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Regulatory Mechanisms of Corticotropin-Releasing Hormone and Vasopressin Gene Expression in the Hypothalamus

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Abstract

Tuberoinfundibular corticotropin-releasing hormone (CRH) neurones are the principal regulators of the hypothalamic-pituitary-adrenal (HPA)-axis. Vasopressin is primarily a neurohypophysial hormone, produced in magnocellular neurones of the hypothalamic paraventricular and supraoptic nuclei, but parvocellular CRH neurones also coexpress vasopressin, which acts as a second 'releasing factor' for adrenocorticotropic hormone along with CRH. All stress inputs converge on these hypothalamic neuroendocrine neurones, and the input signals are integrated to determine the output secretion of CRH and vasopressin. Aminergic, cholinergic, GABAergic, glutamatergic and a number of peptidergic inputs have all been implicated in the regulation of CRH/vasopressin neurones. Glucocorticoids inhibit the HPA-axis activity by negative feedback. Interleukin-1 stimulates CRH and vasopressin gene expression, and is implicated in immune-neuroendocrine regulation. cAMP-response element-binding protein phosphorylation may mediate transcriptional activation of both CRH and vasopressin genes, but the roles of AP-1 and other transcription factors remain controversial. Expression profiles of the CRH and vasopressin genes are not uniform after stress exposure, and the vasopressin gene appears to be more sensitive to glucocorticoid suppression.

When an organism is exposed to a stimulus that threatens its internal milieu (a stressor), defence reactions are aroused within the organism to protect itself ('stress' responses). According to Hans Selye (1), any kind of stressor causes common biological phenomena that mainly result from secretion of adrenal glucocorticoids. Although Selye's principal hypothesis may now need some re-evaluation, the influence of his idea is considerable, even beyond the biomedical world.

The hypothalamus is the integrating centre for stress responses, and corticotropin-releasing hormone (CRH) and vasopressin neurones convert stress signals to hormonal outputs. Tuberoinfundibular CRH neurones are present in the parvocellular subdivisions of the paraventricular nucleus of the hypothalamus (PVN). Vasopressin is coexpressed in some of these CRH neurones, and CRH and vasopressin are coreleased into the pituitary portal circulation (2). However, most vasopressin neurones are present in the magnocellular subdivisions of the PVN and the supraoptic nucleus, and these neurones project to the posterior pituitary. This review discusses how 'stress inputs' are handled by CRH/vasopressin neurones, focussing on (i) the intracellular regulatory mechanisms for CRH and vasopressin gene expression; (ii) neural and humoral factors to regulate CRH and vasopressin neurones; and (iii) implications of colocalization of CRH and vasopressin.

Regulation of CRH gene expression

CRH genes, peptides and receptors

The nucleotide sequence of the CRH gene is highly conserved throughout all vertebrates, including teleost fish, toad, chicken, golden hamster, rat, mouse, cow, sheep, pig, dog and humans. The CRH gene comprises two exons and one intron, and the entire coding region of the CRH precursor is present in exon 2. The human CRH gene is mapped on the long arm of chromosome 8 (2). Putative regulatory elements, including the CRE, AP-1, Brn-2, NGFI-B, GRE and ERE, are present in the 5′-flanking DNA sequence of the CRH gene, but the extent to which these elements are functionally involved in transcriptional regulation (*vide infra*) remains to be fully understood. Human CRH (41-amino-acid peptide, the carboxyl terminal amidated) is produced from a 196-amino-acid precursor, prepro-CRH. Two types of CRH

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receptors, CRH-R1 and CRH-R2, have been discovered (3). Two splice variants of the latter, CRH-R2α and CRH-R2β, are found in rodents and humans, and a third *N*-terminal splice variant, CRH-R2γ, has been reported in humans (4). CRH is a potent agonist for CRH-R1, and the hypophysiotropic effect of CRH is mediated by the CRH-R1 (3). Recently, three CRH-like peptides (urocortin, urocortin II or stresscopin-like peptide, urocortin III or stresscopin) have been discovered. Urocortin is a potent agonist for both receptors, and urocortin II and III are specific agonists for CRH-R2 (5). All CRH-like peptides and CRH receptors show distinct distributions in the brain, and the physiological implications of the multiple CRH system are currently under vigorous investigation (3).

CRH-immunoreactive cell bodies are primarily localized in the medial parvocellular division of the PVN in rodents. A few magnocellular neurones in the PVN and supraoptic nucleus may also be immunoreactive for CRH (2). The cytoarchitectonics of the primate hypothalamus is different from that of rodents, and parvocellular and magnocellular neurones are interspersed within the same area (Fig. 1). CRH-immunoreactivity was present in parvocellular neurones in the human PVN, and did not appear to be present in magnocellular neurones in either the PVN or supraoptic nucleus (Fig. 1) (6).

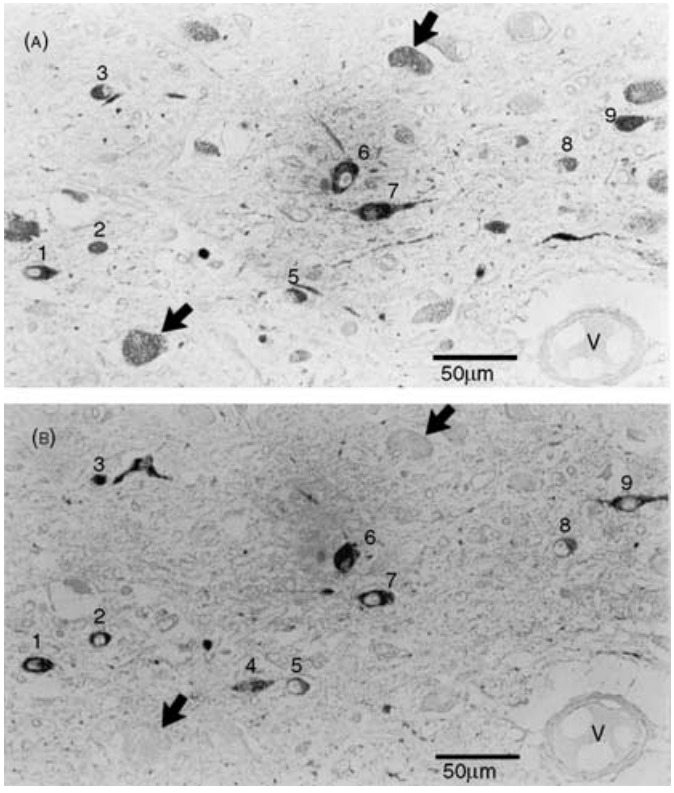

FIG. 1. Immunocytochemical localization of vasopressin- and/or corticotropin-releasing hormone (CRH)-containing neurones in the human paraventricular nucleus of the hypothalamus. Paraffin-embedded hypothalami were sectioned by a microtome, and 2.5-μm adjacent sections were stained with specific antibodies against either (A) vasopressin or (B) CRH. (A) Vasopressin-like immunoreactivity (LI) was present in both the parvocellular (#1–3 and #5–9) and magnocellular neurones (arrows). The cell #4 was not present on this section, but was demonstrated to be vasopressin-positive on a third section adjacent to (B) (not shown). (B) CRH-LI was present in parvocellular neurones (#1–9) but not in magnocellular neurones demonstrated to be vasopressin-positive in (A) (arrows). V, A blood vessel is shown for orientation. Reproduced with permission (6).

The protein kinase A (PKA) pathway and protein kinase C (PKC) pathway

The cyclic AMP (cAMP)-dependent PKA and cAMP-response element (CRE)-binding proteins (CREB) play a major role in transcriptional regulation of CRH gene expression (2). This pathway has been extensively studied *in vitro*. Forskolin or cAMP unequivocally stimulate CRH gene expression, and the cAMP-induced activation is profoundly diminished by deletion of the 5′-flanking regions containing the CRE sequence, or by mutation of CRE sequence (7). More recently, this pathway has been explored *in vivo*, using micro-injection to apply chemicals directly into the PVN of conscious rats. 8-Br-cAMP increased CRH mRNA expression in the hypothalamus, and antisense oligonucleotides against CREB attenuated insulin-induced activation of CRH mRNA expression (8). Rapid phosphorylation of CREB after ether stress, with a time course parallel to that of CRH primary transcript (heteronuclear RNA, hnRNA), is also consistent with the proposal that the PKA pathway is important for stress-induced activation of CRH expression (9).

It remains unconfirmed whether the TPA-response elements in the 5′-flanking region of the CRH gene are involved in transcriptional regulation. Application of a phorbol ester, 12-*O*-tetradecanoyl phorbol 13-acetate (TPA), stimulated CRH mRNA expression in NPLC human hepatoma cells (10), or in BE(2)M-17 and BE(2)C human neuroblastoma cells (Itoi and Seasholtz, unpublished observations) that produce CRH peptide intrinsically. However, no transcriptional activation of CRH promoter-reporter was observed after TPA treatment in other cell lines (e.g. AtT20 cells). It is therefore possible that the action of TPA on CRH expression is cell-type specific.

Glucocorticoids

CRH expression is markedly increased in the rat PVN after bilateral adrenalectomy, and this increase can be suppressed by glucocorticoids (11). The glucocorticoid suppression is specific for the PVN because CRH mRNA expression is unaffected or up-regulated in, for example, the central nucleus of the amygdala or the supraoptic nucleus (12). The molecular mechanisms for negative glucocorticoid regulation of CRH expression are not fully understood. Glucocorticoid receptors (GRs) are required, but their interactions with specific DNA-regulatory sequences and other transcription factors may be cell-type specific. Guardiola-Diaz *et al*. (13) showed that deletion and/or mutation of potential GR-binding sites, identified by DNase I protection, failed to block the negative glucocorticoid regulation. They propose that mechanisms other than direct binding of GRs to DNA may be important, and that interactions, either direct or indirect, between GR and CREB proteins may be involved. Mediation by direct binding of GRs to the DNA sequence has been proposed by another group when additional GRs are expressed in transfected AtT20 cells (14).

Determination of cell-specific expression of CRH

DNA sequences that determine tissue-specific expression of CRH have been explored using transgenic mice, and large regions of 5′- and/or 3′-flanking DNA sequences appear to be important for cell-specific expression and developmental regulation of CRH expression (15). Cell-specific expression improved significantly

with the inclusion of 5′- and 3′-flanking sequences, but even with the inclusion of 21-kb of 5′-flanking CRH DNA, the CRH transgene was not properly expressed compared to the endogenous CRH gene (16). The CRH gene contains a highly conserved intronic element sharing close similarity to the repressor element-1/neurone-restrictive silencing element (RE-1/NRSE). This element can act either as an enhancer or as a repressor of CRH gene transcription (17), but its involvement in the cell-specific expression of CRH gene remains elusive.

Regulation of vasopressin expression

Vasopressin genes, peptides, and receptors

The vasopressin gene is conserved throughout all mammalian species including rat, mouse, guinea-pig, whale, dog, cat, pig, cow, sheep and human. The arginine residue is present in the side chain of the vasopressin molecule in all these species except for the pig, in which it is substituted by lysine (lysine vasopressin). The human vasopressin gene is linked to the oxytocin gene and mapped on the short arm of chromosome 20, consisting of three exons and two introns (18). The vasopressin precursor, prepro-vasopressin, contains two additional peptides, neurophysin II and *C*-terminal glycopeptide (copeptin) in addition to vasopressin, and a signal peptide (18). Neurophysin II is important for the packaging of vasopressin in secretory granules, but the vasopressin domain itself is also crucial for correct trafficking of the prohormone through the secretory pathway (19). The role of copeptin is unknown.

Three types of vasopressin receptors, V1a, V1b and V2, have been discovered. V1a is mainly expressed in vascular walls, V1b in pituitary corticotrophs, and V2 in the kidney. V1a and V1b, but not V2, receptors are distributed in discrete brain regions (20).

The PKA/CREB pathway and PKC/AP-1 pathway

The principal role of vasopressin in magnocellular neurones is to maintain plasma osmolality, and the synthesis and secretion of vasopressin are enhanced when an animal is dehydrated, or hypertonic saline is infused. Vasopressin also helps to maintain blood pressure and to prevent further loss of body fluid under severe hypovolaemia and/or hypotension. Vasopressin expression is stimulated quite rapidly under these circumstances and a significant increase in vasopressin hnRNA expression is observed as early as 10 min after hypovolaemic stimuli (21). The vasopressin promoter region, 5′-flanking the gene, contains several cAMP-response elements, and the involvement of CREB and/or other structurally related transcription factor(s) is suggested by examination *in vitro* (22, 23). CREB can also be phosphorylated by the calcium/calmodulin kinase pathway after depolarization of vasopressin neurones and resultant increase in intracellular calcium. Immediate–early gene products, including members of the Fos/Jun family, may also activate vasopressin expression via a putative AP-1 site in the promoter region.

Glucocorticoids

The vasopressin gene, coexpressed in CRH neurones, is regulated negatively by glucocorticoids (11). This action of glucocorticoids appears to be different from that on the CRH gene, and is discussed later. Magnocellular vasopressin neurones do not express GRs under normally hydrated conditions. However, GR expression is induced in magnocellular neurones in hypo-osmotic states, and glucocorticoid/GR may help to suppress vasopressin synthesis in hyponatraemic conditions to prevent inappropriate antidiuresis (24).

Cell-specific expression of vasopressin

DNA sequences and tanscription factor(s) responsible for the tissue-specific expression of vasopressin remain elusive. Results from transgenic animal studies suggest that a *cis*-acting element(s) resides within 3-kb of both 5′- and 3′-flanking regions of the gene (25). Phenotypic analyses using mutant mice, lacking one of the putative transcription factors, revealed crucial roles of the Otp, Sim1, Arnt2 and Brn-2 in the development of vasopressin neurones (26). It is not clear whether these factors also regulate vasopressin expression in mature neurones. Neurone-specific repressors, REST/NRSF, may also be involved in the cell-specific expression of vasopressin (27).

Neural inputs and humoral factors regulating CRH/vasopressin neurones

Aminergic neurones

The PVN and SON receive large inputs from the noradrenaline- and adrenaline- containing neurones of the lower brain stem. The medial parvocellular division of the PVN, the main source of the tuberoinfundibular CRH fibres, is innervated by the A_2-noradrenergic and C_{1-3}-adrenergic cell groups of the medulla oblongata. The A_6-noradrenergic cell group also innervates the parvocellular PVN, whereas the A_1-noradrenergic group mainly innervates the magnocellular division of the PVN and the SON (2).

Despite earlier reports by Ganong and colleagues suggesting an inhibitory role of noradrenaline in regulation of the hypothalamic-pituitary-adrenal (HPA)-axis (28), more recent studies have shown that noradrenaline stimulates CRH neurones (2). Itoi *et al*. (29) reported, for the first time, that micro-injection of noradrenaline directly into the PVN of conscious rats stimulated CRH mRNA expression. The importance of the use of conscious animals needs to be emphasized in examining the action of neurotransmitters, because anaesthetics may modulate or abolish actions of transmitters administered exogenously, including noradrenaline and acetylcholine. In a further study, using hybridization histochemistry with riboprobes corresponding to a CRH intronic sequence, noradrenaline was demonstrated to stimulate CRH gene transcription very rapidly (Fig. 2) (30), and this was confirmed subsequently by Cole and Sawchenko (31). The α_{1B}-adrenergic receptor mRNAs, among those of the three subtypes of α_1-adrenergic receptors (α_{1A}, α_{1B}, α_{1D}), are colocalized with CRH mRNAs in the rat PVN, suggesting that α_{1B}-adrenergic receptors may mediate this action of noradrenaline (32). By contrast to these data obtained *in vivo*, results obtained *in vitro* suggest the involvement of α_1-, α_2-, and β-adrenergic receptors (2). However, isolated CRH neurones or dissected hypothalami may respond differently in the absence of neural inputs from outside the hypothalamus. Actions of adrenaline have been studied less extensively than those of noradrenaline, and the role of adrenaline remains controversial.

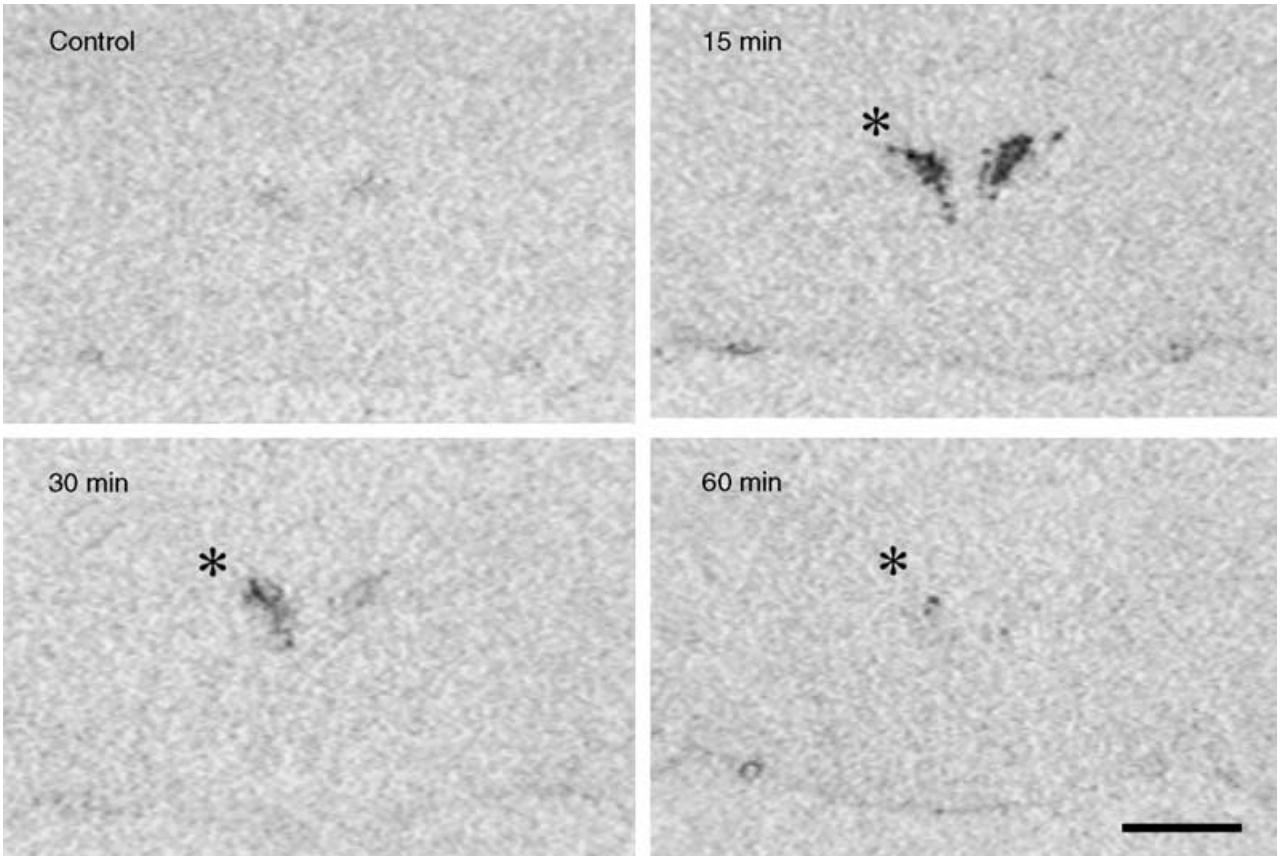

FIG. 2. Rapid induction of corticotropin-releasing hormone (CRH) primary transcript [CRH heteronuclear RNA (hnRNA)] in the paraventricular nucleus of the hypothalamus (PVN) after unilateral micro-injection of noradrenaline into the PVN of conscious rats. CRH hnRNA was almost undetectable in control rats. CRH hnRNA expression increased dramatically 15 min following injection, and the increase subsided by 60 min. The increase in CRH hnRNA expression was observed not only on the injected side, but also on the contralateral side. *Noradrenaline-injected side. Scale bar = 1 mm Reproduced with premission (30).

The PVN is densely innervated with serotoninergic fibres arising from neurones in the B_{7-9} cell groups in the midbrain. Pharmacological data unequivocally support that 5-hydroxytryptamine (5-HT) stimulates secretion and synthesis of CRH (2). The effect of 5-HT on magnocellular vasopressin neurones has been studied less extensively, but a recent study reported that a 5-HT_2 receptor agonist increased vasopressin mRNA expression in the PVN but not in the SON (33). Implications of noradrenergic and/or serotoninergic innervation of CRH neurones are intriguing in relation to the pathophysiology of depressive illness (34). Currently, reuptake inhibitors of these transmitters comprise the most common therapeutic remedies for depressive patients.

Cholinergic neurones

Acetylcholine has been proposed to be one of the transmitters to stimulate CRH neurones in the PVN, but the importance of this 'classical' transmitter has not been recognized by a majority of investigators. Micro-injection of acetylcholine into the PVN of conscious rats increased CRH mRNA expression in the PVN (35). The action of acetylcholine may be mediated by muscarinic receptors because hexamethonium, but not atropine, inhibits acetylcholine-induced adrenocorticotropic hormone (ACTH) secretion. However, in isolated hypothalami, both muscarinic and nicotinic antagonists inhibit the action of acetylcholine on CRH release (2).

Choline acetyltransferase-immunoreactive nerve terminals are present in hypothalamic areas that surround the PVN (e.g. zona incerta, perifornical nucleus and dorsal hypothalamic nuclei), but few if any punctate varicosities are observed in the PVN at the light-microscopic level. Therefore, the cholinergic influence on CRH neurones may be via interneurones, or upon dendrites of CRH neurones that lie outside the PVN. The origin of cholinergic neurones innervating areas adjacent to the PVN is not clear. There is a conspicuous zone of cholinergic neurones in the lateral tegmental area of the rostral rhombencephalon which project to the hypothalamus. This zone includes the pedunclopontine nucleus (also called the parabrachial nucleus) and the laterodorsal tegmental nucleus. Cholinergic fibres from this zone have been traced to the zona incerta and lateral hypothalamic area, adjacent to the PVN, raising the possibility that the pontine nuclei are involved in activation of CRH neurones (2). Dysregulation of cholinergic function has been postulated as an aetiologic factor in depression, because cholinomimetics and muscarinic agonists induce depressive moods both in normal subjects and patients with affective disorders (36).

Interleukins

Inflammation is highly stressful, and interleukin (IL)-1, a cytokine, has been implicated in relaying peripheral inflammatory events to the central stress circuitry. Peripheral administration of

IL-1α or IL-1β stimulates CRH mRNA expression in the PVN as well as ACTH secretion (37). Because IL-1 is a 17-kDa protein, and may not readily penetrate the blood–brain barrier, it is unclear how circulating IL-1 gains access to the brain parenchyma. Circumventricular organs such as the organum vasculosum of lamina terminalis (OVLT) or the area postrema, which lack a functional barrier, may convey IL-1-mediated signals (38, 39). However, IL-1 receptor mRNA was not observed in the OVLT, and ablation of the area postrema did not significantly inhibit IL-1β-induced activation of CRH neurones (40). Ericsson *et al.* (40) proposed that IL-1 may stimulate perivascular cells in the medulla oblongata, in which IL-1 receptors are expressed abundantly, and that these perivascular cells may activate ascending aminergic neurones to eventually stimulate CRH neurones. Induction of cyclooxygenase (COX)-2 expression by IL-1 was also reported in perivascular cells (41), and systemic administration of indomethacin, a COX inhibitor, attenuated IL-1 effects in the hypothalamus and medulla (40). Other cytokines (e.g. IL-6, tumour necrosis factor-α) are also reported to stimulate the HPA-axis (38), but their actions on the CRH gene expression are not yet reported. Cytokines are generated within the brain, and their possible roles in the HPA-axis stimulation have been postulated (38, 42).

GABA, glutamate, and other possible transmitters or modulators

GABA is a major inhibitory neurotransmitter in the hypothalamus, as in other parts of the brain. Bilateral micro-injection of mucimol (a GABA-A channel ligand) into the PVN attenuates acute stress-induced ACTH secretion (43). The PVN contains intrinsic GABA interneurones but, alternatively, GABA neurones from the bed nucleus of the stria terminalis (BNST) may mediate negative glucocorticoid feedback via the hippocampal formation because neurones in the ventral subiculum innervate BNST neurones that project to the PVN (44).

Micro-injection of kynurenic acid, an ionotropic glutamate antagonist, inhibits restraint-stress-induced activation of the HPA-axis, suggesting a stimulatory role of glutamate input to CRH neurones (45). However, direct injection of glutamate into the PVN had no clear stimulatory effect on the HPA-axis (31). This may be due to the concurrent activation of local inhibitory neurones because glutamate elicits Fos induction in GABA neurones adjacent to the PVN (31).

CRH/vasopressin neurones are innervated by a variety of peptide-containing neurones, but the physiological roles of these inputs are mostly obscure. Among these peptides, stimulatory peptide candidates for CRH neurones are neuropeptide Y, angiotensin II, cholecystokinin (CCK), enkephalin, activin and orexin. Inhibitory peptide candidates are β-endorphin, dynorphin, substance P, somatostatin and galanin (2).

Colocalization of CRH and vasopressin

Colocalization of CRH with vasopressin and other neuropeptides

A small population of parvocellular neurones stains for both CRH and vasopressin in colchicine-pretreated rats. In the external zone of the median eminence, where CRH axons make terminals at the capillary walls, more profound colocalization is observed, and nearly all CRH-positive axon terminals become vasopressin-

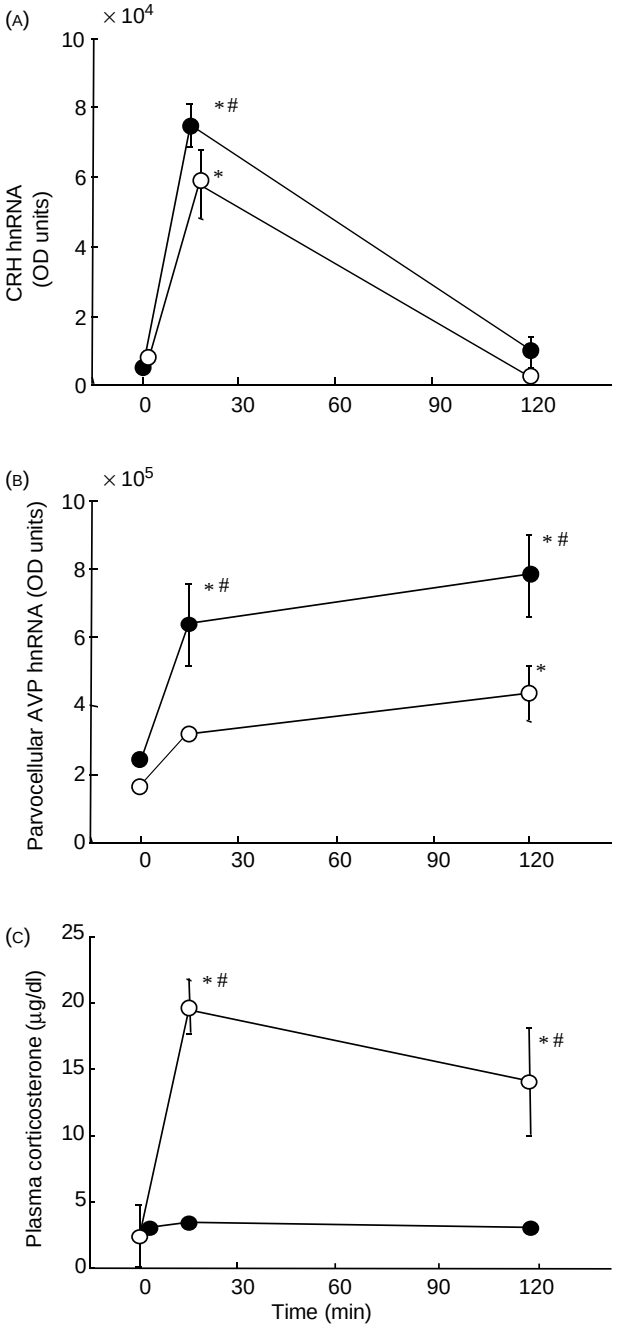

FIG. 3. Time courses of (A) corticotropin-releasing hormone (CRH) heteronuclear RNA (hnRNA), (B) vasopressin hnRNA in the parvocellular paraventricular nucleus of the hypothalamus (PVN) and (C) plasma corticosterone after noradrenaline micro-injection into the PVN in control rats (open circle) and adrenalectomized rats implanted with low-dose corticosterone pellets (closed circle). Control rats were sham-operated and implanted with cholesterol pellets. Plasma concentrations of corticosterone increased rapidly at 15 min and lowered slightly at 120 min in the control group, whereas the corticosterone levels were clamped at a low level in the adrenalectomized/low-dose corticosterone group (C). CRH hnRNA increased rapidly and the increase subsided by 120 min in both groups (A). The peak value was slightly but significantly greater in the adrenalectomized/low-dose corticosterone group (A). Vasopressin hnRNA in the parvocellular division increased rapidly and sustained in the adrenalectomized/low-dose corticosterone group, but it increased only slightly at 120 min in controls (B). OD, Optical density. $^{*}P < 0.05$ versus time = 0 in the same group; $\#P < 0.05$ versus the value in the counterpart at the same time point. Redrawn with permission (48).

positive after repeated exposure to immobilization stress (46). Virtually all CRH neurones in the PVN may therefore have the potential to produce vasopressin. In human PVN, all CRH-positive parvocellular neurones stained for vasopressin (Fig. 1) (6), but the content of vasopressin in human samples may vary depending on the pre-mortem conditions, including the severity of stress, pain and glucocorticoid medication, as well as the post-mortem time length and fixation methods. Besides CRH/vasopressin colocalization, some CRH neurones contain enkephalin, peptide histidine isoleucine (PHI), CCK or galanin (47). The physiological implications for colocalization of multiple peptides in hypothalamic CRH neurones remain elusive.

Glucocorticoid effects on vasopressin expression in CRH neurones

Although most CRH neurones do not express vasopressin in the presence of circulating corticosterone in rat PVN, bilateral adrenalectomy markedly increases the number of CRH neurones that coexpress vasopressin. Furthermore, overexpression of vasopressin after adrenalectomy was suppressed by supplementation with glucocorticoids in parallel with CRH, indicating a potent inhibitory effect of glucocorticoid on vasopressin expression (11).

Helmreich *et al*. (48) examined the possibility that elevation of circulating corticosterone after acute stress could inhibit transcriptional activation of the vasopressin gene in the parvocellular PVN using bilaterally adrenalectomized rats implanted with a low-dose corticosterone pellet. Vasopressin hnRNA expression increased significantly at a rapid phase after noradrenaline injection into the PVN in adrenalectomized rats in which plasma corticosterone was clamped at a low level (comparable to early morning), and the increase in vasopressin hnRNA was sustained at 2 h after noradrenaline injection (Fig. 3). In control rats with intact adrenals, vasopressin hnRNA expression did not increase significantly at the rapid phase when plasma corticosterone increased to a 'highly stressed' level, and increased only slightly at the delayed phase. This implies that, in normal conditions, vasopressin expression is suppressed by stress-induced increase in circulating corticosterone, and that, without this increase in corticosterone, vasopressin expression can be activated by stress at the rapid phase (Fig. 3). More surprisingly, the CRH hnRNA profile was not very different between the adrenalectomized/low-dose corticosterone and control groups, suggesting that an acute increase in plasma corticosterone does not inhibit CRH gene transcription markedly (Fig. 3). Because CRH gene transcription subsided in the adrenalectomized/low-dose corticosterone rats as in the control rats, glucocorticoid negative feedback may not be the major shut-off mechanism of CRH gene transcription after acute stress. Kovacs *et al*. (49) compared the temporal profiles of CRH and vasopressin hnRNA expression between adrenalectomized/low-dose corticosterone rats and control rats following ether stress, confirming the data by Helmreich *et al*. (48).

CRH and vasopressin responses in acute and chronic stress

Vasopressin is released into the portal circulation with CRH in response to stress and potentiates CRH-induced ACTH secretion; thus, vasopressin and CRH are both endogenous releasing peptides for ACTH (2). However, it is not clear why we need both peptides for the regulation of glucocorticoid secretion. Kovacs and Sawchenko (9) reported that CRH hnRNA expression in the parvocellular PVN increased rapidly after ether inhalation, reaching a peak at 5 min, whereas vasopressin hnRNA expression increased later, peaking at 120 min. Itoi *et al*. (30) compared also the time courses of CRH and vasopressin hnRNA expression following noradrenaline micro-injection into the PVN. CRH hnRNA expression increased markedly, and subsided within a short period of time. Parvocellular vasopressin hnRNA expression did not increase in parallel with CRH hnRNA expression; thus, noradrenaline affected transcriptional regulation of the CRH and vasopressin genes differentially. In subsequent studies, a modest but significant increase in parvocellular vasopressin hnRNA was observed at a delayed phase following noradrenaline injection (31, 48), in variance with earlier results (30). This discrepancy may be due to the difference in the exposure time and/or quantification method of *in situ* hybridization. The rapid activation of CRH gene transcription and the delayed activation of vasopressin gene transcription in the parvocellular PVN were also observed in other different stress paradigms [e.g. hypertonic saline (50) and forced swimming (51)].

It is not clear what this differential regulation of vasopressin and CRH means to an organism in the face of acute stressors, but studies performed by Ma and Lightman (52) provide some indication. These authors showed that the magnitude of the parvocellular vasopressin mRNA response increased with repeated exposure to restraint stress, despite the diminution of CRH hnRNA and mRNA responses. Furthermore, exposure to a second novel stressor (hypertonic saline) elicited an amplified vasopressin hnRNA and mRNA responses in rats previously exposed to restraint compared to naïve rats (53). According to Grinevich *et al.* (42), repeated injection of lipopolysaccharide (LPS) attenuated basal CRH mRNA values in the PVN, as well as their increase after a heterotypic stress (restraint). However, the basal vasopressin mRNA values in the parvocellular PVN were enhanced after repeated LPS injection and, furthermore, the vasopressin mRNA response after the restraint stress was as prominent as that in control animals (42). Strengthening the vasopressin gene expression may comprise the mechanism by which the HPA-axis maintains glucocorticoid secretion to protect the organism from long-lasting homotypic stressors and/or superimposed heterotypic stressors.

Conclusions

CRH was isolated from ovine hypothalamus and the amino acid sequence was determined in 1981 by Vale *et al*. (54). The primary nucleotide structure of prepro-vasopressin-neurophysin II was first elucidated in a bovine cDNA clone in 1982 by Richter *et al*. (55), the same year in which Hans Selye, the giant who coined the word 'stress', passed away. To understand how these peptides participate in maintaining the homeostasis of an organism, extensive studies have been carried out using chemical, physiological, pharmacological, histological and molecular biological methods. Currently, in addition to the medical and biological theme, stress is a social problem. The causative relationship between stress and the affective disorders has been recognized, and dysregulation of the HPA-axis is a well-known phenomenon observed in depressive patients (34). Elucidation of the regulatory mechanisms for CRH and vasopressin expression will help in the understanding of the pathophysiology of affective disorders and

provide an eventual benefit in terms of developing means for the therapy and prophylaxis of these disorders. Conditional knockouts of genes involved in the principal stress-circuitry will give us a more precise functional map of the transmission of stress responses in the brain. Comprehensive analyses of mRNAs and/or proteins expressed in specific neurones may lead to the discovery of novel molecules essential for maintaining the HPA-axis. Thus, there is the challenge of revealing the principles of the stress responses underneath the apparently complicated phenomena, as obtained in previous research work, by employing more specific and powerful approaches currently available.

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