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Prenatal stress, anxiety and depression: a mechanism involving CRH peptide family

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Abstract

Prenatal stress (PNS) is associated with increased biological risk for mental disorders such as anxiety and depression later in life, and stress appear to be additive to the PNS influences. Among the most widely cited and accepted alternative hypotheses of anxiety and depression is dysfunction of the HPA axis, a system that is central in orchestrating the stress response. Therefore, understanding how PNS exerts profound effects on the HPA axis and stress-sensitive brain functions including anxiety and depression has significant clinical importance. In this mini-review, we will focus on novel and evolving concepts regarding the potential mechanisms underlying the short and long-term effects of PNS involving CRH peptide family. We present evidence demonstrating prenatal hypoxia exposure induced anxiety-like behavior in adult male rat offspring and CRHR1 in PVN of the hypothalamus is involved.

STRESS, HPA AXIS AND CRH FAMILY

Stress is defined as a state that threatens or is perceived by the individual to threaten his physiological equilibrium, as well as the behavioral and neurochemical reaction. One of the hallmarks of the stress response has long been considered the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The anatomical mediators of the HPA response to stress comprise the hypothalamic paraventricular nucleus (PVN), the anterior pituitary gland, and the adrenal cortex. Neurons in the PVN of the hypothalamus secrete corticotropin-releasing factor (CRH), which stimulates the synthesis and release of ACTH from the anterior pituitary. ACTH then stimulates the synthesis and release of glucocorticoids (GCs, cortisol in humans, corticosterone in rodents) from the adrenal cortex, and thus initiate responses to stress. GCs then executed negative feedback to inhibit the HPA axis at the pituitary, and at brain sites including the PVN, hippocampus and prefrontal cortex, respectively, helping to maintain homeostasis (Fink 2000).

The stress response is essential for adaptation, maintenance of homeostasis, and survival. However, chronic stress can accelerate disease processes, cause neural degeneration, and lead to anxiety, depression or other affective disorders (Nestler *et al.* 2002). In generally, hypothalamic CRH activation is a pivotal signaling molecule in

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the regulation of the HPA axis in particular and of the stress response. Therefore, comprehension of the mechanism responsible for the negative feedback regulation of CRH is of paramount importance. Indeed, dysregulation of the HPA axis and CRH signaling have been associated with development of mood disorders (Arborelius *et al.* 1999; Nemeroff 1992; Reul & Holsboer 2002).

CRH, a 41-amino acid neuropeptide, belongs to a growing family of ligands and receptors. These ligands include CRH, urocortinI (Ucn 1), Ucn 2, and Ucn 3. Although differ in their tissue distribution and pharmacology function, the members of the CRH ligand family are functionally bound through the activation of their two known distinct receptor subtypes, namely, CRHR1 and CRHR2, which belong to the class B1 subfamily of seven transmembrane G protein-coupled receptors. These neuropeptides and receptors were initially shown to be an important regulator of the endocrine stress response, and are now known to be involved in diverse roles necessary for homeostasis maintenance and important in rapid mobilization of resources and behaviors for responses to stress. CRHR1 is believed to be crucial in stress-induced HPA responsiveness and anxiety-like effects, in contrast, CRHR2 seems to be important in dampen HPA axis activity and mediate anxiolytic-like effects, as implied by the phenotypes of the CRHR1 or CRHR2 knockout mice (Timpl *et al.* 1998; Smith *et al.* 1998; Bale *et al.* 2000; Bale *et al.* 2002) and antagonists studies (Bale & Vale 2004). Dysfunction of the CRH and CRH receptor systems has been implicated in a closely link with psychiatric disorders such as anxiety and depression (Bale 2005; Hillhouse & Grammatopoulos 2006; Hauger *et al.* 2006; Hauger *et al.* 2009; Kehne & Cain 2010).

PRENATAL STRESS, ANXIETY AND DEPRESSION

Prenatal stress (PNS) is a term that stress experienced by the pregnant mother which affects the development of the offspring. Although the etiology of anxiety and depression disorders are at present unknown, it is generally accepted that the incidence of these mental illnesses could relate to early adverse life events. To account for such long-term effect, the concept of "fetal programming" for persistent organizational changes in the central nerve system (CNS) stress responses has been proposed. In 1957, Thompson firstly reported that prenatal maternal anxiety influences emotionality in young rats (Thompson 1957). Since then, a considerable number of manipulations in early development have been shown to permanently modify the development and subsequent function of HPA function in newborn, juvenile and adult offspring of many species (Phillips 2007; Fumagalli *et al.* 2007; Chung *et al.* 2005; Vallee *et al.* 1997; Richardson *et al.* 2006; Brunton & Russell 2010).

A growing body of evidence has shown that various neuropathological and psychopathological disorders are associated with adverse prenatal environments in animals and humans. In several models of recurrent or chronic PNS rodents, changes were evident at every level of the HPA axis. In these models, basal plasma CORT and ACTH levels were significantly elevated, indicative of chronic stress. In addition, the activation of this system were enduring, as plasma ACTH and CORT levels response to subsequent stress were higher in rats that had experienced PNS and remained elevated longer than their control counterparts (Vallee *et al.* 1997; Maccari *et al.* 1995; Brunton & Russell 2010). This may indicate decreased negative feedback to the HPA axis after PNS (Chung *et al.* 2005; Maccari *et al.* 1995; Brunton & Russell 2010).

Behaviorally, adult prenatal stressed rats (three 45 min periods per day during the last week of gestation) showed high anxiety-like behavior, expressed as a more time spent in the corners of the open field and a less time (%) spent in the open arms of the elevated plus-maze than rats of the control group. Physiologically, although there was no effect of the perinatal manipulations on the basal level or the stress-response peak of CORT levels, prenatal stress prolonged the stress-induced CORT response and the return to baseline values of CORT after stress was impaired in prenatal stressed rats (Vallee *et al.* 1997). When pregnant mice were exposed to repeated restraint stress from 8.5 d after pregnancy to parturition (19.5 or 20.5), their adult male offspring exhibited anxiogenic behavioral under stressful condition, which was completely blocked by CRH receptor antagonist in a dose-dependent manner. In addition, CRH contents in the hypothalamus and amygdala were significantly higher, indicating an involvement of a hyperactive CRH system, while GR mRNA levels in the hypothalamus and hippocampus were markedly lower, suggested a dysfunction in negative feedback inhibition of the HPA axis could be deteriorated by chronic stress in maternally stressed male mice (Chung *et al.* 2005). Adult male rats born to maternal experienced social defeat stress exposure during the last week of pregnancy displayed increased anxiety behaviour on the elevated plus maze. Systemic interleukin (IL)-1β or restraint increased ACTH and CORT secretion in male and female control rats, whereas HPA responses were greatly enhanced and peak hormone responses to IL-1β were greater in PNS offspring. GR mRNA expression was modestly reduced in the CA2 hippocampal subfield while significantly increased in CeA and CRH mRNA was also markedly increased in PNS offspring compared to controls. These data indicated PNS programmes anxiety behavior and HPA axis responses to stress and attenuated GCs feedback mechanisms in the limbic system may underlie HPA axis hyper-reactivity to stress in PNS offspring (Brunton & Russell 2010).

NEUROBIOLOGICAL MECHANISMS OF PNS

It has been suggested that the effects of PNS probably underlie the altered fetal physiological function firstly, and the resetting of stress system by PNS may be one of mechanisms linking early life experiences with mental disorders such as anxiety and depression later in life. Importantly, studies have shown that the HPA axis is highly susceptible to programming during development and that there are strong correlations between plasma CORT concentrations and the development of anxiety (Vallee *et al.* 1997). Thus, intrauterine programming of the HPA axis may be a mechanism underlying the observed associations between PNS and increased risk for disorders. Moreover, postnatal stress appear to be additive to the PNS influences, manifestion in sensitized HPA axis, dysfunction of brain CRH system, and impairment of brain neurocircuitry, suggesting that mental disorders are more likely to present following stress in those individuals who have experienced PNS, as has proposed of the stress-diathesis hypothesis, which suggests that exposure to stressors later in life course can lead to a maladaptive cascade of events and an increased triggering anxiety and depression episodes in individuals who have experienced PNS and dysregulated HPA function (Hellemans *et al.* 2010).

Impairment of maternal placental 11β-HSD-2 function

It has been suggested that the effects of PNS probably underlie the altered fetal physiological function, and the resetting of stress system by PNS may be one of mechanisms linking early life experiences with mental disorders such as anxiety and depression later in life. Importantly, studies have shown that the HPA axis is highly susceptible to programming during development () and that there are strong correlations between plasma CORT concentrations and the development of anxiety (Vallee *et al.* 1997). Thus, intrauterine programming of the HPA axis may be a mechanism underlying the observed associations between PNS and increased risk for disorders.

Glucocorticoids, steroid hormones produced predominantly by the adrenal gland, are key mediators of stress responses. Although whilst glucocorticoids are important during fetal development for the maturation of tissues and organs, promoting cellular differentiation, and most notably acting during late gestation to stimulate surfactant production by the lung, and these actions are critical to prepare the fetus for extrauterine life, excessive glucocorticoid exposure is associates with susceptibility to later emotional related disorders. Maternal-to-fetal transfer of glucocorticoids is predominantly regulated by a placental enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). In the placenta of rats (Waddell *et al.* 1998) and humans (Stewart *et al.* 1995), 11β-HSD2 catalyses the conversion of active corticosterone into inert 11-dehydrocorticosterone (cortisone). This enzyme normally protects the fetus from relatively high levels of maternal glucocorticoids. In contrast, placental 11β-HSD type 1 is expressed in decidua and other maternal components of the rat placenta and acts in the reverse (reductase) direction, increasing local glucocorticoid levels (Waddell *et al.* 1998). Physiologically, the glucocorticoid concentration in maternity is much higher (about 10-folds) than that in fetus (Campbell & Murphy 1977; Dalle & Delost 1976), and the placenta works as a barrier between the mother and the fetus. Normally, the fetus is protected, at least in large part, from the much higher levels of glucocorticoids in the maternal blood by placental 11β-HSD2.

Chronic restraint stress during late gestation, placental expression and activity of the glucocorticoid "barrier" enzyme 11β-HSD2 was strongly reduced (Mairesse *et al.* 2007). Pregnant mice with ethanol exposure from day 11 to 17 of gestation significantly reduced expression of placental 1β-HSD2, maternal serum corticosterone level was elevated (Liang *et al.* 2011). These results suggest that prenatal ethanol exposure induces maternal HPA axis activation and high glucocorticoid condition, which impair the placental barrier, and lead to an overexposure of elevated maternal glucocorticoid to fetus. In a recent study, 11β-HSD2$^{-/-}$ offspring of either$^{+/-}$or$^{-/-}$mothers exhibited greater anxiety than 11β-HSD2$^{+/+}$ littermates, as shown in the EPM anxiety test, 11β-HSD2$^{-/-}$ mice made significantly fewer entries into the more anxiogenic open arms and spent less time there, indicating elevated anxiety. Moreover, 11β-HSD2$^{-/-}$ mice exhibited significantly fewer crossings into the anxiogenic inner area compared with wild-type C57BL/6J mice in the open field test. This provides clear evidence for the key role of fetoplacental 11β-HSD2 in prenatal glucocorticoid programming (Holmes *et al.* 2006).

But how are PNS effects signal transduced from mother to fetus? Three major hypotheses have been proposed to underlie these associations between adverse conditions in utero and fetal HPA axis function. Exposure to elevated levels of glucocorticoids during a time of rapid brain development may be one of the major factors (Welberg *et al.* 2001; Barbazanges *et al.* 1996). Although the placenta forms a structural and biochemical barrier to many of these maternal hormones to reach the fetus, and access of maternal CORT to the fetus is regulated by the 11β-HSD2. However, this protection is incomplete and a number CORT will still enter the fetus (Barbazanges *et al.* 1996), resulting in elevated levels of fetal hormones and produced deleterious effects on brain development. When the excess stress-induced release of maternal CORT was prevented by adrenalectomy and administration of the steroid at a constant normal level, the stress response of the HPA axis in the adult offspring was normalized (Barbazanges *et al.* 1996). Moreover, elevated maternal glucocorticoids can stimulate the production of placental CRH which is identical to hypothalamic CRH in

structure, immunoreactivity, and bioactivity, placental CRH may therefore enter the fetus and affect the HPA axis (Sandman *et al.* 1997; Robinson *et al.* 1988). In addition, excess maternal CRH released by the hypothalamus during times of stress may cross the placenta and would impair the fetal HPA axis (Williams *et al.* 1998). Lastly, not all, but at least in the later gestation time, the adverse signal of maternal stress may transduce to the fetus and influence its HPA system directly. Indeed, in late gestation of rats, the fetal HPA axis and the negative feedback mechanism of glucocorticoids in the fetal brain have been shown to be functioning (Ohkawa *et al.* 1988). Hyperproduction of glucocorticoids in stressed females may, therefore, affect the development of embryonic adrenal function in their offspring. The foetus has been shown to respond to maternal stress by releasing CRH and β-endorphin from the hypothalamus and increasing plasma levels of ACTH and CORT during the late gestational period (Ohkawa *et al.* 1988). Moreover, a 30-min restraint stress in the mother on gestational day 15–17 increased the expression of CRH mRNA in the foetal PVN (Fujioka *et al.* 1999). This may permanently sensitise the foetal brain to the action of peptides or glucocorticoids released in subsequent stressful situations. Such sensitisation can have long-lasting consequences and may explain the precipitation of anxiety and depressive symptoms at adulthood by psychosocial stress.

Dysfunction of the HPA axis development later in life

Although it is not known to date whether the HPA abnormalities are a primary cause of anxiety and depression, represent an illness marker, or are secondary to another initiating cause, a pioneering work reported by Board and colleagues in 1957 that increased adrenocortical activity in psychotic, manifest as increased basal cortisol levels (Board *et al.* 1957), suggested increased HPA drive and deficits in feedback regulation in anxiety and depressed patients. Since then, a growing body of evidence has shown that dysfunction of the HPA axis is an important biomarkers for the neuropathological and psychopathological disorders such as anxiety and depression. Results from the preclinical models studies indicated circulating hormones such as ACTH and CORT in plasma or adrenal gland weight are significantly increased in depressed patients (Cryan & Mombereau 2004). In direct support of the hypothesis of CNS CRH hypersecretion in depression is the well-documented increase in CRH concentrations in lumbar cerebrospinal fluid (CSF) of depressed patients compared with healthy control subjects and patients with other psychiatric disorders in a series of studies (Nemeroff *et al.* 1984; Banki *et al.* 1992; Banki *et al.* 1987; Kasckow *et al.* 2001; Keck & Holsboer 2001). Increased CRH concentrations were also observed in cisternal CSF of suicide victims, most of whom presumably suffered from depression (Arato *et al.* 1989). Depressed patients also show increased increased CRH mRNA expression and CRH concentrations in the hypothalamic PVN and the locus coeruleus (Bissette *et al.* 2003; Raadsheer *et al.* 1994; Raadsheer *et al.* 1995). Blunted ACTH responses to CRH stimulation provide indirect evidence for CRH hypersecretion in patients with depression (Gold *et al.* 1986; Holsboer *et al.* 1984). Escape from cortisol suppression in the combined dexamethasone/CRH test, as recently observed in humans in relation to PNS, is believed to be the most sensitive marker for HPA axis dysfunction in major depression and also detects vulnerability to depression in first-degree relatives of depressed patients (Holsboer *et al.* 1995). Moreover, in nonhuman primates, early-life stress leads to persistent elevations of cerebrospinalfluid (CSF) CRH concentrations at both the juvenile and adult phases of development (Coplan *et al.* 2005). Clinical research has revealed CSF CRH elevations in depressed patients with perceived early-life stress (Carpenter *et al.* 2004; Coplan *et al.* 2011), and in patients with border line personality disorder exposed to early-life trauma (Lee *et al.* 2005). These findings are concordant with the hypothesis that increased CRH neurons activity in CNS and thereby CRH hypersecretion is, at least in part, responsible for the hyperactivity of the HPA axis characteristic of major depression.

A number of different manipulations in early development can program HPA function in adult rodents and primates. In the animals born to PNS dams, basal or stress-induced plasma ACTH and CORT levels are elevated (Samuelsson *et al.* 2004; Richardson *et al.* 2006; Koenig *et al.* 2005). In addition, PNS is generally associated with increased peak and/or extended pituitary–adrenal response duration, as was seen in the greater or more prolonged elevation of plasma ACTH and/or CORT (Vallee *et al.* 1997; Samuelsson *et al.* 2004; Richardson *et al.* 2006; Koenig *et al.* 2005; Chung *et al.* 2005; Coe *et al.* 2003; Clarke *et al.* 1994), and the effect is most marked in the later poststress samples, suggesting impaired negative feedback regulation of the HPA axis (Maccari *et al.* 1995). PNS rats also had adrenal hypertrophy, which may have resulted from chronic overstimulation of the adrenal gland by ACTH (Ward *et al.* 2000). Interestingly, prenatal exposure to a varied, unpredictable pattern of stressors did not have as much effect on HPA function as those repeated exposure to the same stressor in adult offspring (Richardson *et al.* 2006). Surprisingly, repeated exposure to the same open field increases plasma CORT in both control and PNS rats, but controls shown habituation and no longer released significant amounts of CORT after the fourth exposure, whereas the PS rats continued to release high amounts even after eight consecutive days (Fride *et al.* 1986). This indicated that PNS altered the rats' susceptibility to the later stressful testing situation. It is likely that such rats would also fail to adapt to other experimental situations to which they are exposed such as the elevated plus maze (EPM) or even to handling and cage cleaning by different personnel. PNS, but not

control rats, which had been subjected to a single 5 min exposure to novel, intimidating situations like the open field or EPM, showed higher morning resting plasma levels of CORT 3 weeks later (Weinstock *et al.* 1998). Thus, they behaved like other control rats that had been repeatedly stressed and showed higher morning levels of CORT. PNS rats also showed higher plasma CORT levels at the peak of the diurnal rhythm (Koehl *et al.* 1999).

At the level of GC feedback, several groups have shown that PS causes alterations in adult hippocampal CORT receptor density (Vallee *et al.* 1997; Chung *et al.* 2005). In a recent study reported that MR and GR mRNA expression was reduced in the hippocampus in adult rats born to both early and late IL-6 exposure dams. In adult offspring, prenatal dexamethasone treatment in the last third of pregnancy increased anxiety and depression, with a significant reduced hippocampal glucocorticoid- and mineralocorticoid receptor mRNA expression in these animals (Welberg *et al.* 2001). Hippocampal MR are involved in the control of basal HPA activity and, with GR, coordinate negative-feedback regulation after stress (Spencer *et al.* 1998; Ratka *et al.* 1989), These results suggested that permanently reduced hippocampal negative-feedback may be responsible for the increased poststress CORT levels observed in adult PS rats. In addition, baseline CRH mRNA and CRH level was increased in the hypothalamus PVN and CeA, and CRH receptor type 1 mRNA expression was increased in the pituitary in these animals (Samuelsson *et al.* 2004; Welberg *et al.* 2001), suggested that PNS exposure programs mood disorders perhaps via increased PVN and CeA CRH levels. A latest study reported that mice with forebrain-restricted inducible expression of CRH. After transient elevation of CRH during development only, behavioral testing in adult mice revealed a persistent anxiogenic and despair-like phenotype. These behavioral changes were associated with changes in CRHR1 expression. Furthermore, the despair-like changes were normalized with antidepressant treatment, the results suggest that forebrain-restricted CRH signaling during development can permanently alter stress adaptation leading to increases in maladaptive behavior in adulthood (Kolber *et al.* 2010). Overall, in this regard discussed above, one research direction is to evaluate the therapeutic potentials of weakening of the functions of the HPA axis. The obvious targets are CRH receptors expressed in the pituitary and glucocorticoid receptors expressed in the hippocampus and other brain regions, because those receptors are core components in the HPA axis and the associated feedback loop (Todorovic *et al.* 2009; Holsboer 2000; Brown *et al.* 2004).

Impairment of brain neuronal circuits

Evidence from research and clinical investigations demonstrates that the development of depression encompasses a profound neuronal circuits failure. The brain regions involved in this dysregulation may include the hypothalamus, hippocampus, amygdala, dorsal raphe nucleus (DRN) and locus coeruleus (LC) (Nestler *et al.* 2002; Liotti & Mayberg 2001).

The HPA axis forms a feedback loop via certain brain regions, one is the hippocampus, which exerts an inhibitory influence on hypothalamic CRH-containing neurons via a polysynaptic circuit, the other is the amygdale, which exerts a direct excitatory influence. It was reported that hypercortisolemia, a persistent upregulation of blood glucocorticoid levels, increases the excitotoxicity of CA3 pyramidal neurons in the hippocampus, resulting in dendritic atrophy, reduction in spinogenesis, apoptosis of neurons, and possibly inhibition of adult neurogenesis (McEwen 2007). Prenatal exposed to restraint stress from the last week pregnancy (three times a day, and each time lasted 45 min) significantly decreased the total length of the apical dendrites and the number of branch points of the apical dendrites of pyramidal neurons in hippocampus CA3 in female rats offspring, suggested PNS increases the apical dendritic atrophy (Jia *et al.* 2010). These functional abnormalities of hippocampal neurons caused by chronic stress can reduce the inhibitory tone on the HPA axis, which results in hyperactivity of the HPA-axis (Parker *et al.* 2003). Notably, hyperactivity of HPA axis is evident in approximately half of depressed patients and chronic treatment with antidepressants often reverses this phenomenon (Parker *et al.* 2003; Raison & Miller 2003). Furthermore, evidence from animal studies suggests that chronic treatment with antidepressants appears to contribute to the recovery of the abnormal function of the hippocampus by increasing neurogenesis (Tsankova *et al.* 2006; Scaccianoce *et al.* 2006). Possible interactions of CRH pathways with serotonin (5-HT) neurotransmission may be key factors influencing depression development. Electrophysiological, biochemical, and anatomical localization studies have shown direct input and potent activation of CRH fibers in the 5-HT producing DRN (Kirby *et al.* 2000; Price *et al.* 1998). CRH directly affects 5-HT release to both the striatum and lateral septum as well as alters DRN neuronal activity. Low doses of CRH were found to inhibit 5-HT release, and high doses were shown to either increase or have no effect (Valentino *et al.* 2001). These results may be attributable to the heterogeneity of the DRN or to the specific CRH receptors being activated (Valentino *et al.* 2001; Hammack *et al.* 2002). Both CRHR1 and CRHR2 have been detected in the DRN and may have opposing roles for 5-HT release (Commons *et al.* 2003; Van Pett *et al.* 2000). As CRH has a 10-fold higher affinity for CRHR1 than CRHR2, low doses of CRH in the DRN may preferentially activate CRHR1, where higher doses could potentially stimulate neurons expressing both receptors. Thus, one may hypothesize then that activation of CRHR1 inhibits 5-HT release while activation of CRHR2 may augment its release (Hammack *et al.* 2002; Valentino *et al.* 2001). Certainly, a growing body

of evidence now supports this hypothesis and has demonstrated CRH receptor specific effects on 5-HT fibers. These studies suggest that CRH pathway dysregulation could impact multiple sites within the brain to influence stress feedback systems, stress responses, and the sensitivity to further stress inputs, allowing for a proposed model of how stress influences mood disorder development (Herman *et al.* 1995). Taken together, the specific neurobiological alterations that occur as a consequence of PNS may result in effects that ultimately trigger depression after additional stress: a sensitized feed-forward cascade between the CRH and NE systems, in concert with altered hippocampal function, would drive the HPA axis, resulting in enhanced and sustained cortisol responses, which may cause further brain damage and impairment of neurogenesis, leading to further disinhibition of stress responses. Relative hypocortisolism before a given stressor might have permissive effects towards the activation of central stress responses. Alterations in protective neurotransmitter systems, as a consequence of PNS, may further accelerate this cascade.

HYPOXIA, HPA AXIS AND CRH FAMILY

Oxygen is an essential element in the survival of complex organisms, however, low levels of oxygen in tissues (hypoxia) can be the consequence of a number of pathophysiological conditions including ischemic disorders (cerebral or cardiovascular), diabetes, atherosclerosis, inflammatory diseases, psoriasis, pre-eclampsia, cancer, chronic obstructive pulmonary disease and sleep apnea (Kalaria *et al.* 2004; Veasey *et al.* 2004; Brahimi-Horn & Pouyssegur 2007). In addition, there are large populations of people live at high altitude, and many others like to visit for trekking and climbing or athletic training (Hainsworth *et al.* 2007).

Hypoxic stress may be acute, chronic or intermittent. Studies from our laboratory have specifically focused on investigating the effects of hypobaric hypoxia on HPA axis activity and central CRH family members functional regulation in adult rats (Xu *et al.* 2005; Wang *et al.* 2004; He *et al.* 2008; Chen *et al.* 2004). In these studies, as a measure of HPA axis stress physiology, we examined hypoxia intensity (2, 5, 7km), time (1, 2, 5, 15, 25d) and models (acute, chronic, intermittent) course of CORT levels, and as expected plasma CORT levels showed a substantially higher following the hypoxia than controls (normxia), suggested the HPA axis activation caused by hypoxia. In addition to the physiological HPA axis stress response, we also have examined the role of hypoxia on known CRH peptides immunoactivity in PVN, and CRHR1 and CRHR2 mRNA expression in pituitary. We found rats exposed to 5 or 7 km altitude of continual hypoxia significantly enhanced CRH release in the ME and the PVN (Chen *et al.* 2004). We also found rats exposed to continual hypobaric hypoxia at altitude of 5 km showed a significant increase CRH immunoreactivity and CRH mRNA in PVN (Xu *et al.* 2005; He *et al.* 2008). Rats exposed to simulated continuous hypoxia at 5 km altitude in a hypobaric chamber for 1, 2, 5, 10, 15 or 25 days, the results showed that 5km hypoxia caused a significant decrease CRH on days 1 and 2 while it increased on days 5 and 10. 5km induced increase in CRH and CRH mRNA in PVN at 5 days, and the effects were significantly reversed by treatment with a CRHR1 antagonist, CP-154,526, suggested continuous hypoxia stimulates CRH and CRH mRNA, and CRHR1 evidently modulates CRH release and CRH mRNA activation caused by continuous hypoxia (He *et al.* 2008).

Rats exposed to an altitude of around 2 km (16.0% O2) or 5 km (10.8% O2) intermittent hypoxia for 4 h/d caused a biphasic change in both CRHR1 and CRHR2 mRNA, there being an initial significant decline on day 1 and then an enhancement by day 2. The increase of both receptor subtypes mRNA was relatively well maintained up to 15 days in rats exposed to 2 km intermittently. CRHR2 mRNA in rats exposed to 5 km, after peaking at day 2 therefore declined and was not different to controls at 15 days. Plasma CORT levels during 5 km intermittent hypoxia for 2 days (4 h/day) were significantly increased when compared with controls. These results show that the acute response to intermittent hypoxia is a decrease in the CRH receptor mRNA whereas longer exposure is needed to provoke an increase. This may have important consequences for adaptation to high altitude (Wang *et al.* 2004). The significant differences between the expression of CRHR1 mRNA and CRHR2 mRNA in response to the hypoxia stimuli might suggest that the two receptors in the pituitary play different roles in behavior.

PRENATAL HYPOXIA AND ANXIETY

Clinical studies have demonstrated that hypoxia/ischemia during pregnancy occurs in many pathological conditions, including maternal anemia, hypertension disorder complicating pregnancy, obstructive sleep apnea syndromes, umbilical cord occlusion, reduced placental size and decreased utero-placental blood flow (Golan & Huleihel 2006). Hypoxia also occurs in some physiological conditions in pregnant women, including living, visiting or training at high-altitude hypoxia, maternal smoking and alcohol consumption. Maternal hypoxia in pregnancy has been reported to be one of the most important putative noxious signals occurring during development, which has long lasting consequences for the fetus, infant and adult (Pearce 2006; Vannucci & Hagberg 2004).

In our recently established model of prenatal intermittent hypoxia (PIH) exposure (Fan *et al.* 2009), pregnant dams are assigned to one of three treatment groups daily for 4 h throughout pregnancy (E1–E21). The PIH treatment group is placed carefully into a hypobaric chamber (simulating hypoxia at 5 km alti-

tude, 10.8% O2, 54.02 kPa). The restraint (R) group is kept individually in a transparent plastic cylinder in the animal cages. The combined group (PIH+R) is placed individually in a transparent plastic cylinder and then transferred into a hypobaric chamber for hypoxia. Each of the stress treatments was imposed once daily from 07:00 to 11:00 h. The rats of the control group were kept under sea level conditions (20.9% O2, 100.08 kPa) and left undisturbed. Behaviorally, we found PIH, R, and PIH+R combined stress during pregnancy markedly increased anxiety-like behavior, as manifested by a significant decrease in the percentage of entries into the open arms of the elevated-plus maze when compared with the control group, respectively. In addition, the percentage of time (%) into the open arms was dramatically reduced for PIH+R when compared with the control group, PIH or R alone, respectively. Physiologically, PIH, R, and PIH+R combined stress treatment during pregnancy sensitized the HPA activity, as showed by a significant increase plasma CORT and ACTH levels and adrenal weight, enhance CRHR1 mRNA and CRHR2 mRNA expression in the anterior pituitary, and increased CRH and CRHR1 expression but decreased CRHR2 in the PVN of the hypothalamus in these offspring. Furthermore, NE and DA levels were significantly increased in the LC. In all the above effects, the combination-induced effect was stronger than each stressor alone.

To address the underlying neurobiological mechanisms, we performed a series of experiments to characterize whether CRHR1 in the PVN of the hypothalamus is involved in the regulation of anxiety-like behavior in the PIH offspring. The role of CRHR1 was assessed with bilateral PVN microinjection of (1) CRHR1 selectively antagonist, antalarmin (0.5 μg) or (2) CRHR1 selectively agonist, hCRH (0.5 μg), and (3) pretreated with antalarmin followed by hCRF microinjection. The open field and elevated-plus maze were applied to test the anxiety-like behavior. The results showed that bilateral PVN microinjection of antalarmin significantly increased the distance and time to enter the central portion of the open field and the time (%) into the open arms of the elevated-plus maze only in PIH offspring, suggest the decrease of anxiety-like behavior in these animals. However, there was no effect on those of the control group. When compared with the offspring in control group, the entries and time (%) into the open arms of the EPM were markedly decreased in those of the PIH group, suggest the increase of anxiety-like behavior, and pretreated with antalarmin significantly attenuated the anxiogenic effect of hCRH in these animals. These results suggest CRHR1 in PVN of the hypothalamus, is involved in the anxiogenic effect of PIH in adult male rats offspring both in basal and mimic stress (hCRH) conditions. (Fan *et al.* 2013). We also found PIH induces anxiety-like behavior in the adult male offspring is associated with the sex-dependent expression of CRHR1 mRNA in the PVN but not in the CeA in adult male and female offspring, and the sexually differential epigenetic modification of CRHR1 genomic regions are implicated in the regulation of stress pathway programming specific to the maternal stress response (Wang *et al.* 2013). Morphology, the result of in situ hybridization combined with double immunofluorescence strengthened that CRHR1 and CRHR2 are coexpressed in CRH-specific neurons, suggest the colocalization of CRHR1 and CRHR2 in the CRH-specific neurons in PVN of the hypothalamus might be the neural basis of these effects and may be participate coordinately in the regulation of CRH neuronal activity involvement in stress responses and behavior to external stressful stimuli (Fan *et al.* 2014).

It is reported that perinatal hypoxia (12% O_2 from E19 to PD14) augmented the CORT response to restraint stress and increased basal CRH mRNA levels in the parvocellular portion of the PVN in adult male rats offspring. However, there was no effect on the levels of anterior pituitary CRHR1 mRNA or hippocampus GR mRNA, suggested that perinatal hypoxia programs the HPA axis to sensitise to acute stress in adulthood and this probably from drive from the parvocellular CRH neurons (Raff *et al.* 2007).

EFFECTS OF ENRICHED ENVIRONMENT (EE) IN POSTNATAL

The physiological and structural development of an organism is subject to complex environmental influences, and a number of studies from human and animal have demonstrated that environmental stimulation plays a critical role in neural circuit formation and function. Adverse life events induce a series of defects for the neurobiological of brain function (McEwen 2000), whereas favorable conditions such as environmental enrichment (EE) manipulations increase the brain plasticity and benefit for the psychological and behavioral response and counteract the negative effects come from the adverse environments. Studies demonstrate that pre- or postnatal stress impaired learning and memory performance of rats, however, pre- or postnatal EE housing improved behavioral performance, suggest EE treatment counteracts cognitive deficits induced by early life stress in animals (Guilarte *et al.* 2003), rescues abnormal behaviors such as emotional reactivity, motor skills and spatial learning induced by prenatal stress (Chapillon *et al.* 2002), facilitated long-term potentiation (LTP) but decreased long-term depression (LTD) in the hippocampal CA1 region (Yang *et al.* 2007). Furthermore, EE treatment in the early postnatal stage counteracts prenatal stress-induced deficits in hippocampus neurogenesis (Lemaire *et al.* 2006).

EE is also considered to improve the negative feedback function at the hippocampus. EE stimulation after weaning or during adulthood can elicit structural and functional changes in the nervous system, including increases in dendritic arborization and spine density,

synaptic protein expression, synaptic plasticity, and neurogenesis (Sale *et al.* 2009; van Praag *et al.* 2000). Rearing in EE during the first 2 weeks of mouse postnatal development promotes GABAergic neurotransmission and accelerates maturation of GABAergic and glutamatergic synapses in the hippocampal, an important regulator of early development (He *et al.* 2010).

Although rearing animals in an EE is a classic paradigm that has been used extensively in juvenile and adult rodents for studying the effect of a combination of pre- and/or postnatal life events, and EE is known to have an anxiolytic effect in several animal models (Ilin & Richter-Levin 2009; Vallee *et al.* 1997), the molecular mechanisms underlying these behavioral changes are not understood. As a critical role in initiating the cascade of biological events during the stress response, amelioration of CRH system and HPA axis activity maybe good a candidate. Rats reared in EE showed a more exploratory behavior and higher number of entries in the open arms of the EPM, suggesting a greater motivation to explore and an anxiolytic effect of EE. In addition, EE resulted in an altered daily pattern of CORT and a lower hormone response to a novel environment (hole board) (Pena *et al.* 2009). Juvenility stressed rats showed anxiety- and depressive-like behaviors and altered HPA axis activity in adulthood, which was reversed by EE manipulation both at the behavioral, endocrine and at the biochemical levels (Ilin & Richter-Levin 2009). A recent study reported that the decrease in anxiety-like behavior after housing in enriched conditions was associated with very low levels of CRHR1 mRNA expression in the basolateral amygdala of C57BL/6 mice, and was further confirmed by using a lentiviral-based system of RNA interference, that knockdown of CRHR1 mRNA expression in the basolateral amygdala induces a significant decrease in anxiety levels, similar to those achieved by EE nurture, which strongly suggest that reduced expression of CRHR1 mRNA levels in the basolateral amygdale mediates the effect of EE on anxiety-like behavior (Licinio 2010). In PNS rats there is evidence of enhanced anxiety-like behavior and increased peak and prolonged CORT secretion in response to restraint stress, but both these effects were markedly reversed following postnatal EE treatment (Vallee *et al.* 1997; Morley-Fletcher *et al.* 2003).

CONCLUSIONS

Environment during development produces sustained effects on cellular function and physiology; these effects in turn appear to form a basis for the developmental origins of vulnerability to mental health disorders such as anxiety and depression (Meaney *et al.* 2007). In the nearly three decades since CRH was characterized, a vast body of studies have demonstrated the importance of the involvement of CRH ligand and receptors in development of stress related mood disorders, with supporting evidence from behavioral, physiological, molecular and biochemical studies. From the initial agonist and antagonist infusion studies to the more recent transgenic mouse models and clinical trials, results have further strengthened an involvement of this family in the endocrine and behavioral responses to stress (Hillhouse & Grammatopoulos 2006; Hauger *et al.* 2006; Hauger *et al.* 2009; Kehne & Cain 2010). Little is known to date as to whether certain treatments can alter or reverse the neurobiological consequences of PNS. One recent study showed that mice with forebrain-restricted transient elevation of CRH during development exhibited a persistent anxiogenic and despair-like behavior and were associated with long-term increases in CRHR1 expression. Furthermore, the behavioral changes and CRHR1 mRNA expression can be reversed with imipramine antidepressant treatment (Kolber *et al.* 2010), suggested forebrain-restricted CRH signaling during development can permanently alter stress adaptation leading to increases in maladaptive behavior in adulthood. In order to improve treatment of depression, it will probably be imperative to develop treatment strategies that directly target the differential etiological and neurobiological pathways to depression. Drugs that directly target the neuronal circuits and mechanisms that are modified by PNS, such as CRH receptor antagonists, might be particularly effective in the treatment or prevention of depression related to PNS, in addition to psychotherapy, and clinicians should explore the use of pharmacological blockade of CRH receptors in a prophylactic manner before high-risk patients develop psychiatric illness.

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