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Chronic social defeat up-regulates expression of norepinephrine transporter in rat brains

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ABSTRACT

Stress has been reported to activate the locus coeruleus (LC)–noradrenergic system. However, the molecular link between chronic stress and noradrenergic neurons remains to be elucidated. In the present study adult Fischer 344 rats were subjected to a regimen of chronic social defeat (CSD) for 4 weeks. Measurements by *in situ* hybridization and Western blotting showed that CSD significantly increased mRNA and protein levels of the norepinephrine transporter (NET) in the LC region and NET protein levels in the hippocampus, frontal cortex and amygdala. CSD-induced increases in NET expression were abolished by adrenalectomy or treatment with corticosteroid receptor antagonists, suggesting the involvement of corticosterone and corticosteroid receptors in this upregulation. Furthermore, protein levels of protein kinase A (PKA), protein kinase C (PKC), and phosphorylated cAMP-response element binding (pCREB) protein were significantly reduced in the LC and its terminal regions by the CSD paradigm. Similarly, these reduced protein levels caused by CSD were prevented by adrenalectomy. However, effects of corticosteroid receptor antagonists on CSD-induced down-regulation of PKA, PKC, and pCREB proteins were not consistent. While mifepristone and spironolactone, either alone or in combination, totally abrogate CSD effects on these protein levels of PKA, PKC and pCREB in the LC and those in the hippocampus, frontal cortex and amygdala, their effects on PKA and PKC in the hippocampus, frontal cortex and amygdala were region-dependent. The present findings indicate a correlation between chronic stress and activation of the noradrenergic system. This correlation and CSD-induced alteration in signal transduction molecules may account for their critical effects on the development of symptoms of major depression.

1. Introduction

Over the past several decades, numerous investigations with human subjects have demonstrated a close correlation between stressful life events and the onset of an episode of major depression (Brown, 1998; Brown et al., 1994). Moreover, a large body of animal studies (Cui and Vaillant, 1996; Kessler, 1997; Paykel, 1994; Rosenblum et al., 1994; Tennant et al., 1981) has revealed striking parallels in the neurobiological abnormalities caused by stress and those found in depressive patients. These studies convincingly suggest a causal role of stress in the development of depression, in which prolonged stress-induced hypersecretion of glucocorticoids may form part of the intrinsic mechanism underlying the development of depression (Carrasco and Van de Kar, 2003).

On the other hand, a functional disturbance in the central noradrenergic system has also been implicated in the development of depression (Bunney and Davis, 1965). For instance, drugs that selectively antagonize norepinephrine (NE) transporter (NET) and α_2-adrenergic receptors serve as effective antidepressants. Moreover, a more direct measure of brain NE levels by placing catheters in the jugular veins of patients suffering from depression revealed a deficit in brain NE levels in these patients (Lambert et al., 2000). In addition, NE depletion leads to a relapse of depressive symptoms in depressed individuals who responded to antidepressants that inhibit NE reuptake (Berman et al., 1999; Delgado and Moreno, 2000).

Since both chronic stress and dysfunctional noradrenergic systems are involved in the development of depression, their interaction may contribute to the pathophysiology of depression. Animal studies have shown that the brain noradrenergic system is rapidly activated by different stressors (Abercrombie and Jacobs, 1987; Anisman and Sklar, 1979; Korf et al., 1973; Ritter et al., 1998), which results in an increase in NE release from terminal regions of noradrenergic nerves (Pacak et al., 1995; Rosario and Abercrombie, 1999; Smagin et al., 1997), and finally can lead to a reduction of brain NE

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levels (Glavin et al., 1983; Kvetnansky et al., 1977; Nakagawa et al., 1981; Zigmond and Harvey, 1970). Discovering molecular links underlying this interaction has important implications for elucidating the biological basis of depression and identifying new treatments. The NET may be one of target proteins involved in these interactions for its special role in the noradrenergic system.

The NET is a membrane protein primarily located on presynaptic terminals of noradrenergic nerves. In neuronal tissues, reuptake of NE by the NET is the primary mechanism by which NE transmission is inactivated at the synapse (Barker and Blakely, 1995). NET expression has not been found in the serotonergic and dopaminergic neurons. This unique distribution has led to the recognition that the presence of NET serves to identify noradrenergic neurons. Given that NET's function is related to the antidepressant effects of certain drugs and NET is one of the key proteins in the regulation of noradrenergic transmission, its expressional changes have been related to the development and symptoms of depression. For example, NET knockout mice display significantly less depressive-like behaviors than wild type controls (Xu et al., 2000), are more aggressive in early phases of stress and demonstrate inhibition of depressive-like behavior in chronic stress models (Haller et al., 2002). These findings suggest that depressive behavior in stressed animals requires functional NET and that abnormal NET expression and function could contribute to depressive symptoms (Haenisch et al., 2009; Haller et al., 2002). Likewise, the involvement of NET in stress has been reported previously (Hwang et al., 1999; Rusnak et al., 2001; Zafar et al., 1997). However, effects of stress on NET expression are inconsistent and stressor-dependent. Furthermore, there are very few reports investigating the possible regulatory mechanisms mediating the effects of stress on NET expression *in vivo*. Thus, it is crucial to evaluate the effects of chronic stress on the expression of NET in the brain and investigate the possible mechanisms by which their interaction may affect the development of depressive symptoms.

In the present study we used a rat model of chronic social defeat (CSD) to examine the effects of this chronic stressor on the expression of NET in the locus coeruleus (LC) and its projections to such terminal areas as the hippocampus, frontal cortex and amygdala. Furthermore, we investigated the expression of protein kinase A (PKA), protein kinase C (PKC), and phosphorylated cAMP-response element binding protein (pCREB) in brain regions of CSD rats to explore the possible involvement of these signal transduction-related proteins in the regulation of NET by chronic stress. Our results reveal that CSD upregulated the expression of NET in the LC and its terminal areas through effects on corticosteroid receptors. Also, PKA, PKC and pCREB proteins may be involved in this regulation. The present findings reveal an interaction between chronic stress and the noradrenergic system, which may account for their putative role in the etiology of affective psychiatric disease.

2. Materials and methods

2.1. Animals

Male Fischer 344 rats, weighing 200–250 g at the beginning of the experiment, Long-Evans retired male breeder and ovariectomized female rats were purchased from Harlan Laboratories Inc. (Indianapolis, IN, USA). All animal procedures were approved by the Animal Care and Use Committee of East Tennessee State University, and complied with the NIH Guide for the Care and Use of Laboratory Animals. Rats were maintained on a 12-h light/dark cycle (lights on at 07:00 h) with ad libitum access to food and tap water except as specifically described below. After an acclimation period of 5 days, rats were randomly assigned to experimental groups.

2.2. Chronic social defeat paradigm and drug treatment

This protocol is similar to that reported previously with minor modification (Becker et al., 2008). Each pair of Long-Evans rats (larger retired male breeders and sterile female rats) was placed in individual cages for 7 days to establish their territorial "resident" status before the beginning of experiments. Adult male Fischer 344 rats serve as the experimental "intruder" in antagonistic encounters. The experiment began by exposing an "intruder" rat to the home cage of the "resident" after the female rat had been removed. After being attacked and defeated, as shown by freezing behavior and full submissive posture, usually within 2 min, the "intruder" was rescued and placed into a small protective cage within the resident's cage, which allows unrestricted visual, auditory, and olfactory contacts with the resident but precludes further physical contact. The "intruder" was left in the cage of "resident" for one and a half hour in the protective enclosure, and then returned to its home cage where it was subsequently housed individually for the rest of the experimental period. Similarly, some male Fischer 344 rats (control) are given access to the entire resident home cage in the small protective cage when the residents have been removed. Therefore, these rats are never physically attacked and defeated by the residents. This exposure was repeated four times in the first and fourth weeks, and two times in the second and third weeks. One group of rats were adrenalectomized (ADX) by Harlan Laboratories Inc. before shipping to the animal facility of East Tennessee State University. To control for the loss of adrenal steroids, ADX rats were provided with 25 μg/ml corticosterone in their drinking water immediately after rats arrived at the animal facility and during remaining experimental period. This small replacement dose of corticosterone has been shown to be adequate for prevention of post-adrenalectomy alterations in the central nervous system (Pace et al., 2009). The sham operation for corresponding ADX was performed by opening and closing the abdomen without adrenal removal. There are two types of corticosteroid receptors in the central and peripheral nervous systems: mineralocorticoid receptors (MRs, or Type I) and glucocorticoid receptor (GRs, or Type II) (Reul and de Kloet, 1985; Spencer et al., 1990). Corticosterone binds these two types of receptors but with a difference in affinity of an order of magnitude (Spencer et al., 1993). In order to examine whether these receptors are involved in the CSD-induced regulation of NET expression, relatively specific MR antagonist spironolactone and GR antagonist mifepristone were utilized in these animals. Therefore, some groups of rats were treated with mifepristone (10 mg/kg, daily, s.c.) or spironolactone (15 mg/kg, daily, s.c.), either alone or in combination. The doses of these antagonists are chosen on the basis of previous reports (Haller et al., 1998; Macunluoglu et al., 2008; Ni et al., 1995; Ratka et al., 1989) and our preliminary experiments. All these compounds were purchased from Sigma–Aldrich (St. Louis, MO, USA). Rats in the untreated control and CSD alone groups were injected with similar volumes of vehicle in the same manner. Corticosteroid receptors antagonists or vehicle were injected 10 min prior to the CSD regimen.

2.3. Sucrose consumption test

All rats were given a free choice between two bottles, one containing a 1% sucrose solution and one containing tap water, 72 h before starting of CSD regimen to familiarize rats with the test condition. At 08:00 am of the third day, all drinking solutions were removed and at 06:30 pm all rats were again allowed access to two freshly prepared bottles of drinking solution for 1 h. Water and sucrose solution consumption was measured by comparing bottle weight before and after the 1 h window as the consumption amount. The regular water bottle was then provided after the test.