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Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress

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Abstract

Stress-associated disorders such as melancholic depression are characterized by persistent hypothalamic–pituitary–adrenocortical (HPA) axis activation and intensive anxiety. Corticotropin-releasing hormone (CRH) appears to play an essential role in pathophysiology of such disorders. In an attempt to elucidate possible mechanisms underlying persistent activation of CRH in the central nervous system (CNS), we examined responses of hypothalamic and extrahypothalamic CRH systems to the stressors (immobilization stress or psychological stress) and interactions between these CRH systems and glucocorticoids in rats. We propose multiple feedback loops activating central CRH system: (1) attenuation of glucocorticoid-induced negative feedback on the activity of the hypothalamic and brainstem nuclei during chronic stress, (2) autoregulation of CRH biosynthesis in the hypothalamic paraventricular nucleus (PVN) through up-regulation of Type-1 CRH receptor (CRHR-1), and (3) glucocorticoid-mediated positive effects on the amygdaloid CRH system. Stress initially activates the hypothalamic CRH system, resulting in the hypersecretion of glucocorticoids from the adrenal gland. In addition, the psychological component of the stressor stimulates the amygdaloid CRH system. In the chronic phase of stress, down-regulation of GR in the PVN and other brain structures such as the locus coeruleus (LC) fails to restrain hyperfunction of the HPA axis, and persistent activation of the HPA axis further up-regulates the amygdaloid CRH system. Thus, the hypothalamic and the amygdaloid CRH systems cooperatively constitute stress-responsive, anxiety-producing neurocircuitry during chronic stress, which is responsible for the clinical manifestations of stress-associated disorders. Effects of tricyclic antidepressants (TCAs), which appear to mitigate the above mentioned multiple feedback loop forming the vicious circle to activate central CRH systems, will also be discussed.

Keywords: Corticotropin-releasing hormone; Paraventricular nucleus; Amygdala; Bed nucleus of the stria terminalis; Vasopressin; Corticosteroid receptor; Neuropeptide Y; Antidepressant

1. Introduction

Corticotropin-releasing hormone (CRH) is a key neuropeptide integrating hormonal, autonomic and behavioral responses to stress (Brown and Fisher, 1990; Menzaghi et al., 1993; Whitnall, 1993). CRH, which is synthesized in the paraventricular nucleus (PVN) of the hypothalamus, is released in the hypophyseal portal circulation to activate the pituitary–adrenocortical axis during stress (Whitnall, 1993). Glucocorticoids, final products of the hypothalamic–pituitary–adrenocortical (HPA) axis, are secreted from the adrenal cortex and exert a negative feedback effect on the biosynthesis and release of CRH in the PVN and ACTH in the anterior pituitary (AP), resulting in the termination of stress-induced HPA axis activation (Dallman et al., 1992).

Chrousos and Gold (1992) have described the behavioral and physical adaptation during stress. Behavioral adaptation includes arousal, vigilance, focused attention and suppression of vegetative function such as feeding and reproductive behavior. On the other hand, physical adaptation includes increased blood pressure and heart rate, inhibition of the growth and reproductive system, and containment of immune responses. The most important adaptational response, however, is the containment of the stress responses mainly through glucocorticoids. They defined stress syndrome as a failure of adequate counterregulation (i.e., maladaptational responses to stress), and divided stress-associated disorders into two categories (Chrousos and Gold, 1992). One is associated with increased stress system activity, including melancholic depression, anorexia nervosa, panic anxiety,

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Table 1
Peripheral and central responses to repeated immobilization for 7–14 days

Food intake	↓
Body weight	↓
Plasma corticosterone	↑↑
Plasma ACTH	↑
PVN	
CRH mRNA	↑
AVP mRNA	↑↑
GR mRNA	↓↓
MR mRNA	→
CRHR-1 mRNA	↑
CRHR-2 mRNA	→
Anterior pituitary	
POMC mRNA	↑
GR mRNA	→
MR mRNA	→
CRHR-1 mRNA	↓
Locus coeruleus	
TH mRNA	↑
NPY mRNA	↑
GR mRNA	↓↓
Hippocampus (CA1–3, DG)	
GR mRNA	↓↓
MR mRNA	→
Arcuate nucleus	
NPY mRNA	↑
POMC mRNA	↓
Galanin mRNA	→
Ventromedial hypothalamus	
CRHR-2 mRNA	↓

The arrows denote an increase (↑), decrease (↓), or no change (→) relative to the control group. Data are taken from Makino et al. (1995a,b, 1999a,b, 2002 (in press)).

and so on; the other includes diseases with decreased stress system activity, such as atypical depression and posttraumatic stress disorder.

In this short review, we focus on the former type of stress-associated disorders such as melancholic depression. Thus, stress-associated disorders in humans, categorized as increased stress system activity, are characterized by persistent HPA axis activation and intensive anxiety (Gold and Chrousos, 1998). Patients with such disorders appear to escape from glucocorticoid negative feedback, as indicated by centrally mediated hypercortisolemia and lack of dexamethasone suppressiveness. Since maladaptational stress responses resemble the effects of central administration of CRH, CRH is thought to play an essential role in stress-associated disorders such as melancholic depression and anorexia nervosa (Gold and Chrousos, 1998).

In rats, repeated immobilization is one chronic stress in which the HPA axis is persistently activated, homologous to stress-associated disorders. We attempt to reveal mechanisms that maintain HPA axis activation in the central nervous system (CNS) using repeated immobilization as a chronic stress model. Immobilization stress originated from Kvetnansky and Mikulaj (1970), placing the rats' head through two stainless steel loops and taping the limbs to a stainless platform with their dorsal surface up. Peripheral and central responses to repeated immobilization are summarized in Table 1. We found that multiple feedback loops activate the central CRH system by: (1) attenuation of glucocorticoid-induced negative feedback on the activity of the hypothalamic and brainstem nuclei, (2) autoregulation of CRH biosynthesis in the PVN through up-regulation of Type-1 CRH receptor (CRHR-1), and (3) glucocorticoid-mediated positive effects on the CRH system in the amygdala (Fig. 1). We also discuss the therapeutic effects of antidepressants on neuropeptides involved in the central stress responses, such as arginine vasopressin (AVP), neuropeptide Y (NPY), as well as CRH. Tricyclic antidepressants (TCAs) appear to mitigate the abovementioned multiple feedback loop forming the vicious circle to activate central CRH systems. Based on our own work, we make a brief review of the relevant articles (for reviews, see Koob, 1999; Korte, 2001; Schulkin et al., 1998).

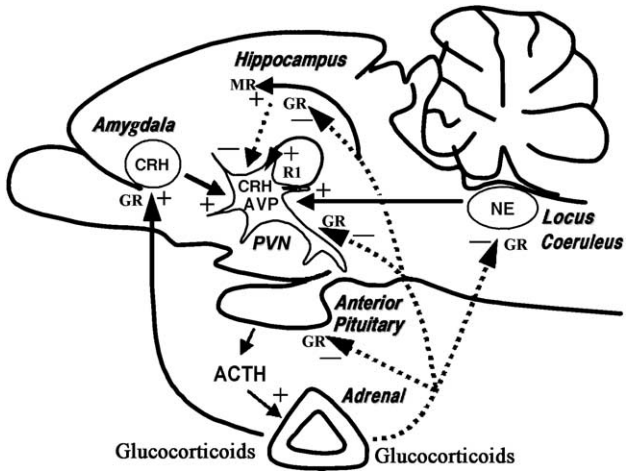

Fig. 1. Multiple feedback loops activating CRH systems during chronic stress. Stress initially activates the hypothalamic CRH system (i.e., CRH in the PVN), resulting in the hypersecretion of glucocorticoids from the adrenal gland. In addition, the psychological component of the stressor stimulates the amygdaloid CRH system (i.e., CRH in the central nucleus of the amygdala). Glucocorticoids exert GR-mediated negative feedback effects on the biosynthesis and release of CRH in the PVN and ACTH in the AP directly or indirectly through the brainstem catecholaminergic nuclei such as the LC, resulting in the termination of stress-induced HPA axis activation. In the chronic phase of stress, down-regulation of GR in the PVN and other brain structures such as the LC fails to restrain hyperfunction of the HPA axis. Increased CRH in the PVN also induces a putative ultrashort positive feedback effects on its own biosynthesis through up-regulation of PVN CRHR-1. The persistent activation of the HPA axis further up-regulates the amygdaloid CRH system involved in the expression of fear and anxiety, and the amygdala may have stimulatory effects on the HPA axis. Thus, the hypothalamic and the amygdaloid CRH systems cooperatively constitute stress-responsive, anxiety-producing neurocircuitry during chronic stress.

2. Attenuation of glucocorticoid-induced negative feedback on the activity of the hypothalamic and brainstem nuclei

Melancholic depression is a representative disorder associated with psychological and/or physical stressors in the daily life. The manifestations of this type of depression appear to be related to centrally mediated hypercortisolemia. Patients with melancholic depression often show increased CRH concentration in the cerebrospinal fluid and a lack of dexamethasone suppressiveness (Nemeroff et al., 1984; Gold et al., 1988), indicating the persistent activation of the central CRH system that has escaped from containment by high circulating cortisol. Which brain sites are involved in the activation of the CRH system? One of the most probable sites is the PVN, because PVN CRH neurons are known to stimulate the pituitary–adrenocortical axis. In fact, human studies revealed increased CRH and CRH/AVP-expressing neurons and elevated CRH mRNA levels in the PVN in depressed patients (Raadsheer et al., 1994, 1995). Then, why and how does the PVN CRH system escape from glucocorticoid-mediated negative feedback that normally exerts itself to limit overshoot of the HPA axis responsiveness to the stressor?

To elucidate this 'paradoxical' rise of the PVN CRH system during chronic stress, we compared the differential central and peripheral responsiveness to acute or repeated immobilization stress in sham vs. adrenalectomized, corticosterone replaced (ADX+CORT) rats (Makino et al., 1995a,b, 2002 (in press)). We found that responses of CRH mRNA in the PVN and tyrosine hydroxylase (TH), a rate-limiting enzyme of catecholamine biosynthesis mRNA in the locus coeruleus (LC), increased following acute and repeated immobilization both in sham and ADX+CORT rats. The magnitude of the increases in CRH and TH mRNAs was smaller in sham rats than in ADX+CORT rats following acute stress, indicating an intact glucocorticoid feedback inhibition on these mRNAs. In contrast, the magnitude of the increases in CRH and TH mRNAs was similar in both sham and ADX + CORT rats following repeated stress, suggesting an attenuation of glucocorticoid feedback inhibition during chronic stress. Interestingly, a reduction of GR mRNA in the hippocampus, the PVN, and the LC was observed following repeated stress only in sham (adrenally intact) rats. The results suggest that the glucocorticoid-dependent reduction of GR mRNA in multiple regions in the brain is associated with a decrease in the capacity of glucocorticoids to restrain the hypothalamic secretagogues that activate the pituitary corticotroph cells during chronic stress. Acute immobilization caused a significant increase in AVP mRNA in the PVN in ADX+CORT rats, but not in sham rats, whereas repeated immobilization resulted in a robust increase in PVN AVP mRNA both in sham and ADX+CORT rats. These data indicate that PVN AVP mRNA levels are more sensitive to glucocorticoid negative feedback than are the levels of CRH mRNA. During chronic or repeated stress, a relative shift could occur to AVP-mediated pituitary–adrenal activation (Hashimoto et al., 1988; Whitnall, 1989, 1993; De Goeij et al., 1991, 1992; Scaccianoce et al., 1991; Bartanusz et al., 1993) as a consequence of a reduction in the critical negative feedback stimuli to a highly sensitive target. Furthermore, a greater responsivity of AVP than CRH to chronic stimulatory stress input (Whitnall et al., 1993) (e.g., catecholaminergic input from the brainstem nuclei such as the LC) may contribute to the shift from CRH to AVP in the PVN.

In the above mentioned report (Makino et al., 1995a,b), we found an association in sham rats exposed to repeated stress between (1) decreases in GR mRNA levels in the hippocampus and the PVN, and (2) robust responses of PVN CRH and AVP mRNA levels to repeated stress despite high plasma CORT levels. Our data appeared to be compatible with two other separate findings: (1) stress down-regulates hippocampal GR (Sapolsky et al., 1984; Jacobson and Sapolsky, 1991; Chao et al., 1993; Brooke et al., 1994; Johren et al., 1994; Herman et al., 1995) and (2) hippocampal lesions increase CRH and AVP secretion in the PVN (Sapolsky et al., 1989; Herman et al., 1989, 1992; Jacobson and Sapolsky, 1991). These two findings, however, now need to be revisited.

First, our data suggest that down-regulation of hippocampal GR mRNA during repeated immobilization is glucocorticoid-dependent (Makino et al., 1995b). Consistent with this, several in vitro studies have shown a glucocorticoid-induced decrease in GR transcription (Okret et al., 1986; Dong et al., 1988; Vedeckis et al., 1989). Changes in hippocampal GR, however, depend on the type of the stressors, but not on the elevated plasma CORT per se (Herman, 1993; Herman et al., 1999; Clark et al., 1994; Kabbaj et al., 1996; Kitraki et al., 1999). Recently, we have also reported lack of decrease in hippocampal and hypothalamic GR mRNA during starvation despite a robust increase in plasma CORT, suggesting that hippocampal GR mRNA is not solely regulated by circulating CORT (Makino et al., 2001). A wide variety of neurotransmitters, which are regulated differentially in distinct stressors, may be involved in the regulation of hippocampal GR mRNA and/or binding (Herman, 1993). A recent study has shown the regulation of hippocampal GR or GR mRNA by catecholamines (through a β-adrenergic receptor), NMDA, or GABA-A receptors (Tritos et al., 1999).

Second, effects of hippocampal GR on HPA axis feedback regulation are now being revised. Accumulating evidence suggests that hippocampal MR activation maintains hippocampal excitability that relays GABAergic inhibitory tones to the PVN (Joels and deKloet, 1992; Herman and Cullinan, 1997; De Kloet et al., 1998); therefore, hippocampal MR appears to mediate the inhibitory effect of glucocorticoids in maintaining a basal HPA tone. In contrast, GR activation suppresses the hippocampal output (Joels and deKloet, 1992; De Kloet et al., 1998), theoretically resulting in the disinhibition of the HPA axis.

van Haarst et al. (1997) demonstrated that an intracerebroventricular injection of GR antagonist increased plasma ACTH and CORT levels at the diurnal peak, whereas the intrahippocampal injection of GR antagonist produced an opposite, inhibitory effect, indicating a positive glucocorticoid feedback influence on the HPA axis through hippocampal GR. They proposed the importance of GR in the PVN itself in the glucocorticoid-mediated restraint of the activity of PVN CRH and AVP neurons, based on previous reports showing suppression of CRH biosynthesis by local administration of glucocorticoids (Kovacs and Mezey, 1988; Kovacs et al., 1986; Sawchenko, 1988), and suggested that feedback inhibition through GR in the PVN may override a positive feedback effect through hippocampal GR. In this context, decreased GR mRNA levels in the PVN rather than in the hippocampus are important for the attenuation of glucocorticoid-induced negative feedback on the activity of PVN CRH and AVP neurons during repeated immobilization stress. In line with this notion, genetically impaired GR function is associated with hyperfunction of PVN CRH neurons (Tronche et al., 1999), although this is not always the case (van Haarst et al., 1996; Dijkstra et al., 1998).

Third, in addition to GR, an involvement of brain MR in the pathophysiology of melancholic depression has also drawn attention. Chronic antidepressant treatment raises hippocampal MR density (as well as hippocampal GR density), which appears to be associated with attenuation of CRH in the PVN and the HPA axis activity (Brady et al., 1991; Seckl and Fink, 1992; Reul et al., 1993, 1994; Bjartmar et al., 2001). As noted above, hippocampal MR is thought to play a principal role in the negative feedback effects of glucocorticoids primarily on the basal HPA tone at both the trough and peak of the diurnal cycle (Dallman et al., 1989; Ratka et al., 1989; Bradbury et al., 1994; van Haarst et al., 1997). Recently, Reul and Holsber's group extended this notion and showed the inhibitory role of MR in the HPA axis during stress. Reul et al. (1997) reported that, using intracerebroventricular injection of antisense oligonucleotide against MR for 1 week, down-regulation of brain MR produced an enhanced responsiveness of plasma ACTH following social defeat. Likewise, Gesing et al. (2001) found that psychologically stressful events such as novelty and forced swimming increased hippocampal MR and the rise in MR was associated with a tonic inhibition of the HPA axis activity following stress. They also suggested the possible link between CRH system activation and increased MR function during stress. However, there is limited evidence showing decreased MR (either mRNA, number, binding, or function) during chronic or persistent stress (Herman et al., 1999) and attenuation in the inhibitory effects of MR on the HPA axis during stress. The exact role of MR vs. GR in dysregulation of the HPA axis during chronic stress and that in the pathophysiology of stress-associated disorders has yet to be elucidated.

3. Autoregulation of CRH biosynthesis in the PVN through up-regulation of CRHR-1

Ono et al. (1985) reported that an intraventricular injection of ovine CRH applied 5 min prior to stress significantly enhanced increase in plasma ACTH following ether stress. They postulated that the enhancement of ACTH response to stress was attributable to the activation of CRH neurons in the PVN by applied CRH. Such a putative ultrashort positive feedback of CRH on its own biosynthesis within the PVN has recently drawn attention since the cloning of classical CRH receptor in 1993 (Perrin et al., 1993). It was termed CRHR-1 after the discovery of a novel subtype of CRH receptor designated Type-2 CRH receptor (CRHR-2) (Kishimoto et al., 1995; Lovenberg et al., 1995; Perrin et al., 1995; Stenzel et al., 1995). We, and others, have found that CRHR-1 mRNA in the PVN increases during various types of the stressor (Luo et al., 1994; Makino et al., 1995a; Rivest et al., 1995). Since a central administration of CRH increases both CRH mRNA and CRHR-1 mRNA in the PVN (Imaki et al., 1996; Mansi et al., 1996), CRH may be capable of up-regulating CRHR-1, resulting in enhancing its own biosynthesis in the PVN in a paracrine or autocrine manner. In this context, positive effects of CRH on its own receptor (CRHR-1), and vice versa, may represent one mechanism of persistent activation of CRH neurons in the PVN during stress. However, up-regulation of CRHR-1 through CRH has not definitively been proven, because we have found that adrenalectomy, which is known to increase CRH biosynthesis dramatically, decreases CRHR-1 mRNA in the PVN (Makino et al., 1995a, 1997).

The regulations of CRHR-1 mRNA in the PVN by molecules other than CRH have been examined by several investigators. We have already found that brainstem hemisection attenuated immobilization stress-induced increase in CRHR-1 mRNA ipsilaterally in the PVN (Makino et al., 1995a). Since our hemisection damages at least ascending noradrenergic bundle and results in the significant reduction of noradrenaline concentration ipsilaterally in the PVN (Pacak et al., 1993; Palkovits et al., 1999), our finding may reflect the up-regulation of PVN CRHR-1 mRNA by noradrenergic input from the brainstem during stress. However, this is not always the case (Kiss et al., 1996).

4. Glucocorticoid-mediated positive effects on the amygdaloid CRH system

The amygdala is part of the neural circuit involved in the expression of conditioned fear and anxiety (LeDoux, 1992; Davis, 1998; Fendt and Fanselow, 1999). Among the amygdaloid subnuclei, the central nucleus of the amygdala (CEA) is another main source of CRH-producing cells in the brain (Gray, 1990). Accumulating evidence suggests that activation of CRH receptors in the CEA and/or CRH pathways emanating from the CEA plays an important role in

fear-related behaviors. Electrical lesions of the CEA abolished the behavioral effects of centrally administered CRH such as conditioned startle reflex (Liang et al., 1992), while chemical lesions of the CEA blocked fear-potentiated startle reflex (Lee and Davis, 1997). The direct injection of CRH antagonist, α-helical CRH, into the CEA also diminished the stress-evoked freezing (Swiergiel et al., 1993) or reduced emotionality in socially defeated rats (Heinrichs et al., 1992). Interestingly, Pich et al. (1995) reported that pharmacological blockade of voltage-dependent K_A-channel applied to the CEA neurons increased both CRH levels in the extracellular fluid and several behavioral indices of arousal, indicating the possible link between CRH in the CEA and fear-related behavior. These functional data suggest that activated amygdaloid CRH system contributes to intensive anxiety in many depressive patients.

Alterations in the amygdaloid CRH system vs. alterations in the hypothalamic CRH system in the variety of situations are shown in Table 2. Kalin et al. (1994) reported that acute restraint increased CRH mRNA in the CEA using both RNase protection assay and in situ hybridization histochemistry (ISHH) (Hsu et al., 1998). A study using in vivo microdialysis demonstrated increased CRH levels in the CEA following restraint stress or ethanol withdrawal (Pich et al., 1995; Merali et al., 1998; Richter et al., 2000). Although we failed to show a significant change in CEA CRH mRNA following acute immobilization (Pacak et al., 1996), we found that psychological stress resulted in a dramatic increase in both CRH mRNA levels, as assessed by ISHH, and CRH content, as assessed by micropunch RIA, in the CEA (Makino et al., 1999b). Similarly, subordinate rats in the visible burrow system model of chronic social stress showed increased CRH mRNA expression in the CEA (Albeck et al., 1997). It should be noted that stressors, which contain more physical or metabolic components, such as salt-loading (Watts, 1992), cold (Makino et al., 1994a), or starvation (Makino et al., 2001) can lead to decreased CEA CRH mRNA. Thus, psychological components of the stressor could activate the amygdaloid CRH system, but the balance between the psychological and the physical component of the stressor may determine the responsivity of the amygdaloid CRH system. Greater responsiveness of the amygdaloid CRH system to a psychological component of the stressor supports the hypothesis provided by Herman and Cullinan (1997) that limbic stress pathways are sensitive to stressors involving higher-order sensory processing, but are insensitive to simple physical threats. Alternatively, stimulation of CRH in the CEA may require prior conditioning by virtue of pairing with an aversive event rather than unconditioned anxiogenic effect of the stressor (Davis, 1998). Nevertheless, in view of stimulatory effects of psychological component of the stressor on the amygdaloid CRH system, it is of interest that chronic administration of the triazolobenzodiazepine agonist alprazolam decreased CRH mRNA in the CEA and CRHR-1 mRNA expression and receptor binding in the basolateral amygdala (Skelton et al., 2000).

We have also shown that a high dose of CORT replacement increased CRH mRNA in the CEA, whereas it reduced CRH mRNA expression in the medial parvocellular part of the PVN (Makino et al., 1994b). This "positive" effect of glucocorticoids has also been reported by a number of laboratories (Swanson and Simmonds, 1989; Watts, 1996). A decrease in CRH mRNA in the CEA following adrenalectomy has also been shown (Swanson and Simmonds, 1989; Watts and Sanchez-Watts, 1995; Palkovits et al., 1998). Although direct effects of glucocorticoids on cultured amygdalar neurons are not evident (Kasckow et al., 1997), Shepard et al. (2000) recently demonstrated that stereotaxic delivery of corticosterone to the amygdala increased basal CRH mRNA levels in the CEA and increased indices of anxiety on the elevated plus-maze. Mechanisms underlying positive vs. classical negative glucocorticoid effects are uncertain, but differential combinations of glucocorticoid-responsive neurotransmitters or transcription factors in the CEA vs. the PVN may be responsible for it.

The amygdaloid CRH system may have a direct stimulatory effect on the HPA axis (Allen and Allen, 1975; Beaulieu et al., 1986, 1989; Van de Kar et al., 1991; Feldman et al., 1994), potentially activating a positive feedback loop between hypercortisolism and amygdala-mediated fear responses. Alternatively, a recent report has shown the innervation of CRH neurons in the CEA to the LC (Van Bockstaele et al., 1998). A stress-induced increase in CRH release from the nerve terminals emanating from the

Table 2
Responses of amygdala vs. hypothalamic CRH

Treatment	CRH in PVN	CRH in CEA	References
Immobilization	↑	→	(Pacak et al., 1996)
Restraint	↑	↑	(Hsu et al., 1998; Kalin et al., 1994; Merali et al., 1998; Pich et al., 1995; Richter et al., 2000)
Psychological stress	→	↑	(Makino et al., 1999b)
Chronic social stress (subordinates)	→ or ↓	↑	(Albeck et al., 1997)
Hypertonic saline	↓	↓	(Watts, 1992)
Cold exposure	→	↓	(Makino et al., 1994a,b,c)
Starvation	↓	↓	(Makino et al., 2001)
Glucocorticoids	↓↓	↑	(Makino et al., 1994b; Shepard et al., 2000; Swanson and Simmonds, 1989; Watts, 1996)
Antidepressants	↓	??	(Brady, 1994; Brady et al., 1991)
Benzodiazepines	↓	↓	(Skelton et al., 2000)

The arrows denote an increase (↑), decrease (↓), or no change (→) relative to the control group.

CEA could potentially increase CRH content in the LC, which activates LC neurons resulting in the stimulation of ACTH secretagogues such as CRH and AVP in the PVN and the stimulation of arousal-producing pathways (Chappell et al., 1986; Butler et al., 1990; Koegler-Muly et al., 1993; Valentino et al., 1993).

Another brain site, which is anatomically and functionally related to the amygdala (thus called the extended amygdala), is the bed nucleus of the stria terminalis (BNST) (Rosen and Schulkin, 1998). Davis (1998) has proposed that highly processed explicit cue information activates the CEA, whereas less explicit information activates the BNST; the CEA or the BNST in turn activates hypothalamic and brainstem target areas involved in conditioned fear or anxiety, respectively. There have also been several reports showing the involvement of the BNST in unconditioned fear (Henke, 1984; Casada and Dafny, 1991; Gray et al., 1993) or in HPA axis regulation (Dunn, 1987; Feldman et al., 1990; Herman et al., 1994). Among subdivisions in the BNST, the dorsolateral part of the BNST (BSTLD) is anatomically associated with the CEA (Gray, 1989). Interestingly, as with in the CEA, CRH mRNA in the BSTLD is positively regulated by glucocorticoids (Makino et al., 1994b,c) and is elevated following psychological stress (Makino et al., 1999b). The parallel changes in CRH mRNA in the CEA and the BSTLD have also been observed in response to cold (Makino et al., 1994a) and salt-loading (Watts, 1996). Since the CRH neurons in the BNST also target the LC dendrites, the BNST CRH neurons could be part of the neurocircuitry involved in the HPA hyperactivity during chronic stress through eliciting LC neuronal activity (Van Bockstaele et al., 1998; Koob, 1999).

Similarly, it is of great interest that glucocorticoids increased CRH mRNA expression in the dorsal parvocellular part of the PVN (Swanson and Simmonds, 1989), which project to the autonomic cell groups in the brainstem and the spinal cord including the LC and the NTS (Swanson and Sawchenko, 1983; Sawchenko, 1989; Van Bockstaele et al., 1998). It is possible that the descending CRH-nergic projection from the PVN to the LC also participates in the part of the neural circuits that continuously activates the LC neurons during chronic stress. The possible functional connection between the CEA, BNST, PVN and the LC is shown in Fig. 2.

Taken together, stress initially activates the hypothalamic CRH system, resulting in the hypersecretion of glucocorticoids from the adrenal gland. On the other hand, the psychological component of the stressor stimulates the amygdaloid CRH system (in the CEA and the BSTLD). In the chronic phase of stress, down-regulation of GR in the PVN fails to restrain hyperfunction of the HPA axis, and persistent activation of the HPA axis in turn up-regulates the amygdaloid CRH system. Thus, the hypothalamic and amygdaloid CRH systems cooperatively constitute stress-

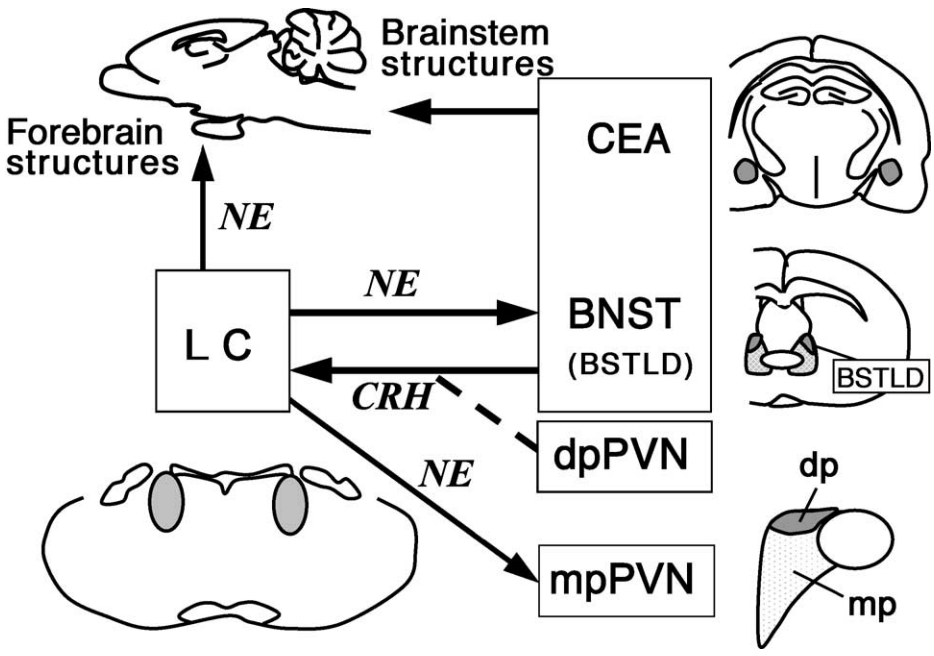

Fig. 2. The possible functional connection between the CEA, the BNST, the hypothalamic PVN, and the LC. CRH neurons emanating from the CEA and the BSTLD, and possibly from the dorsal parvocellular part of the PVN (dpPVN), innervate the LC dendrites. Released CRH elicits the LC neuronal activity which in turn activates the target forebrain structures including CRH neurons in the CEA, the BNST, and the medial parvocellular part of the PVN (mpPVN) through NE neurotransmission. Such a putative closing loop could explain the potentiation of stress responses during chronic stress and thus the pathophysiology of stress-associated disorders such as melancholic depression. Note that the projections from the LC to the forebrain structures and those from the CEA and the BNST to the brainstem structures are involved in the behavioral responses such as fear and anxiety, whereas the LC–PVN loop maintains the persistent activation of the HPA axis.

responsive, anxiety-producing neurocircuitry during chronic stress, which is one mechanism responsible for the pathophysiology of stress-associated disorders such as melancholic depression.

5. CRH, NPY, and antidepressants

The classical mechanism of action of TCAs is to inhibit the uptake of norepinephrine (NE) and/or serotonin. Chronic administration of TCAs decreases NE biosynthesis, TH activity, and TH mRNA in the LC (see, for review; Brady, 1994). Consequently, chronic TCAs treatment decreased basal CRH mRNA in the PVN and the pituitary–adrenocortical axis (Brady et al., 1991). We have also shown that CRH and AVP mRNA responses to immobilization were reduced following chronic desipramine treatment (Makino et al., unpublished observations). On the other hand, it has been proposed that a novel mechanism to decrease the HPA axis by TCAs is an up-regulation of corticosteroid receptors in the various brain regions, resulting in the potentiation of glucocorticoid-mediated negative feedback on the ACTH secretagogues in the PVN (Barden et al., 1995). It is of interest that these mechanisms of TCAs' action are opposite to the effects of chronic stress; the possible effects are to mitigate the above mentioned multiple feedback loop forming the vicious circle to activate central CRH systems, although its effects on the amygdaloid CRH system are presently uncertain.

NPY is another key molecule involved in the regulation of food intake and the modulation of the HPA axis (Dallman et al., 1993), thus, is potentially related to the pathophysiology of stress-associated disorders. We found that repeated immobilization stress resulted in the pronounced reduction of food intake and body weight (Makino et al., 1999a). As noted, CRH mRNA in the PVN increased following repeated immobilization, presumably causing appetite loss in repeatedly stressed rats. During repeated immobilization for 7 days, NPY mRNA in the hypothalamic arcuate nucleus (ARC) also increased, suggesting a compensatory response to retain appetite (Makino et al., 1999a). Although 4 days of repeated immobilization did not induce a significant increase in NPY mRNA in the ARC, treatment of TCAs, desipramine, potentiated the stress-induced rise in ARC NPY mRNA expression (Makino et al., 2000). Our findings suggest that a failure of stress-induced activation of ARC NPY could contribute to the clinical manifestation of stress-associated disorders, especially anorexia. Furthermore, in the light of the data indicating that NPY can exert anxiolytic effects via Y1 receptors (Heilig et al., 1989, 1994; Wahlestedt et al., 1993), a possible deficiency in response of ARC NPY may exacerbate hyperarousal and anxiety. In mitigating the pathological hyperarousal and anorexia of stress-associated disorders, TCAs seem able to shift the hypothalamic neuroendocrine function from a predominant CRH-mediated catabolic effector status to a more NPY-mediated anabolic effector status.

6. Perspectives

In the 1990s, a CRH-related peptide, urocortin (Vaughan et al., 1995), and a novel subtype of CRH receptor, CRHR-2 (Kishimoto et al., 1995; Lovenberg et al., 1995; Perrin et al., 1995; Stenzel et al., 1995), were subsequently discovered. Selective nonpeptidergic CRHR-1 antagonists have also been developed (Arborelius et al., 2000; Habib et al., 2000; Heinrichs and DeSouza, 1999; Higuchi et al., 2000; Keck et al., 2001; Okuyama et al., 1999; Otagiri et al., 2000) and have been on trial in humans (Willenberg et al., 2000; Zobel et al., 2000). Recently in 2001, novel CRH-related peptides, urocortin-II (Reyes et al., 2001), urocortin III (Lewis et al., 2001), or stresscopin (Hsu and Hsueh, 2001), have been identified as selective agonists of CRHR-2. On the other hand, selective CRHR-1 (Smith et al., 1998; Timple et al., 1998; Contarino et al., 1999; Bradbury et al., 2000; Contarino et al., 2000; Muller et al., 2000a,b) or CRHR-2 (Bale et al., 2000; Coste et al., 2000; Kishimoto et al., 2000) knock-out or compound CRHR1/CRHR2 mutant (Bale et al., 2002; Preil et al., 2001) has been developed in mice. These discoveries may provide new means to differentiate exact roles of CRHR-1 and CRHR-2 in the CNS (Smagin and Dunn, 2000), and provide new insights into the involvement of CRH and CRH-related peptides in the stress-associated disorders. Furthermore, revealing more precise mechanisms of persistent activation of systems of central CRH and CRH-related peptides could lead to the development of more effective drugs for stress-associated disorders by shutting down the vicious circle via multiple feedback loops upon these systems, by the functional modulation of either corticosteroid receptors or CRH receptors. In this respect, apart from genetic predisposition, early environmental and maternal care are known to affect brain corticosteroid receptor numbers and the HPA axis responsiveness in adulthood in rodents (see, for review, Caldji et al., 2000; Meaney, 2001). This indicates the importance of early life events not only as forming individual differences, but also as a prophylaxis against stress-associated disorders.

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