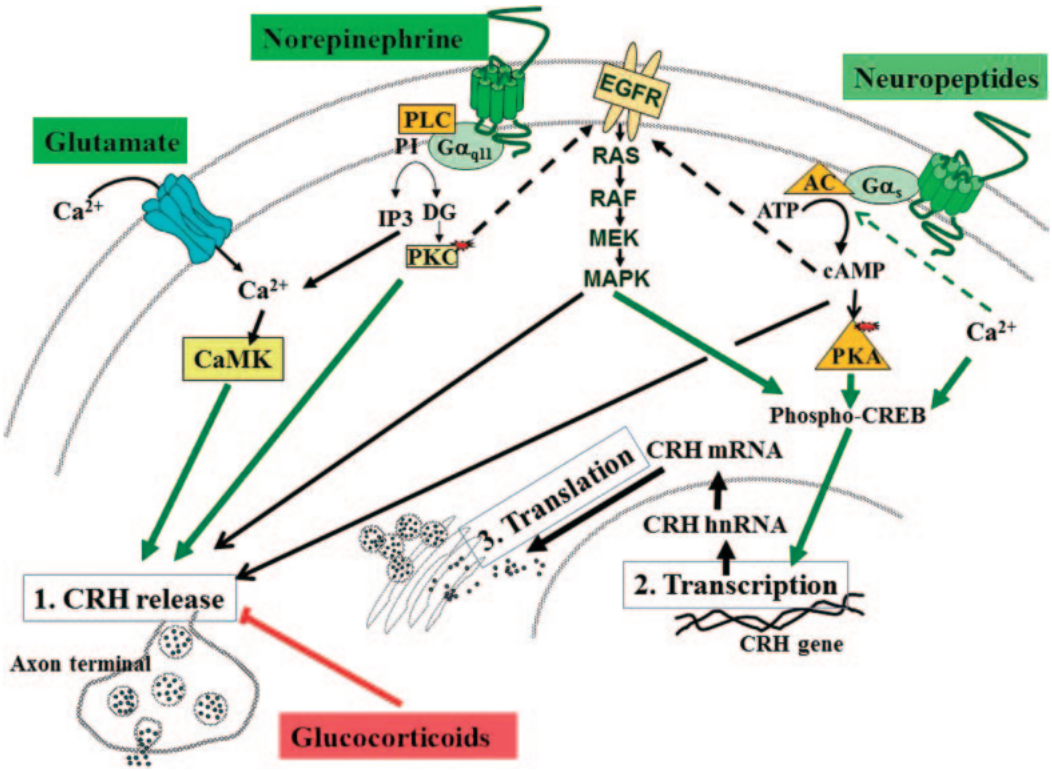

Fig. 2. **Regulators and signaling-transduction systems controlling CRH expression and secretion in the PVN** Norepinephrine secreted by ascending projections to the CRH neuron act on alpha adrenergic receptors coupled to the guanyl nucleotide binding protein q/11 (Gq11) and phospolipase C (PLC), resulting in increase in intracellular calcium (Ca^{2+}) and protein kinase C (PKC) activity. Glutamate secreted by from multisynaptic projections from the limbic system interacts with NMDA and GluR5 receptors leading to increases in intracellular calcium. Both cyclic AMP and calcium phospholipid dependent pathways transactivate the MAP kinase pathway. These pathways mediate rapid CRH release from axonal terminals within seconds (CRH release). Secretion is followed by activation of CRH transcription with production of primary transcript or heteronuclear RNA (hnRNA) within minutes, a process which requires cyclic AMP. The mechanism for cyclic AMP production is may involve activation of neuropeptide receptors coupled to adenylate cyclase, such as CRHR1, PACAP and GLP1, or/and activation of a calcium dependent adenylate cyclase (indicated by the broken arrow). Transcription increases CRH mRNA levels and translation to the CRH precursor protein and packing into secretory granules at the Golgi. The signaling mechanisms controlling translation and processing of the precursor protein to CRH have not been fully elucidated.

in situ hybridization studies have shown the presence of the calcium-dependent adenylate cyclase 8 in the PVN of the mouse brain [24].

2. Basal and stress regulated CRH expression

CRH neurons in the PVN are a major target of the neural circuitry activated during stress. In experimental animals and humans, a proportion of CRH neurons also express vasopressin (VP), which is co-released with CRH and contributes to HPA axis regulation. The number of CRH neurons expressing VP increases following acute and chronic stress [25]. While low levels of CRH output appear to be sufficient for basal HPA axis activity [26, 27], appropriate increases in CRH expression and secretion are required for HPA axis responses to most stressors [28]. Acute stress induces rapid and transient activation of parvocellular CRH neurons leading to rapid release of CRH into the pituitary portal circulation [29]. This is associated with rapid increases in CRH transcription, as shown by rapid (within minutes) and transient increases in primary transcript or heteronuclear RNA (hnRNA), followed by elevation of steady-state mRNA levels and translation to the precursor peptide, pro-CRH [28] (Fig. 2). Consistent with the primary role of CRH regulating ACTH secretion, changes in CRH expression during chronic stress parallel the pattern of ACTH secretion, with habituation or sustained activation depending on the stress paradigm. Confirming the critical role of CRH as stimulator of HPA axis activity during stress, genetically modified mice lacking of CRH or CRH receptors have blunted or absent glucocorticoid responses to most stressors [30].

3. Molecular mechanisms regulating hypothalamic CRH transcription

Stress causes rapid depletion of releasable pools of CRH from parvocellular neuron projections in the median eminence, and maintenance of peptide stores requires de novo synthesis of peptide. In resting conditions there are

relatively high levels of mature CRH mRNA allowing rapid initiation of translation, which is associated with mRNA degradation. The rapid transcriptional activation following stress has no impact on the immediate secretory response to stress, but it is necessary for restoring mRNA levels.

The CRH gene has two exons and one short intron located in the 5′ untranslated region (Fig. 3-A). The sequence of the CRH gene including the proximal 5′flanking region is highly conserved between species, and it is almost identical for human and rat. It contains two TATA binding protein sites and a number of responsive elements, including a cyclic AMP responsive element (CRE) at position −247, a C/EBP site, an atypical GRE/AP1 element located at −249 to −248, half estrogen responsive elements (ERE), and a neuron-restrictive silencing element, (RE-1/NRSE) element located in the intron [28]. Some of these sites including the CRE, GRE/AP1 and NRSE are functional in reporter gene assays in heterologous cell lines, but with exception of the −247 CRE, their importance in physiological conditions remains unclear. The CRE has been the most studied in vitro and in vivo and it is clearly essential for regulation of CRH transcription. In addition, the proximal promoter contains potential sites for the POU homeodomain protein, Brn-2, which is required for the development of CRH neurons [31].

3.1 Mechanisms of activation of CRH transcription

Activation of the CRH neuron during stress is associated with induction of a number of immediate early genes, such as c-fos, Fra-2, zif-268/Egr-1, the Nr4a family factors, Nur-77 and Nor-1, NGF1B, as well as nuclear translocation of phopho-CREB and the MAP kinase, ERK, in CRH neurons [32]. Although all of these transcription factors are potentially involved in transcriptional regulation of CRH, with the exception of CREB, the exact interaction of these factors with response elements in the CRH promoter remains unknown.

Activation of CRH transcription depends on cyclic AMP/protein kinase A (PKA) signaling, leading to phosphorylation of cyclic AMP response element binding protein (pCREB) and its recruitment by the CRE at position −247 of the CRH promoter [18, 33, 34]. Blockade of CREB activity prevents cyclic AMP-dependent activation of CRH transcription indicating that it is essential for this process (Fig. 3-B). A number of signaling

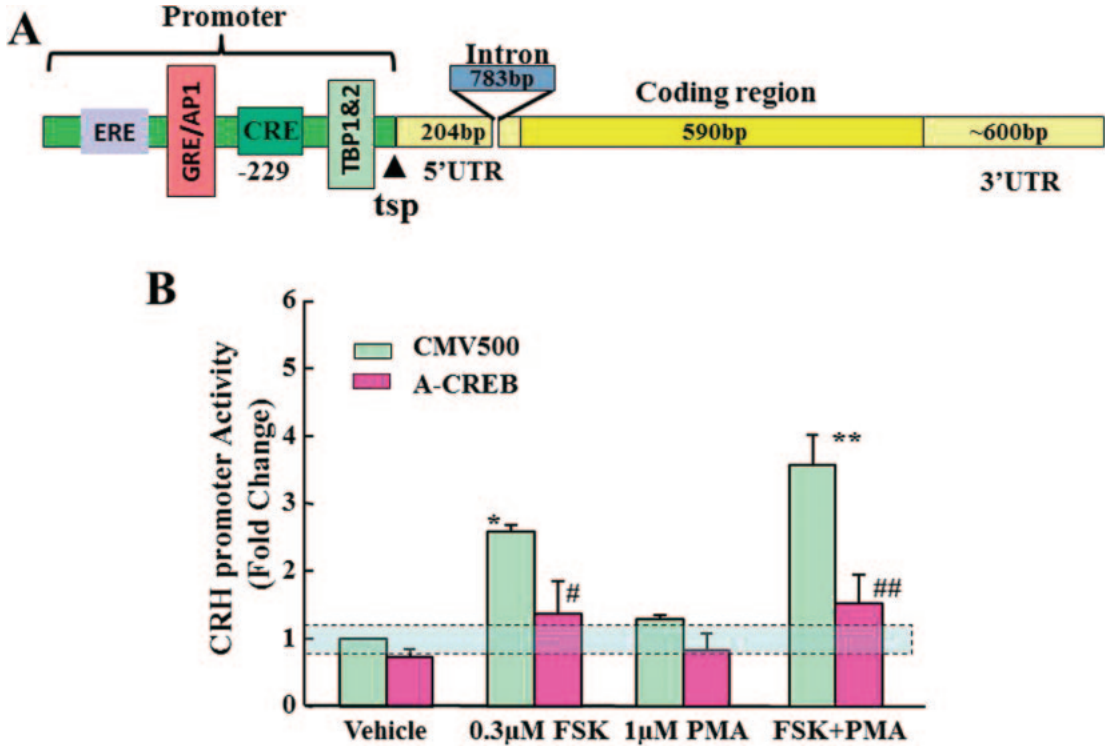

Fig. 3. **Structure of the CRH gene and cyclic AMP/CREB-dependent transcriptional regulation of CRH** (A) The CRH gene has two exons (shown in yellow) and one intron (shown in blue) located in the 5′ untranslated region (5′UTR). The 5′ flanking region, shown in green, has 2 TATA boxes or TATA binding protein elements (TBP1&2), a consensus CRE at −229, a combined glucocorticoid and AP1 response element (GRE/AP1), and an a half estrogen response element (ERE). These elements have been characterized using reporter gene assays. In addition, there are several potential sites for the POU homeodomain protein, Brn-2, which is essential for CRH neurons differentiation (not shown). The cyclic AMP response element (CRE) at −229 has been well characterized and it is essential for transcriptional activation. (B) The cyclic AMP stimulator, forskolin (FSK) but not the PKC activator, PMA, activates CRH promoter activity in a CRH promoter-luciferase reporter gene assay in the hypothalamic cell line 4B. However, PMA potentiates forskolin-stimulated transcription (green bars). Cotrasnfection of the CREB dominant negative A-CREB completely blocks the stimulatory action of forskolin alon or in combination with PMA (fuchsia bars). *, $P < 0.01$ vs vehicle; **, $P < 0.05$ vs FSK alone; #, $P < 0.05$ vs empty vector (CMV500); ##, $p < 0.01$ vs CMV500.

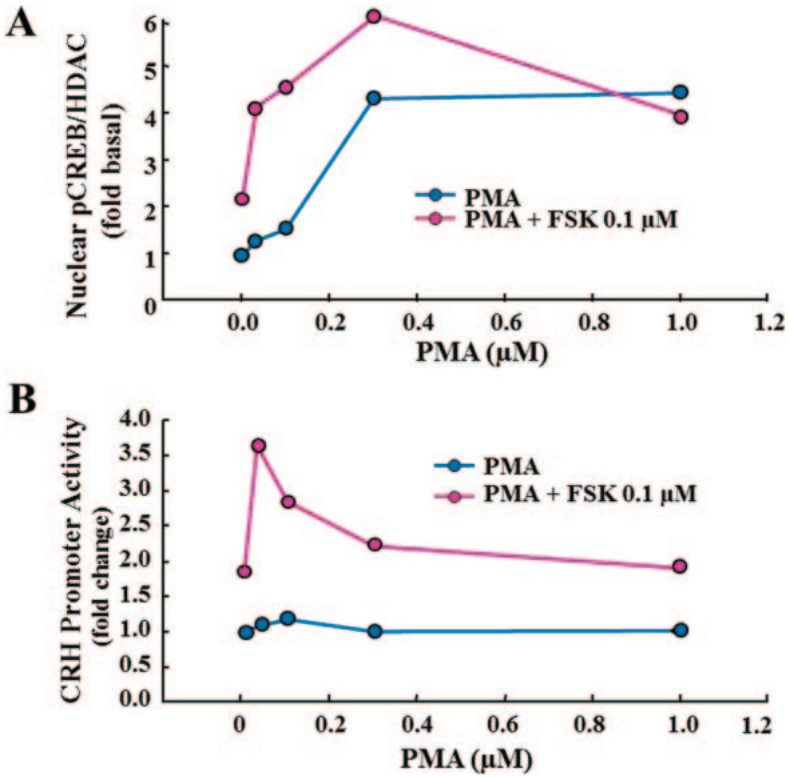

Fig. 4. **Phosphorylation of CREB is not sufficient to initiate CRH transcription** Dose responses for the effect of the phorbol ester, PMA, alone or in the presence of the adenylate cyclase stimulator, forskolin (FSK), on nuclear phospho-CREB, measured by western blot (A) and on CRH promoter activity in the hypothalamic cell line, 4B. PMA alone causes a dose-dependent increase in phospho-CREB and potentiates the stimulatory effect of FSK. PMA alone has no effect on CRH promoter activity in spite of the marked increses in phospho-CREB indicating that phopho-CREB is not sufficient for driving CRH transcription.

transduction pathways lead to CREB phosphorylation but CRH transcription requires cyclic AMP production and PKA activation, indicating that CREB is required but not sufficient to activate transcription (Fig. 4).

More recently it has been shown that the CREB co-activator, Transducer of Regulated CREB Activity (TORC), also known as CREB-regulated transcription co-activator (CRTC) is required for CREB-dependent activation of CRH transcription [35, 36]. There are three TORC subtypes, TORC 1, 2 and 3, encoded by different genes. In basal conditions, TORC remains inactive in the cytoplasm, phosphorylated and bound to the scaffolding protein 14-3-3. TORC phosphorylation is mediated by members of the AMP-activated protein kinase (AMPK) family of Ser/Thr protein kinases, including salt-inducible kinase, (SIK) [37]. Protein kinase A inactivates these kinases and prevents TORC phosphorylation. Dephosphorylation releases TORC from 14-3-3, allowing its translocation to the nucleus and binding to the dimerization domain of CREB, which is necessary for CREB mediated transcription in a number of genes [38]. In addition to cyclic AMP-dependent inhibition of TORC kinases, the calcium/calmodulin dependent phosphatase, calcineurin, dephosphorylates and therefore facilitates TORC activation. TORC 1 is the most abundant and widely distributed in the brain, but all three TORC subtypes are present in the PVN [39].

Concerning the role of TORC on CRH transcription, most in vivo and in vitro studies have focused on TORC 2 but there is also evidence for the involvement of TORC 3 [35, 36]. Studies in rats show co-localization of TORC 2 immunostainining in 100% of CRH neurons in the PVN. In basal conditions TORC 2 immunostaining is cytoplasmic, while following 30 min restraint stress undergoes nuclear translocation. In vitro, overexpression of TORC 2 or TORC 3 in the hypothalamic cell line 4B potentiates the effect of the cyclic AMP stimulator, forskolin, on CRH promoter activity. Moreover, knockdown of endogenous expression of TORC isoform using silencing RNA (siRNA) inhibits the stimulatory effect of forskolin on CRH transcription in a reporter gene assay in 4B cells, or CRH hnRNA production in primary cultures of hypothalamic neurons. Combined knockdown of TORC 2 and TORC 3 completely blocked cyclic AMP-stimulated CRH promoter activity without affecting CREB phosphorylation. In addition, immunohistochemistry and western blot studies have shown that cyclic AMP-induction of CRH transcription is associated with rapid TORC translocation to the nucleus. On the other hand, the inability of CREB phosphorylation by phorbolesters to induce CRH transcription is associated with cytoplasmic sequestration and hyperphosphorylation of TORC. Co-immunoprecipitation experiments in 4B cells demonstrate that forskolin but not phorbolesters induce association of TORC 2 protein with CREB. Finally, chromatin immunoprecipitation studies reveal increased association of TORC 2 and CREB proteins with the CRH promoter after treatment of 4B cells with forskolin but not with PMA. In keeping with the physiological importance of TORC on CRH transcription in vivo, immunoprecipitation of hypothalamic chromatin of

rats subjected to restraint stress, revealed recruitment of TORC 2 and phospho-CREB by the CRH promoter at 30 min, but 3 h after stress, when CRH transcription has returned to basal, only phospho-CREB remained associated with the CRH promoter [35, 36].

The mechanism of activation/inactivation of TORC in CRH neurons involves salt inducible kinase (SIK) [40]. There are two major isoforms of SIK, SIK1 and SIK2 and both are present in the dorsomedial PVN, site corresponding to the location of CRH neurons. Restraint stress causes dramatic induction of SIK1 mRNA and smaller induction in SIK2 in this region. Overexpression of either SIK1 or SIK2 in the hypothalamic cell line 4B reduces nuclear TORC 2 levels and inhibited forskolin-stimulated CRH transcription. Conversely, the SIK inhibitor, staurosporine, increases nuclear TORC 2 content and stimulates CRH transcription. Specific shRNA knock down of endogenous SIK2 induces nuclear translocation of TORC 2 and CRH transcription, in spite of a compensatory increase of SIK1. Suppression of SIK1 has no effect on TORC translocation or CRH transcription. The SIK overexpression experiments indicate that both SIK1 and SIK2 can inhibit TORC translocation and CRH transcription, but the lack of effect of SIK1 blockade and the fact that increases in SIK1 due to SIK2 knock down cannot compensate for the lack of SIK2, suggest that SIK2 is responsible for maintaining phosphorylated TORC in the cytoplasm. The temporal pattern of induction of SIK1, reaching a maximum at the time when TORC content in the nucleus and CRH transcription declines, suggests that SIK1 induction and activation limits transcriptional responses of the CRH gene. The overall evidence indicates that the CREB co-activator, TORC is essential for activation of CRH transcription, and that regulation of the SIK/TORC system by stress-activated signaling transduction pathways acts as a sensitive switch mechanism for rapid activation and inactivation of CRH transcription (Fig. 5).

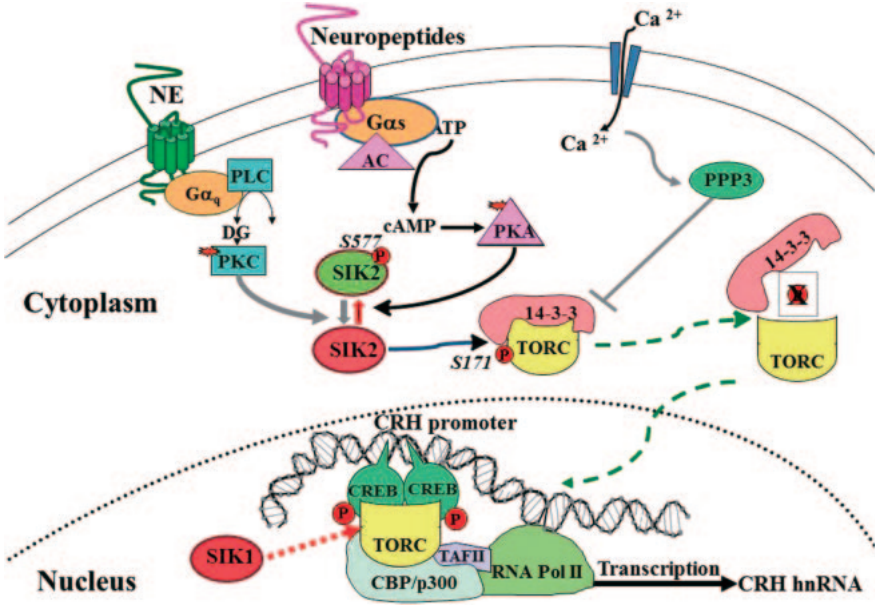

Fig. 5. **Diagram representing the signaling pathways regulating TORC translocation to the nucleus and activation of CRH transcription** In basal conditions TORC is in the cytoplasm, in an inactive state phosphorylated at Ser 171 and bound to the scaffolding protein 14-3-3. Based on the described effect of the phorbol ester, PMA, on TORC phosphorylation, and known ability of salt inducible kinase (SIK) to phosphorylate TORC, it is likely that protein kinase C (PKC) activates SIK, which in turn maintains TORC phosphorylated in the cytoplasm. Stimulation of adenylate cyclase (AC) by forskolin or a G-protein coupled receptor (not shown) leads to cyclic AMP production and stimulation of protein kinase A (PKA), which phosphorylates SIK at Ser 577 and inactivates it, blocking TORC phosphorylation. In addition, increase in intracellular calcium stimulates the phosphatase, calcineurin (PPP3), which has been shown to dephosphorylate TORC. Upon dephosphorylation, TORC dissociates from 14-3-3 nad translocates to the nucleus (indicated by the green dotted lines) where it interacts with CREB at the cyclic AMP response element in the CRH promoter and facilitates the recruitment the transcription initiation complex including CREB binding protein (CBP)/p300, TATA binding protein (TBP), TBP associated factors (TAFII) and RNA polymerase II (Pol II).

3.2 Negative regulation of CRH expression

Since excessive production of CRH can lead to disease through over stimulating HPA axis activity and direct effects in the brain, a prompt termination of activation of the CRH neuron following stress response is also essential for homeostasis.

3.2.1 Glucocorticoid feedback inhibits CRH secretion and expression

Glucocorticoid feedback plays a critical role in preventing HPA axis hyperactivity and the hypothalamic CRH neuron is an important target. The steroid inhibits CRH secretion and expression through a number of mechanisms.

First, the rapid inhibition of CRH secretion depends on non-genomic actions, mediated partly by retrograde inhibition of glutamaergic transmission by endocannabinoids in the PVN [41]. In vitro and in vivo evidence indicates that glucocorticoids and stress induce rapid synthesis of anandamide and 2-arachidonoylglycerol in CRH neurons of the PVN, and act as retrograde messengers by binding to presynaptic CB1 receptors. Pharmacological of genetic disruption of CB1 function increases CRH expression in the PVN and plasma levels of ACTH and corticosterone. Some reports suggest that this non-genomic effect of glucocorticoids depends on a G-protein coupled receptor [42]. However, the lack of rapid effects of dexamethasone in hypothalamic slices of mice bearing targeted deletion of GR in the PVN, strongly suggests that the classical GR mediates non-genomic effects [43]. Supporting the involvement of the classical GR in mediating the rapid effects of glucocorticoids, immunohistochemical and biochemical studies have shown membrane localization of GR in a number of systems [26, 44–47].

A second mechanism involves glucocorticoid-induced activation of GR in limbic areas, such as the hippocampus and the frontal cortex, resulting in stimulation of inhibitory neural pathways to the CRH neuron [48]. This has been demonstrated by the fact that glucocorticoid implants in the medial frontal cortex inhibit HPA axis responses to psychogenic stressors. Moreover, mice with selective GR deletion in the cortex, hippocampus and amygdala have elevated basal and prolonged corticosterone responses to psychogenic stress, as well as reduced HPA axis inhibition following exogenous glucocorticoid administration [49].

Thirdly, there is evidence that glucocorticoids modulate stimulatory catecholaminergic and peptidergic afferents pathways to the PVN. Microdialysis studies in rats show that peripheral administration of glucocorticoids inhibits stress-induced norepinephrine release in the PVN [50]. Glucocorticoids also influence the neurotransmitter receptors in the PVN, as adrenalectomy increases and glucocorticoid administration decreases the content of alpha adrenergic receptors in the PVN. Another target of glucocorticoid feedback is the excitatory peptide GLP-1, produced by non-catecholaminergic neurons of the nucleus of the solitary tract and released in the PVN during stress. Stress-induced glucocorticoid surge or glucocorticoid injection in rats causes rapid decreases of the GLP-1 precursor protein, pre-pro-glucagon mRNA, suggesting an increase in translation, and increases in hnRNA indicating transcriptional activation [23].

Glucocorticoids also inhibit CRH transcription but most evidence indicates that in physiological conditions indirect mechanisms mediate this effect [51, 52]. First, suppression of the glucocorticoid surge by adrenalectomy does not affect the declining phase of stress induced CRH hnRNA, indicating that factors other than glucocorticoids are responsible for the rapid decline of CRH transcription during persistent stress. Second, injection of high doses of corticosterone at the time of stress exposure does not prevent the increases in CRH hnRNA induced by stress [53]. Third, glucocorticoids do not directly affect cyclic AMP induced CREB phosphorylation and nuclear accumulation of the co-activator TORC in vitro [52]. Fourth, GR immunoprecipitation in hypothalamic chromatin from rats injected with corticosterone show a lack of GR recruitment by the CRH promoter, in spite of marked recruitment by the glucocorticoid-dependent, period 1 gene [52]. Previous reports have shown that a conserved sequence upstream of the essential −247 CRE is capable of binding GR in gel shift assays and mediates glucocorticoid dependent repression in reporter gene assays [54, 55]. However, the lack of GR recruitment by the CRH promoter in vivo is against a direct interaction of the GR with this atypical GRE. Although GR interaction at distal promoter sites or with other transcriptional proteins may play a role in the minor inhibition of CRH transcription observed in vitro, it appears that the repressor effects of glucocorticoids on CRH expression in vivo are predominantly indirect, through modulation of pathways regulating CRH neuron function.

3.2.2 Intracellular feedback mechanisms limiting CRH transcription

In addition to long-loop feedback, most intracellular mechanisms are autoregulated at the level of signaling cascades leading to gene induction or post-translational modifications of existing proteins. There is evidence for involvement of at least two intracellular control mechanisms regulating CRH transcription.

First, the rapid induction of CREB phosphorylation, TORC translocation and CRH hnRNA in CRH neurons of the PVN by stress, is followed by delayed increases in expression of the transcriptional repressor, Inducible Cyclic AMP Early Repressor (ICER) [53, 56, 57]. ICER, a product of activation of the second promoter of the CREM gene, represses cyclic AMP-induced gene transcription in several neuroendocrine tissues, by inhibiting the effect of phospho-CREB at the CRE [58]. Gel shift assays and chromatin immunoprecipitation experiments reveal ICER recruitment by the CRH promoter paralleling decreases in Pol II binding. Thus, delayed induction of ICER and binding to the CRE in the CRH promoter probably contribute to the declining phase of stress-induced CRH transcription.

Second, another potential intracellular mechanism limiting the activation of CRH transcription during stress is regulation of the activity of the CREB co-activator, TORC by salt inducible kinase 1 and 2. Activation of TORC by PKA-induced inactivation of SIK2 allows TORC translocation to the nucleus and initiation of CRH transcription during stress. Stress also causes rapid induction and activation of SIK1 which would mediate phosphorylation and export of TORC from the nucleus and termination of CRH transcription [40].

4. Concluding remarks

Corticotropin releasing hormone is the major regulator of HPA axis activity, as well as a mediator of behavioral and

autonomic responses to stress. The neurons responsible for the hypophyseotrophic actions of CRH are located in the parvocellular region of the PVN. Activation of afferent projections to these neurons during stress triggers signaling transduction mechanisms leading to rapid secretion of CRH, followed by increases in CRH gene transcription, translation and processing of newly synthesized peptide. Transcriptional activation of the CRH gene is mediated by cyclic AMP/protein kinase A/phospho-CREB dependent pathways, and requires the recruitment of phopho-CREB by the proximal CRH promoter and protein-protein association of phopho-CREB with the co-activator, Transducer of regulated CREB activity (TORC).

While activation of the CRH neuron is required to restore mRNA and peptide levels, termination of the response is essential to prevent pathology associated with chronic elevations of CRH and HPA axis activity. Elevated plasma levels of adrenal glucocorticoids, as a consequence of HPA axis activation, plays an important role in limiting the magnitude and duration of the activation of CRH neurons during stress. Intracellular feedback mechanisms at the signaling transduction level also contribute to limiting the transcriptional response of the CRH gene. This includes a delayed induction of the repressor, inducible cyclic AMP early repressor (ICER), and possibly rapid sequential activation and inactivation of salt-inducible kinase (SIK) plays a primary role on CRH transcription by regulating the activity of the CREB co-activator, TORC. Understanding the mechanisms regulating the expression of CRH is essential for a better understanding of the mechanism involved in the pathogenesis of stress related disease as well as contributing to the development of new diagnostic and therapeutic tools for these disorders.

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REFERENCES

[1] Vale W, Spiess J, Rivier C, Rivier J. (1981) *Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin.* Science **213**: 1394-1397.

[2] Gold PW, Chrousos G, Kellner C, Post R, Roy A, Augerinos P (1984) *Psychiatric implications of basic and clinical studies with corticotropin-releasing factor.* Am J Psychiatry. **141**: 619-627.

[3] Müller M, Wurst W (2004) *Getting closer to affective disorders: the role of CRH receptor systems.* Trends in molecular medicine. **10**: 409-415.

[4] Swanson LW, Sawchenko PE, Rivier J, Vale WW (1983) *Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study.* Neuroendocrinology **36**: 165-186.

[5] Herbert J, Goodyer IM, Grossman AB, Hastings MH, de Kloet ER, Lightman SL, Lupien SJ, Roozendaal B, Seckl JR (2006) *Do corticosteroids damage the brain?* J Neuroendocrinol **18**: 393-411.

[6] Holsboer F, Gerken A, Steiger A, Benkert O, Müller OA, Stalla GK (1984) *Corticotropin-releasing-factor induced pituitary-adrenal response in depression.* Lancet **1**: 55.

[7] Palkovits M, Baffi JS, Pacak K (1999) *The role of ascending neuronal pathways in stress-induced release of noradrenaline in the hypothalamic paraventricular nucleus of rats.* J Neuroendocrinol **11**: 529-539.

[8] Ulrich Lai Y, Herman J, *Neural regulation of endocrine and autonomic stress responses.* Nature reviews. Neuroscience **10**: 397-409.

[9] Lefkowitz RJ, Caron MG (1988) *Adrenergic receptors. Models for the study of receptors coupled to guanine nucleotide regulatory proteins.* J Biol Chem **263**: 4993-4996.

[10] Day HE, Campeau S, Watson SJ, Akil H (1999) *Expression of alpha(1b) adrenoceptor mRNA in corticotropin-releasing hormone-containing cells of the rat hypothalamus and its regulation by corticosterone.* J Neuroscie **19**: 10098-100106.

[11] Pieribone VA, Nicholas AP, Dagerlind A, Hökfelt T (1994) *Distribution of alpha 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes.* J Neuroscie **14**: 4252-4268.

[12] Lerea LS (1997) *Glutamate receptors and gene induction: signalling from receptor to nucleus.* Cell Signalling, 1997 **9**: 219-226.

[13] Ziegler D, Cullinan W, Herman J (2005) *Organization and regulation of paraventricular nucleus glutamate signaling systems: N-methyl-D-aspartate receptors.* J Comp Neurol **484**: 43-56.

[14] Hsu D, Price J (2009) *Paraventricular thalamic nucleus: subcortical connections and innervation by serotonin, orexin, and corticotropin-releasing hormone in macaque monkeys.* J Comp Neurol **512**: 825-848.

[15] Berg KA, Clarke WP, Sailstad C, Saltzman A, Maayani S (1994) *Signal transduction differences between 5-hydroxytryptamine type 2A and type 2C receptor systems.* Mol Pharmacol **46**: 477-484.

[16] Adler GK, Smas CM, Fiandaca M, Frim DM, Majzoub JA (1990) *Regulated expression of the human corticotropin releasing hormone gene by cyclic AMP.* Mol Cell Endocrinol **70**: 165-174.

[17] Itoi K, Horiba N, Tozawa F, Sakai Y, Sakai K, Abe K, Demura H, Suda T (1996) *Major role of 3′,5′-cyclic adenosine monophosphate-dependent protein kinase A pathway in corticotropin-releasing factor gene expression in the rat hypothalamus in vivo.* Endocrinology, 1996. **137**: 2389-2396.

[18] Nikodemova M, Kasckow J, Liu H, Manganiello V, Aguilera G (2003) *Cyclic adenosine 3′,5′-monophosphate regulation of corticotropin-releasing hormone promoter activity in AtT-20 cells and in a transformed hypothalamic cell line.* Endocrinology **144**: 1292-1300.

[19] Grinevich V, Fournier A, Pelletier G (1997) *Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on*

corticotropin-releasing hormone (CRH) gene expression in the rat hypothalamic paraventricular nucleus. Brain Res **773**: 190-196.

[20] Luo X, Kiss A, Makara G, Lolait SJ, Aguilera G (1994) *Stress-specific regulation of corticotropin releasing hormone receptor expression in the paraventricular and supraoptic nuclei of the hypothalamus in the rat.* J Neuroendocrinol **6**: 689-696.

[21] Nakade Y, Tsukamoto K, Iwa M, Pappas T, Takahashi T (2007) *Glucagon like peptide-1 accelerates colonic transit via central CRF and peripheral vagal pathways in conscious rats.* Autonomic neuroscie: basic & clinical **131**: 50-56.

[22] Stroth N, Liu Y, Aguilera G, Eiden LE (2011) *Pituitary adenylate cyclase-activating polypeptide controls stimulus-transcription coupling in the hypothalamic-pituitary-adrenal axis to mediate sustained hormone secretion during stress.* J Neuroendocrinology **23**: 944-955.

[23] Tauchi M, Zhang R, D'Alessio D, Seeley R, Herman J (2008) *Role of central glucagon-like peptide-1 in hypothalamo-pituitary-adrenocortical facilitation following chronic stress.* Exp Neurol **210**: 458-466.

[24] Conti AC, Maas JW, Muglia LM, Dave BA, Vogt SK, Tran TT (2007) *Distinct regional and subcellular localization of adenylyl cyclases type 1 and 8 in mouse brain.* Neuroscience **146**: 713-729.

[25] Aguilera G (1994) *Regulation of pituitary ACTH secretion during chronic stress.* Front Neuroendocrinol **15**: 321-350.

[26] Deng Q, Riquelme D, Trinh L, Low M, Tomic M, Stojilkovic S, Aguilera G (2015) *Rapid Glucocorticoid Feedback Inhibition of ACTH Secretion Involves Ligand-Dependent Membrane Association of Glucocorticoid Receptors.* Endocrinology, **In press**.

[27] Walker JJ, Terry JR, Tsaneva Atanasova K, Armstrong SP, McArdle CA, Lightman SL (2010) *Encoding and decoding mechanisms of pulsatile hormone secretion.* J Neuroendocrinol **22**: 1226-1238.

[28] Aguilera G, Liu Y (2012) The *molecular physiology of CRH neurons.* Front Neuroendocrinol **33:** 67-84.

[29] Plotsky PM (1991) *Pathways to the secretion of adrenocorticotropin: a view from the portal.* J Neuroendocrinol **3**: 1-9.

[30] Bale TL. Vale WW (2004) *CRF AND CRF RECEPTORS: Role in Stress Responsivity and Other Behaviors.* Ann Rev Pharmacol Toxicol **44**: 525-557.

[31] Ingraham HA, Albert VR, Chen R, Crenshaw EB, Xi He HPE, Kapiloff MS (1990) *A Family of Pou-Domain and Pit-1 Tissue-Specific Transcription Factors in Pituitary and Neuroendocrine Development.* Ann Rev Physiol **52**: 773-791.

[32] Khan A, Kaminski K, Sanchez Watts G, Ponzio T, Kuzmiski JB, Bains J, Watts A (2011) *MAP kinases couple hindbrain-derived catecholamine signals to hypothalamic adrenocortical control mechanisms during glycemia-related challenges.* J Neuroscie **31**: 18479-18491.

[33] Liu Y, Kamitakahara A, Kim A, Aguilera G (2008) *Cyclic adenosine 3′,5′-monophosphate responsive element binding protein phosphorylation is required but not sufficient for activation of corticotropin-releasing hormone transcription.* Endocrinology **149**: 3512-3520.

[34] Wolfl S, Martinez C, Majzoub JA (1999) *Inducible binding of cyclic adenosine 3′,5′-monophosphate (cAMP)-responsive element binding protein (CREB) to a cAMP-responsive promoter in vivo.* Mol Endocrinol **13**: 659-669.

[35] Liu Y, Coello A, Grinevich V, Aguilera G (2010) *Involvement of transducer of regulated cAMP response element-binding protein activity on corticotropin releasing hormone transcription.* Endocrinology **151**: 1109-1118.

[36] Liu Y, Knobloch HS, Grinevich V, Aguilera G. (2011) *Stress induces parallel changes in corticotrophin-releasing hormone (CRH) Transcription and nuclear translocation of transducer of regulated cAMP response element-binding activity 2 in hypothalamic CRH neurones.* J Neuroendocrinol **23**: 216-223.

[37] Takemori H, Okamoto M (2008) *Regulation of CREB-mediated gene expression by salt inducible kinase.* J Steroid Biochem Mol Biol **108**: 287-291.

[38] Conkright M, Canettieri G, Screaton R, Guzman E, Miraglia L, Hogenesch J, Montminy M (2003) *TORCs: transducers of regulated CREB activity.* Mol Cell **12**: 413-423.

[39] Watts A, Sanchez Watts G, Liu Y, Aguilera G (2011) *The distribution of messenger RNAs encoding the three isoforms of the transducer of regulated cAMP responsive element binding protein activity in the rat forebrain.* J Neuroendocrinol **23**: 754-766.

[40] Liu Y, Poon V, Sanchez Watts G, Watts A, Takemori H, Aguilera G (2012) *Salt-inducible kinase is involved in the regulation of corticotropin-releasing hormone transcription in hypothalamic neurons in rats.* Endocrinology **153**: 223-233.

[41] Tasker J, Herman J (2011) *Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis.* Stress **14**: 398-406.

[42] Di S, Malcher Lopes R, Halmos K, Tasker J (2003) *Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism.* J Neuroscie **23**: 4850-4857.

[43] Nahar J, Haam J, Chen C, Jiang Z, Glatzer NR, Muglia LJ, Dohanich GP, Herman JP, Tasker JG (2015) Rapid Nongenomic Glucocorticoid Actions in Male Mouse Hypothalamic Neuroendocrine Cells Are Dependent on the Nuclear Glucocorticoid Receptor. Endocrinology **156**: 2831-2842.

[44] Deng Q, Waxse B, Riquelme D, Zhang J, Aguilera G (2015) *Helix 8 of the ligand binding domain of the glucocorticoid receptor (GR) is essential for ligand binding.* Mol Cell Endocrinol **408**: 23-32.

[45] Johnson LR, Farb C, Morrison JH, McEwen BS, LeDoux JE (2005) *Localization of glucocorticoid receptors at postsynaptic membranes in the lateral amygdala.* Neuroscience **136**: 289-299.

[46] Komatsuzaki, Murakami G, Tsurugizawa T, Mukai H, Tanabe N, Mitsuhashi K, Kawata M, Kimoto T, Ooishi Y, Kawato S (2005) *Rapid spinogenesis of pyramidal neurons induced by activation of glucocorticoid receptors in adult male rat hippocampus.* Biochem Biophys Res Com **335**: 1002-1007.

[47] Liposits Z, Bohn MC (1993) *Association of glucocorticoid receptor immunoreactivity with cell membrane and transport vesicles in hippocampal and hypothalamic neurons of the rat.* J Neuroscie Res **35**: 14-19.

[48] Uchoa ET, Aguilera G, Herman JP, Fiedler JL, Deak T, de Sousa MBC (2014) *Novel aspects of glucocorticoid actions.* J Neuroendocrinol **26**: 557-572.

[49] Arnett M, Kolber B, Boyle M, Muglia L (2011) *Behavioral insights from mouse models of forebrain–and amygdala-specific glucocorticoid receptor genetic disruption.* Mol Cell Endocrinol **336**: 2-5.

[50] Pacak K, Palkovits M, Kvetnansky R, Matern P, Hart C, Kopin IJ, Goldstein DS (1995) *Catecholaminergic inhibition by hypercortisolemia in the paraventricular nucleus of conscious rats.* Endocrinology, 1995. **136**(11): p. 4814-4819.

[51] Aguilera G, Kiss A, Liu Y, Kamitakahara A (2007) *Negative regulation of corticotropin releasing factor expression and limitation of stress response.* Stress **10**: 153-161.

[52] Evans A, Liu Y, Macgregor R, Huang V, Aguilera G (2013) *Regulation of hypothalamic corticotropin-releasing hormone transcription by elevated glucocorticoids.* Mol Endocrinol **27**: 1796-1807.

[53] Shepard J, Liu Y, Sassone Corsi P, Aguilera G (2005) *Role of glucocorticoids and cAMP-mediated repression in limiting corticotropin-releasing hormone transcription during stress.* J Neuroscie **25**: 4073-4081.

[54] Malkoski SP, Dorin RI (1999) *Composite glucocorticoid regulation at a functionally defined negative glucocorticoid response element of the human corticotropin-releasing hormone gene.* Mol Endocrinol **13**: 1629-1644.

[55] Malkoski SP, Handanos C, Dorin RI (1997) *Localization of a negative glucocorticoid response element of the human corticotropin releasing hormone gene.* Mol Cell Endocrinol **127**: 189-199.

[56] Liu Y, Aguilera G (2009) *Cyclic AMP inducible early repressor mediates the termination of corticotropin releasing hormone transcription in hypothalamic neurons.* Cell Mol Neurobiol **29**: 1275-1281.

[57] Liu Y, Kalintchenko N, Sassone Corsi P, Aguilera G (2006) *Inhibition of corticotrophin-releasing hormone transcription by inducible cAMP-early repressor in the hypothalamic cell line, 4B.* J Neuroendocrinol **18**: 42-49.

[58] Lamas M, Monaco L, Zazopoulos E, Lalli E, Tamai K, Penna L, Mazzucchelli C, Nantel F, Foulkes NS, Sassone Corsi P (1996) *CREM: a master-switch in the transcriptional response to cAMP.* Philosophical transactions - Royal Soc Biol Scie **351**: 561-567.